University of Crete

School of Medicine

Department of Social Medicine, Preventive Medicine and Nutrition

PhD Thesis

“The effects of fasting of the Christian Orthodox Church on bone development and osteoporosis”

«Οι επιπτώσεις της Νηστείας της Ορθόδοξης Εκκλησίας στην οστική ανάπτυξη και οστεοπόρωση»

Nikolaos E. Rodopaios

PhD Cantidate

Heraklion, Crete
2020

Επιβλέπων Καθηγητής

Λασιθιωτάκης Κωνσταντίνος, Επικ. Καθηγητής

Μέλη Επταμελούς Εξεταστικής Επιτροπής

Γαλανάκης Εμμανουήλ, Καθηγητής

Καφάτος Αντώνιος, Ομότιμος Καθηγητής

Καραντάνας Απόστολος, Καθηγητής

Καλαϊτζάκης Ευάγγελος, Επικ. Καθηγητής

Μπερτσιάς Γεώργιος, Επικ. Καθηγητής

Περδικογιάννη Χρυσούλα, Επικ. Καθηγητής

Acknowledgements

The assignment of this Doctoral Thesis was a challenge for me and I would like to express my warm thanks to all those who contributed to its completion.

I would like to express my sincere gratitude to my supervisor Professor Emeritus, Dr Anthony Kafatos for the continuous support of my PhD study and related research, for his patience, motivation and immense knowledge. I specifically appreciated his extensive personal and professional guidance and provision of precious scientific knowledge.

I am particularly indebted to Adjunct Professor, Dr Konstantinos Lasithiotakis for his insightful comments and encouragement; and to Professor, Dr Emmanouil Galanakis for his advice and scientific support. Without their precious support it would not be possible to conduct part of my research.

Special thank to Professor Maria Hassapidoy and Mrs Alexandra Koulouri, without her precious support it would not be possible to conduct part of my research. I am very grateful to Professor Vasilis Mougios for instrumental support and advice in the development and publication of my research.

I am grateful also to Metropolitan Varnavas of Neapolis and Stavroupolis, Thessaloniki; Metropolitan Georgios of Kitros, Katerini, and Platamon; Hieromonk Father Luke Kipouros of Holy Trinity Monastery, Panorama, Thessaloniki; Nun, Melani Chatzipatera, Mother Superior of Assumption of Virgin Monastery, Panorama, Thessaloniki; Professor Emeritus of the School of Theology, Aristotle University of Thessaloniki, Dimitrios Tselegidis; Archimandrite Nikodemos Skrettas-Plexidas; and Archpriest Athanasios Gikas; Professors of the School of Pastoral and Social Theology, Aristotle University of Thessaloniki, for their help in collecting the study sample. Also I want to thank the Hieromonk Archimandrite Eulogios Tsalapatanis for information about Cristian Orthodox Church fasting.

Nobody has been more important for me in the completion of this project than my family members. I would like to thank my parents and my wife for all their help and support throught this challenging work. Words cannot express how grateful I am to my father and mother for all of the sacrifices they have made on my behalf. Their words of wisdom and prayers were what sustained me thus far. Finally, I wish to give thanks to my three daughters for the inspiration, support and understanding through hard times during my PhD thesis.

To my wife Tatiana

To my daughters: Smaragdi, Christina-Anna and Trifonia

Contents

Abstract

This study is the first to attempt to examine the effects of periodic vegetarianism, through the fasting of Orthodox Church, on bone growth, osteoporosis, bone fractures and bone health in general. It is also the first study to investigate lifelong nutrition through the multi-year use of fasting (at least 10 years) according to the Rules of the Orthodox Church and is the largest sample population to date (400 people) dealing with the issue of fasting of the Orthodox Church.

The low intake of calcium and dairy products observed during fasting periods for an average of 178 (± 19) days a year, does not seem to compromise bone health in older individuals, men and postmenopausal women. Also, young people (18-35 years old) who have been fasting since childhood did not have a problem with height or any of the bone health indicators in adulthood. Therefore, periodic abstinence from dairy products and, in general, animal products for decades, even starting in childhood, does not endanger bone health.

The periodic restriction of the dietary intake of products of animal origin to a slightly hypothermic diet that characterizes the fast of the Orthodox Church has been combined with increased consumption of plant foods, with a wide variety, which seems to beneficially affect musculoskeletal metabolism, perhaps better than complementary nutrition. Despite having lower protein intakes, men and women who had been periodically avoiding animal source foods for a median of 15 years did not differ in bone mineral density (BMD) and bone mass (BMC) or prevalence of bone fracture from controls with no food restrictions. BMD and BMC exhibited only weak (if any) correlations with consumption of protein-rich foods. Thus, continuous protein intake for a along time seems to play a minor (if any) role in bone health. It was found that the individuals following a religious lifestyle had lower vitamin D intake, lower sunlight exposure and, at times, lower serum 25-hydroxyvitamin D concentration than controls, although these differences did not impact bone health. These finding of the present study may prove useful in designing healthy diets for people who wish, or need, to have limited intake of animal and dairy foods. Future studies could employ assessment of fasters during periods of fasting and periods of no food restriction (in addition to the moderate-fasting period chosen in the present study) for a more accurate characterization of the relation between nutrient intake and bone health.

Περίληψη

Η παρούσα μελέτη είναι η πρώτη μελέτη που προσπαθεί να εξετάσει την επίδραση και τις επιπτώσεις της περιοδικής φυτοφαγίας, μέσω της νηστείας της Ορθόδοξης Εκκλησίας, στην ανάπτυξη των οστών, στην οστεοπόρωση, στα οστικά κατάγματα και γενικά στην υγεία των οστών. Επίσης είναι η πρώτη γνωστή μελέτη που ερευνά τη δια βίου διατροφή μέσω της πολυετούς χρήσης της νηστείας (τουλάχιστον 10 έτη) σύμφωνα με τους Κανόνες της Ορθόδοξης Εκκλησίας και είναι η μεγαλύτερη σε πληθυσμό δείγματος έρευνα μέχρι σήμερα (400 άτομα) που ασχολείται με το θέμα της νηστείας της Ορθόδοξης Εκκλησίας.

Η χαμηλή πρόσληψη ασβεστίου και γαλακτοκομικών προϊόντων που παρατηρείται κατά τις περιόδους νηστείας για κατά μέσο όρο 178 (± 19) ημέρες το χρόνο, δεν φαίνεται να βλάπτουν την υγεία των οστών σε ηλικιωμένα άτομα, άνδρες και μετεμμηνοπαυσιακές γυναίκες. Επίσης, σε νεαρά άτομα (18-35 ετών) που νηστεύουν από την παιδική τους ηλικία δεν είχαν πρόβλημα σε ανάστημα ή οποιονδήποτε από τους δείκτες υγείας των οστών κατά την ενηλικίωσή τους. Συνεπώς η περιοδική αποχή από γαλακτοκομικά προϊόντα και, γενικά, ζωικά προϊόντα για δεκαετίες, ακόμη και ξεκινώντας από την παιδική ηλικία, δεν θέτει σε κίνδυνο την υγεία των οστών.

Ο περιοδικός περιορισμός της διατροφικής πρόσληψης ζωικής προέλευσης προϊόντων σε μια ελαφρώς υποθερμιδική δίαιτα που χαρακτηρίζει τη νηστεία της Ορθόδοξης Εκκλησίας, συνδυάστηκε με αυξημένη κατανάλωση φυτικών τροφών, με μεγάλη ποικιλία, η οποία φαίνεται να επηρεάζει ευεργετικά τον μυοσκελετικό μεταβολισμό, ίσως καλύτερα από την αποκλειστική κατανάλωση συμπληρωμάτων διατροφής.

Παρά το γεγονός ότι έχουν χαμηλότερες προσλήψεις σε ζωικές πρωτεΐνες, άνδρες και γυναίκες που αποφεύγουν περιοδικά τροφές ζωικής προέλευσης για μέσο όρο 15 ετών, δεν διέφεραν στην οστική πυκνότητα (BMD) και οστική μάζα (BMC) και στα πέντε σημεία-θέσεις που εξετάστηκαν ή τον επιπολασμό της οστεοπενίας, της οστεοπόρωσης ή του κατάγματος των οστών από την ομάδα ελέγχου, με χωρίς περιορισμούς στα τρόφιμα.

Η κατανάλωση τροφών πλούσιων σε πρωτεΐνες εμφάνισε μόνο ασθενείς συσχετισμούς με BMD ή BMC. Έτσι, η συνεχής πρόσληψη πρωτεΐνης φαίνεται να παίζει δευτερεύοντα ρόλο στην υγεία των οστών.

Διαπιστώθηκε ότι η πολυετή χρήση της νηστείας (τουλάχιστον δέκα χρόνια), εμφάνισε αύξηση κατανάλωσης φρούτων και λαχανικών και αύξηση κατανάλωσης θαλασσινών και ψαριών και σε περιόδους μη νηστείας, επηρεάζοντας και διαμορφώνοντας τις διατροφικές συνήθειες των νηστευόντων, κατά τη διάρκεια όλου του έτους και όχι μόνο στις περιόδους νηστείας, διατροφή που προσεγγίζει την Μεσογειακή διατροφή.

Επίσης, τα άτομα που ακολουθούσαν τη νηστεία βρέθηκαν να είχαν χαμηλότερη πρόσληψη βιταμίνης D, χαμηλότερη έκθεση στο ηλιακό φως και μερικές φορές, χαμηλότερη συγκέντρωση 25-υδροξυβιταμίνης D στον ορό από ότι οι μη-νηστεύοντες, αλλά αυτές οι διαφορές δεν επηρέασαν την υγεία των οστών τους.

Αυτά τα ευρήματα της παρούσας μελέτης μπορούν να αποδειχθούν χρήσιμα στο σχεδιασμό και διαμόρφωση υγιεινών διαίτων ή διατροφικών σχεδίων για άτομα που επιθυμούν ή χρειάζονται περιορισμένη ή χαμηλή πρόσληψη ζωικών και γαλακτοκομικών τροφών.

Μελλοντικές μελέτες θα μπορούσαν να χρησιμοποιήσουν την αξιολόγηση των νηστευόντων κατά τις περιόδους νηστείας και τις περιόδους χωρίς περιορισμό των τροφίμων (εκτός τις ημέρες νηστείας της Τετάρτης και Παρασκευής που επιλέχθηκαν στην παρούσα μελέτη, με τις ημέρες νηστείας της Τετάρτης και της Παρασκευής) για έναν πιο ακριβή χαρακτηρισμό της σχέσης μεταξύ πρόσληψης θρεπτικών συστατικών και υγείας των οστών.

Chaptel 1. Is periodic abstinence from dairy products for half a year, detrimental to bone health of children and adolescents?

[Austin Journal Food Sciences. 2017; 5(3):1094]

Introduction

Proper nutrition is important for the development and conservation of the bone mass. 80-90% of the bone mineral content (BMC) is formed by calcium and phosphorus (1), along with other important molecules and elements (proteins, magnesium, copper, iron) and vitamins (A, C, D, K) (2).

Milk is broadly consumed because of its high nutritional value. It contains various elements such as bioactive peptides, calcium, and growth factors that interfere to the bone metabolism stages, namely bone turnover (3).

The Estimated Calcium Average Requirement (EAR) for the adolescent ranges between 800 to 1050 mg/day while the recommended daily intake (RDI) is 1000 - 1300 mg/day; it's also well understood that EAR as well RDI vary under certain conditions as in pregnancy, lactation, menopause and aging (4).

For more than two decades, USA recommends to children and adults the intake of calcium for prevention of osteoporosis (1, 5-8). They suggest the dairy servings as the best way for that, depending on age, for 4-8 years old is 3 servings and 9-18 years old, 4 servings daily. Three servings of dairy provide 828 mg calcium and 4 servings 1104 mg Ca (9).

In the USA over 120 food products are calcium-fortified. Therefore taking the recommended portions of diary daily plus the calcium from non dairy products far exceeds the RDI intake of calcium. It is also amazing that although the dietary intake of calcium ranks first around the world, representing the 72% of global dietary calcium intake (10), osteoporosis and bone fractures rates are high as they range between 700 and 1000 incidents per 100000 population annually (11, 12). Excessive calcium intake has been questioned on its efficacy in preventing osteoporosis and its potentially negative effects in health (13-17).

Recent epidemiological studies in women (18), children and adolescents (19-22) have questioned the efficacy of dairy products and other calcium-containing products for the good health of the bones (23).

World Health Organization (WHO) recommendations for the prevention of osteoporosis highlight the “calcium paradox” and suggest a minimum intake of 400-500 mg/day of calcium from all sources for individuals above 50 who live in countries with high rate of bone fractures (24). Furthermore, WHO concludes that “there is no chance to globally approximate the calcium intake for the general population”. This statement also includes children and adolescents. On the other hand, European and UK reports suggest dietary reference intake of calcium through dairy products between 800 and 1300 mg/day for all individuals –USA included- for their whole life (25)

Skeletal health indexes such those of bone growth as well of biological maturity of bony structures can be determined through correlation between Bone Mineral Density (BMD) and/or Bone Mineral Content (BMC) outcomes and calcium intake from milk and dairy products (25). The Institute of Medicine (IOM) in order to assess the importance of calcium and vitamin D intake determined the optimum Dietary Reference Intake (DRI) (26).

According to Recommended Dietary Allowances (RDA) recommendations, 700 mg of calcium is the optimum daily dosage for children between 1 and 3 years old, whereas 1000mg for those of 4-8 years old. Adolescents need higher calcium levels, no less than 1300mg per day (26).

However, prospective epidemiological studies have raised questions about the effectiveness of the use of dairy products in the promotion of bone health (23). Over the past two decades an increasing number of studies it has been carried out on religious fasts and their impact on human health (27). The Christian Orthodox Church (COC) diet proposes entirely different dietary habits that are worth to be mentioned because through alternating time-fixed fasting periods they achieve the optimal balance in an admittedly healthy nutritional pattern: the Mediterranean diet.

COC recommends abstaining from dairy, meat, and eggs for about 180 days per year (159-197, SD: 178±19) and instead of 155 days/year from fish for adults and children (Table 1).

For that reason, it may be considered as periodic vegetarianism although seafood allowed in all fasting periods (31-35).

Low consumption of dairy, meat and egg products leads to reduced intake of saturated fats, while the use of olive oil in large quantities has shown positive effects on the prevention of chronic diseases (36). In addition, COC fasts as nutritional health-promotive habits (37, 38) are still the main characteristics of Mediterranean diet in Greece (39-41).

The objective of this study is to review the existing literature on the effects of consumption of dairy products and total dietary calcium intake on bone integrity in children and adolescents, primarily to assess whether the evidence supports the following:

(1) Which one of the two nutritional habits promotes the optimum bone growth during childhood and adolescence: a daily consumption of 3-4 servings of dairy products, or a periodic fasting that includes calcium from non-dairy foods?

(2) Does the statement "the recommended servings of dairy products is the optimal choice for promoting bone health and integrity in contrast to other sources of food or supplements containing calcium" seem to be valid?

(3) Has the 180-day abstinence from dairy products per year, as suggested by the COC, negative effects on bone growth in children and adolescents?

Methods

The MEDLINE, COCHRANE, EMBASE and PUBMED databases were searched. Articles not included in those databases and studies on vegetarian diets as well as studies from the Christian Orthodox Church on fasting were also searched.

Keywords used were: dairy, dairies, dairy products, vegan, fast, fasting, plus bones, bone mineral density (BMD), bone mineral content (BMC), osteoporosis, osteopenia, including only studies and reports to humans, children and teenagers, aging above 3 years-old, published in English language, from 1990 to January 2017.

The research focused on age, sex, and race of the participants and also on the activity level and the pubertal status. Other factors such as socioeconomic status, exposure to the sun or caffeine consumption didn’t be taken into consideration.

As changes in BMD develop slowly (42) we focused on trials and studies having at least one-year follow-up.

Finally, our search yielded to 12 cross-sectional studies; 7 longitudinal prospective studies, 3 randomized and one case-control study.

Results

We studied 22 selected randomized controlled trials of milk and calcium intake for children and adolescents (Table 2).

Kardinaal et al. in cross-sectional study investigated the association between dietary calcium intake and radial bone density among 1116 Caucasian girls (aged 11-15 years) at different levels of calcium intake in six European countries. There was no evidence of a relation between calcium intake and BMD at different levels of intake; although there was a positive association at calcium intake levels <600 mg/day (6.28, p=0.02). Nevertheless, the interaction was not significant and there was no consistent trend over intake categories. These results do not support the hypothesis that dietary calcium acts as a determinant of peak BMD in European young women, for a wide range of intake (15).

Kröger et al. studied the effect of puberty and genetic factors on the development of bone mass and density in a prospective study 65 children and adolescents (aged 7-20 years old). They didn't find any significant relationship between the increment rate of bone density and physical activity or calcium intake (<800 - >1200) (19).

Lloyd et al. studied in two longitudinal studies the relation of exercise and BMD on teenage. The first, one (year 2000) was conducted in 81 girls (20) Cumulative sports-exercise scores between ages 12 and 18 years were associated with hip BMD at age of 18 years (r = .42) but not related to the total body bone mineral gain. Time-averaged daily calcium intake ranged from 500 to 1500 mg/day in this cohort, was not associated with hip BMD at age 18 years or with total body bone mineral gain at age 12 through 18 years.

The second study (2002) included 75 healthy white female adolescents (aged 12-18 years) sports-exercise scores were correlated with BMD at the femoral neck and shaft.

Average total daily calcium intake at age 12-20 years old ranged from 486 to 1958 mg/day and no associations (p < 0.05) were observed between bone measurements and calcium intake (22).

Kohlenberg-Mueller et al. in a cross-sectional study focused on the vegan diet in seven women and one man 19 to 24 years old indicating that neither calcium balance nor a single bone turnover biomarker has been significantly affected. Calcium intake levels (843±140 mg, p<0.01) were adequate despite the significant difference in calcium intake levels between plant foods and dairy (43).

Ho-Pham's et al. cross-sectional study compared bone health in 105 postmenopausal vegan Mahayana Buddhist nuns and 105 omnivorous postmenopausal women 50-85 years old. Nuns, which had been engaged the vegan diet at mean 33 years (range: 10-72 years), demonstrated on average very low (330 ± 205 vs. 682 ± 417 mg/day, p < 0.001mg) calcium daily intake. Lumbar Spine BMD was identical in both groups (0.74±0.14 vs. 0.77±0.14 g/cm2; mean ± SD; p=0.18). Same results in Femoral Neck BMD (0.62±0.11 vs. 0.63±0.11 g/cm2; p=0.35) as well as in Body BMD results were 0.88±0.11 vs. 0.90±0.12 g/cm2; (p=0.31). Prevalence of osteoporosis (T scores ≤−2.5) at the femoral neck in vegans and omnivores was 17.1% and 14.3% (p=0.57), respectively. Therefore, veganism has no adverse effect on BMD (44).

In a randomized case-control study, milk intake of 1.5 L/day for 7 days was associated with higher calcium intake (p<0.0001) but decreased bone turnover in prepubertal 24 boys. Serum osteocalcin (s-OC) and C-terminal telopeptides of type I collagen (s-CTX) were significantly decreased in the milk group (-30.9%; -18.7%, respectively, p≤0.04) as well as bone-specific alkaline phosphatase (s-BAP) (p=0.06) (45).

Another randomized case-control prospective study examined the effects of increased milk consumption on bone (and body) composition in boys engaged with resistance training. Twenty-eight boys (13 to 17 years of age) were randomly assigned to consume, in addition to their habitual diet, 3 servings/day of 1% milk (n=14) or fruit juice not fortified with calcium (n=14) while followed a 12-week resistance training program. Only the “whole-body BMD" was been affected. The milk group showed two-fold greater increase than the juice group (0.028 vs 0.014 g/cm2, respectively). Nevertheless, the juice group showed a greater increase at "whole-body BMC", after 12 weeks (2,667g vs 2,591g respectively, p<0.001). BMD and BMC were increased in both groups after six weeks, and there was a further increase at 12th week (46).

A cross-sectional study of 330 boys and girls aged 8 years no association was found between children‘s current calcium intake (1336 mg per day deriving from milk, fruit and vegetable consumption) and BMD. Only potassium and vegetable intakes, were negatively associated with total-body BMD (-0.14 and -0.15, respectively, p <0.05) (47).

A cross-sectional study of 176 Finnish girls aged 8-20 years-old had a seven-day Calcium intake Diary (CaD). Subjects were classified into three groups according to Tanner stage: prepubertal (Tanner 1; n=41), peripubertal (Tanner 2 and 3; n=54), and postpubertal (Tanner 4 and 5; n=81). No association was found between calcium intake and bone activity variables (BMD, BMC, and BMD femoral neck), but the high level of calcium intake in all age groups of the study T1+1018±361mg/d, T3 and

T4=1059±460mg/d and T4 and T5=1231±565mg/d was likely to explain the lack of association. Namely, the fact that calcium intake that exceeds a certain level does not further contribute to bone mineralization (48).

A cross-sectional study in Central Japan's community (Mie) study the effects of dairy intake on bone health in a representative population sample of Japanese adult women (n = 1252, 19-80 years-old), high-school adolescent girls (n = 2651, 15-18 years-old) and boys (n = 2110, 15-18 years-old). These groups were examined according to the frequency of weekly milk consumption which was set at 4-5 times, 2-3 times and ≤ 1 time a week. Z-score dropped as the frequency of milk intake increased in all women. Specifically, it was 103.4% for once a week intake or less, 102.3% for 2 or 3 times a week, 102.8% for 4 to 5 times a week, and 101.2% for the daily intake. Bone density of the subjects was noticeably lower in the daily intake subgroup than that of the once a week or less, although not statistically significant ($p < 0.1$). This suggests that the bone density of adult women decreases as the frequency of milk intake is increasing. Also, the intake of milk did not affect the bone density of high school girls who avoided physical exercise (Z-score daily 95.3%, ≤1 time 95.2%, $p < 0.1$) (49).

The same study included 2110 Japanese boys, study the effect of milk intake on bone density on those abstained from physical activities in comparison to those systematically exercised was similar ($p < 0.1$). For no-exercise boys, Z-score showed a rise proportionally to the milk consumption, although the alteration was not significant (for daily milk intake was 95.9% and for ≤1 time was 93.3%, $p < 0.1$). In those Japanese boys cheese intake showed that Z-score was lowest in the daily intake group (systematic-exercise boys: daily 99.5% and ≤1 time 100.4%, no-exercise boys: daily 93.6% and ≤1 time 94.4%, $p < 0.1$). Same results for yoghurt in no-exercise high school girls (daily 93.8% and ≤1 time 95.1%, $p < 0.1$). Lastly, the cream seems to be beneficial for the bone health of girls without physical activity ($p = 0.05$) (49).

In a cross-sectional study in Netherlands examined the relation between physical activity, calcium intake from dairy products and BMC in 1359 children aged 7 to 11 years. No significant differences (in adjusted mean levels) in bone mineral content were found when "high" and "low" calcium intake groups were compared ("high" and "low" being defined by the upper and lowest decile of calcium intake). No evidence was found for any association between daily calcium intake and BMC in childhood, concluding that increased BMC was only detected in children with a high level of physical activity (50).

The study of 215 female twin pairs (122 monozygotic and 93 dizygotic) aged 10-26 years demonstrated the roles of constitutional and lifestyle factors on bone mass. 60% of total dietary calcium was derived from dairy products. In post-menarchial pairs, the detected difference in daily calcium intake level of intake (1041±674g/day) was associated with a univariate fashion with a relevant difference in total body BMC, although the robust estimation was considerably smaller. Across all pairs and after adjusting for menarchial status, height, lean mass, and fat mass, the coefficient for calcium intake became 0.04 -/+ 0.09%/100 mg. There were also found no associations within-pair relatively calcium intake and BMD at the lumbar or femoral sites (51).

In a 3-year prospective study of 179 Hong Kong boys and girls aged 12-13 years at baseline - reported that there was no influence of calcium intake (711.8 mg/day for boys and 580.8 mg/day for the girls) on BMD, BMC and bone mineral accretion. During the study period, mean BMC of the dominant forearm increased from 0.61 to 0.75 ($p < 0.001$) in the boys (25%) and from 0.64 to 0.73 ($p < 0.001$) in the girls (12.3%). Similarly, mean BMD of L2-L4 vertebrae was increased for both sexes from 0.63 to 0.77 ($p < 0.001$) in boys (24.2%) and from 0.75 to 0.87 ($p < 0.001$) in girls (14.5%). For girls, a higher age and an advanced pubertal stage at the beginning of the study resulted in a significantly ($p = 0.03$) slower rate of increase in BMC of the forearm and BMD of the lumbar spine when compared with those girls with a less advanced pubertal stage at the beginning of the study. Comparing BMC of the forearm and BMD of the lumbar spine among boys and girls, rates of change in boys were significantly higher than those in girls ($p < 0.01$) (52).

In a study of 162 Icelandic Caucasian girls aged 13 and 15, reported a threshold effect intake on BMD of calcium intake 1293±452 mg/d in 13 year age group and 1082±382 mg/d calcium in 15 year age group (the intake of calcium from dairy products was 16.3% higher in younger girls as compared to older); above this, no further effect has been noticed. Univariate analysis showed no significant correlation between calcium intakes from milk and other dairy products and BMD or BMC in either age group (53).

A case-control study investigated for 2 years the relationship between dairy intake and the risk of fractures in 100 children (74 boys and 26 girls, aging 7-14 years old). They concluded that high consumption of cola sodas and noncarbonated beverages (including fruit juices) are positively and significantly associated with the probability of a fracture. On the other hand, consumption of either non-cola carbonated beverages or dairy products is unrelated. The latter is possibly due to misclassification of dairy intake or/and the fact that consumption of dairy products is generally high in adolescence and early adulthood (54).

A cohort of 581 pupils from Wales aged 7-9 investigated for the effect of milk supplement in childhood during growth on adult forearm bone mineral content (BMC) and density (BMD) in a 14 years follow-up. They were randomized half of them to receive 190 ml of milk daily (or 228 mg of calcium) at their free school meal, regardless of that at home, on each school day for six terms in comparison to a control group given no milk.

The total calcium intake in the two groups was for the Men: 1297±397, 1323±319 (Control) and for the Women: 933±278, 940±317 (Control). They found that BMD was positively associated with body weight ($p < 0.01$) current intakes of calcium (P less than 0.05), vitamin D (P less than 0.01) and sports activity during adolescence; inversely associated with alcohol consumption ($p < 0.05$). In multiple linear regression analysis, body weight and sports activity during adolescence were stronger determinants of female BMD than was diet (55).

In a 3-year prospective study 171 Finnish girls aged 9-15 years were investigated for calcium intake (1575±637 g/day) and BMD measured every 6 months for 3 years. There's no significant correlation between mean dietary calcium & vitamin D intake and BMD or BMAD at the lumbar spine or femoral neck regarding the overall study population. However, lumbar spine BMD was at 27% (0.030g/cm^2) greater in the highest than in the lowest vitamin D intake tertile in the girls with advanced sexual maturation (95% CI: 0.004, 0.056 g/cm^2 - p for trend for all tertiles = 0.016) (56).

Another 15-year longitudinal study (the "Amsterdam Growth and Health Study"), examined 84 males and 98 females (aged between 13 and 28). Multiple-regression analyses incorporating calcium intake by body height (CAIH), weight-bearing activities (WBA), body weight (WT) and BMD indicated that calcium intake during adolescence and young adulthood was not a significant predictor of lumbar BMD at the age of 27 years for both sexes (males $p<0.01$, females $p<0.001$) (57).

The effects of calcium supplementation and dairy products consumption on bone and body composition were studied in 48 of pubertal girls from USA (aged 9 to 13). In this randomized intervention study, the control group consumed their usual diet (728±321 mg/day calcium) and the dairy group's diet was supplemented weekly with dairy products to at least 1200 mg calcium daily (1437±366 mg/day) over a period of 1 year. There were no differences between the two groups during the study as showed by bone mineral values (control group: 1508±167, dairy group: 1490±291gm) and lumbar spine bone density data (control: 0.665±0.077, dairy: 0.663±0.096 gm/cm2). At the end of the year, either average lumbar spine bone density (control group: 0.748±0.084, dairy group: 0.772±0.086) or total body bone mineral content (control: 1617±152, dairy: 1695±317) did not differ significantly between groups (58).

Another randomized intervention study, 82 girls of United Kingdom 12 year old girls treated with daily consumption of 568 ml milk for 18 months follow-up. The control group received an average baseline of 150 ml of daily milk. Both groups demonstrated 9.6% increase for total body BMD of milk group (SD 1.9%; 95% confidence interval 9.0% to 10.2%) and control group 8.5% (2.7%; 7.6% to 9.4%); p=0.017) and total body BMC 27.0% (5.8%; 25.2% to 28.8%) v 24.1% (6.3%; 22.0% to 26.1%); p=0.009). Expressed in absolute terms, the respective increases were 0.090 (0.020; 0.084 to 0.096) v 0.081 (0.025; 0.072 to 0.089) g/cm2 for total body BMD (p=0.021) and 428 (88; 398 to 452) v 391 (107; 358 to 430) g for total body BMC (p=0.035). Short term increases in calcium or dairy food intake in children or adolescents may not be sufficient to sustain an increase in bone mass over several decades (59).

At last, Chryssochoou et al. in a cross-sectional study investigated the relationships between Christian Orthodox Church's fasting and overall nutrition, growth and physical activity in children and adolescents from northern Greece. Among 609 children between 5 and 15.5 years old, 12.1% of them followed all fasting periods (AF) without consuming dairy products (p=0.056) during the last 3.5 years and they didn't demonstrate any significant difference in their height (144.0±1.2 cm) in comparison to those that partially fasted (PF) (145.0±0.6 cm) or to the never fasted (NF) (145.1±0.6) (p=0.730). These children (NF) were found to have significantly higher calcium intake than full-fastened ones (1.151 versus 1.037 mg, respectively, p=0,017) (60).

Discussion

This study summarizes the published data on calcium balance regarding different types of dietary patterns. On the other hand, it presents the current knowledge on Orthodox Christian Church's diet as she combines features of other diets in proper timing as well as in a balanced combination of food ingredients.

Benefits from calcium consumption are well-established regardless the source.

Calcium intake - as the apparent rate of calcium absorption - doesn’t significantly differ between vegan (26%) and lactovegetarian (24%) diet (43). These rates are consistent with other studies on calcium balance that carried out with different kinds of non-vegetarian diet: 15% -36 % (61), 27% (62), 16% -21% (63), 23%-31 % (64) and 16% -24 % (65).

The controversy about calcium's impact on musculoskeletal metabolism and more specifically on bone density shows significant interest. Studies on dairy-free vegetarian diets showed no (statistically significant) difference in BMD or in the degree of bone loss (66).

On the other side, the "Rotterdam study" points that fruit, vegetable, and dairy pattern with 135gr more milk as well as 29gr more yogurt, is responsible for high BMD, bending strength and more stable bones and hence for reduced osteoporotic fracture risk [HR (95% CI): 0.92 (0.89 - 0.96)] and hip fracture [HR (95% CI): 0.81 (0.70 - 0.93)] in women at the age of 55 (67).

Finally, others support that bone density and risk of bone fractures were found to be similar in omnivores and lacto-vegetarians (68, 69).

The amount of calcium uptake is inversely related to the risk of fractures. Indeed sub-Saharan people with around 200 mg calcium daily consumption, they present lower bone fragility than those with double intake levels (i.e. Hong Kong, Singapore and Papua New Guinea) and even more than people who exceed 1000 mg (Norway, Sweden, Denmark, USA, UK, Ireland, New Zealand, Finland, etc.). Reasons for the wide geographic variation of fracture incidence still remain unknown (70) while causality can be found in high animal-derived protein intake, low vegetable and fruit intake, and low physical activity. The negative effect of menopause onto the musculoskeletal metabolism logically it should be accompanied by corresponding changes in bone density, though this has not yet been confirmed in populations where the daily calcium intake is significantly limited about 375 mg on average per day (44).

Even calcium and vitamin D3 daily intake for vegans is 55% and 63% respectively of those of omnivores; this doesn't adversely affect bone density or the frequency of fractures. In contrast, the higher intake of animal protein and lipids is associated with more bone loss than that of vegans (66).

On the daily milk consumption or calcium supplements during childhood and adolescence, some studies support that dietary or supplementary calcium does not affect spine & hip bone density (15, 17-19, 21, 22, 25, 44, 45, 48, 50, 52, 53, 55, 56, 71-92) or bone turnover (45), even when the daily consumption of calcium exceeds 1000 mg (48, 53, 90).

Others studies advocate the opposite; total body BMC/BMD increase range from small (93) to significant (46, 59, 76, 85, 94-97) also they lack sufficient information about that beneficial effect.

Having on mind that the ideal vitamin D intake levels for the children and adolescents are 400-600 IU/day, alterations on its quantity as well as on that of other nutrients (such retinol) along with exercise habits could alter the effects of milk on BMD (18, 87, 89, 98, 99).

High intakes of calcium (>1400 mg/day) in women are associated with higher death rates from all causes and cardiovascular disease (100). Indeed, higher milk intake was associated with higher mortality as disclosed by cohort studies in both sexes with higher fracture incidence in women (101). Moreover, there's a link between habitual consumption of milk & dairy products with increasing risk for prostate cancer (102-104).

Apart from all the above reported on calcium equilibrium and its interactions, non-animal sources of calcium such beverages from soy or rice, cereals, and fruit juices are of great biological and nutritional importance (105).

The content of oxalates or fibers (phytate/dietary) seems to be associated with low calcium intake (106-109). Indeed, high oxalates in spinach and rhubarb show 5% (110) and 9% (111) absorption rates respectively, while other plant foods demonstrate higher: 41% for cabbage (112), 22% for beans (113) or 52% for Chinese cabbage and 48% for broccoli (114).

Regardless of vegan or lactovegetarian diet, calcium equilibrium isn't affected despite foods with high oxalates (spinach, beets), fiber-rich (whole grain bread, oats) or the calcium-rich mineral water (43). Nevertheless, adequate calcium amounts in humans can be also achieved at intake levels far below the recommended (115-118).

Over and above, calcium bioavailability is of importance. In mineral water and milk is almost the same no matter the content (119-120) as a single dose of 172mg/l mineral water's calcium inhibits PTH secretion and bone resorption (121). Higher bioavailability was observed in low-oxalate vegetables as broccoli, kale, cabbage and collards unlike in nuts, dried beans, and spinach (122, 123).

Regular weight-bearing exercise and at least a normal age-related body weight in adolescence and young adulthood are of key importance in reaching the highest lumbar peak bone mass at the age of 27 years, regardless calcium intake (57), protein consumption or vitamin D levels (58).

Alternative nutritional lifestyles such as vegetarianism are popular the trend for any age group. An increasing rate of 2-5% of European adult population adopts a vegetarian eating pattern (124). Concerns about global warming and sustainable food production as well as economic concerns are important incentives for someone to become vegetarian (105, 125, 126). Most of them are young parents who introduce their children to vegan diet (105). Moreover, vegan teenagers live in an otherwise omnivorous family (127); published data (even limited) report no significant effect of vegan or vegetarian diet in growth during adolescence (128).

Among these various diets and under the general acceptance of the healthy eating habits that they introduce, Christian Orthodox Church (COC) diet introduces the "rationale of measure" instead of a complete abstinence from certain kind of foods. Fish is less frequently recommended while dairy and meat products are not allowed.

These fasting periods are well-defined and applied every year. Before long periods of fasting, such the 48-day long Lent, free consumption of all kind of food for three weeks is allowed. At the third week, only dairy products and fish (except meat) are allowed. During the week that follows the end of Lent (Easter Week) and the first 12 days after the Christmas fasting (The Twelve days) any kind of food is allowed to be consummated (Table 1) (28-30).

Papadaki et al. studied data on calcium intake alterations during one week of Lent. A double portion of all foods and drinks were chemically analyzed. Mean daily calcium intake was 533mg all from non-dairy products. Same chemical analysis for the non-fasting week after Easter reported 966mg/day (129).

A study conducted in children who fast, show a lower than the recommended daily intake of vitamin E and magnesium, but not for calcium and other minerals or trace elements which did not differ significantly between fasters (partially or completely) and non-fasters. These latter are ranked higher in daily energy intake, as they consume larger amounts of animal proteins and/or saturated fatty acids (6).

Conclusion

Overall balance and bioavailability of calcium are not significantly affected by the type of diet followed by children and adolescents. Christian Orthodox Church diet with its fasting may have a favorable effect on health as it provides adequate nutrition coverage as well as protection against numerous chronic diseases. In fact, periodic abstinence since childhood from dairy products meat and eggs tends to reduce the risk for future osteoporosis as it positively affects the bone mass density.

Far away from permanent food deprivations, which are not secondary to medical directives, one can argue that COC fasting is an integrated nutritional opportunity for a healthy lifestyle.

References

1. Institute of Medicine. Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board. Washington, DC: National Academy Press. 1997.
2. Ilich JZ, Kerstetter JE. Nutrition in bone health revisited: a story beyond calcium. Journal of the American College of Nutrition 2000; 19(6): 715-737.
3. Aoe S, Koyama T, Toba Y, Itabashi A, Takada Y. A controlled trial of the effect of milk basic protein (MBP) supplementation on bone metabolism in healthy menopausal women. Osteoporosis International 2005; 16(12) :2123-2128.
4. FAO/WHO Expert Group. Calcium requirements. Report of an FAO/WHO Expert Group. Rome, Food and Agriculture Organization of the United Nations, 1962 (FAO Nutrition Meetings Report Series, No. 30. Available at: http://apps.who.int/iris/bitstream/10665/42716/1/9241546123.pdf
5. National Institutes of Health. Consensus Development Panel on Optimal Calcium Intake. NIH Consensus Conference: optimal calcium intake. JAMA 1994; 272(24): 1942-1948.
6. National Institutes of Health. Osteoporosis Prevention, Diagnosis, and Therapy. NIH Consensus Statement. 2000; 17(1): 1-45.
7. The Food Guide Pyramid. US Department of Agriculture. Human Nutrition Information Service. Hyattsville, MD. 1996 (Home and Garden Bull. No. 252).
8. US Department of Agriculture and US Department of Health and Human Services. Nutrition and Your Health: Dietary Guidelines for Americans. 5th ed. Washington, DC. US Department of Agriculture and US Department of Health and Human Services. 2000 [Home and Garden Bull. No 232].
9. National Dairy Council, 2004. Additional resources are available at: www.nationaldairycouncil.org.
10. Gerrior S. Nutrient Content of the US Food Supply. Washington, DC. US Department of Agriculture, Center of Nutrition Policy and Promotion. 2001 (Home Economics Research Report no.53).
11. Abelow BJ, Holford TR, Insogna KL. Cross-cultural association between dietary animal protein and hip fracture: a hypothesis. Calcified Tissue International 1992; 50(1): 14-18.

12. Report of a Joint Food and Agriculture Organization of the United Nations/World Food Organization of the United Nations Expert Consultation. Human vitamin and mineral requirements. Bangkok, Thailand; September 1998. Available at: ftp://ftp.fao.org/es/esn/nutrition/Vitrni/vitrni.html.
13. Avenell A. Bone density and milk. Exercise and body size influence bone density. BMJ 1994; 308(6943): 1566.
14. Hegsted DM. Fractures, calcium, and the modern diet. American Journal of Clinical Nutrition 2001; 74(5): 571-573.
15. Kardinaal AF, Ando S, Charles P, et al. Dietary calcium and bone density in adolescent girls and young women in Europe. Journal of Bone and Mineral Research 1999; 14(4): 583-592.
16. Seppa K. Bone density and milk. Consider fat as well as calcium intake. BMJ 1994; 308(6943): 1566.
17. Weinsier RL, Krumdieck CL. Dairy foods and bone health: examination of the evidence. American Journal of Clinical Nutrition 2000; 72(3): 681-689.
18. Feskanich D, Willett WC, Colditz GA. Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women. American Journal of Clinical Nutrition 2003; 77(2): 504-511.
19. Kröger H, Kotaniemi A, Kröger L, Alhava E. Development of bone mass and bone density of the spine and femoral neck—a prospective study of 65 children and adolescents. Bone and Mineral 1993; 23(3): 171-182.
20. Lloyd T, Chinchilli VM, Johnson-Rollings N, Kieselhorst K, Eggli DF, Marcus R. Adult female hip bone density reflects teenage sports exercise patterns but not teenage calcium intake. Journal of Pediatrics 2000; 106(1): 40-44.
21. Lloyd T, Taylor DS. Calcium intake and peak bone mass. Journal of the American Medical Women’s Association 2001; 56(2): 49-52,72.
22. Lloyd T, Beck TJ, Lin HM, et al. Modifiable determinants of bone status in young women. Bone 2002; 30(2): 416-421.
23. Lanou AJ, Berkow SE, Barnard ND. Calcium, dairy products, and bone health in children and young adults: a reevaluation of the evidence. Journal of Pediatrics 2005; 115: 736–743.
24. Joint Food and Agriculture Organization of the United Nations/World Food Organization of the United Nations Expert Consultation on Diet, Nutrition, and the

Prevention of Chronic Diseases. WHO Technical Report Series 916. Geneva, Switzerland: World Health Organization; 2003. Available at: www.who.int/hpr/NPH/docs/who_fao_expert_report.pdf.

25. Whiting SJ, Healey A, Psiuk S, et al. Relationship between carbonated and other low nutrient dense beverages and BMC of adolescents. Nutrition Research 2001; 21: 1107-1115.
26. Institute of Medicine of the National Academy of Sciences. Dietary Reference Intakes for Calcium and Vitamin D; March 2011.
27. Trepanowski JF and Bloomer RJ. The impact of religious fasting on human health. Nutrition Journal 2010; 9: 57.
28. Βολουδάκης ΕΒ. Νηστειοδρόμιον [Book in Greek]. Εκδόσεις Υπακοή, Έκδοση Β΄, Αθήνα, 1999. ISBN: 9607048091.
29. Καππάης Δ. Η Νηστεία. Σύμφωνα με τη διδασκαλία της Εκκλησίας μας [Book in Greek]. Εκδόσεις Βιβλιοπωλείου Ιεράς Μητροπόλεως Λεμεσού, 1995. ISBN: 9607048091.
30. Κούτσας Συμεών, Αρχιμ. Η Νηστεία της Εκκλησίας. Γιατί, πότε και πως νηστεύουμε [Book in Greek]. Αποστολική Διακονία, Σειρά Θεωρία και Πράξη, Έκδοση Γ΄, Αθήνα, Δεκέμβριος 1991. ISBN: 9963794440.
31. Sarri KO, Tzanakis NE, Linardakis MK, Mamalakis GD, Kafatos AG. Effects of Greek Orthodox Christian Church fasting on serum lipids and obesity. BMC Public Health 2003; 16: 3-16.
32. Sarri KO, Linardakis MK, Bervanaki FN, Tzanakis NE, Kafatos AG. Greek Orthodox fasting rituals: a hidden characteristic of the Mediterranean diet of Crete. British Journal of Nutrition 2004; 92(2): 277-284.
33. Papadaki A, Vardavas C, Hatzis C, Kafatos A. Calcium, nutrient and food intake of Greek Orthodox Christian monks during a fasting and nonfasting week. Public Health Nutrition 2008; 11(10): 1022-1029.
34. Sarri KO, Kafatos AG, Higgins S. Is religious fasting related to iron status in Greek Orthodox Christians? British Journal of Nutrition 2005; 94(2): 198-203.
35. Sarri K, Bertsias G, Linardakis M, Tsibinos G, Tzanakis N, Kafatos A. The effect of periodic vegetarianism on serum retinol and alpha-tocopherol levels. International Journal for Vitamin and Nutrition Research 2009; 79(5-6): 271-280.

36. Hakim I. Mediterranean diets and cancer prevention. Archives of Internal Medicine 1998; 158(11): 1169-1170.
37. White R, Frank E. Health effects and prevalence of vegetarianism. The Western Journal of Medicine 1994; 160(5): 465-471.
38. Whorton JC. Historical development of vegetarianism. American Journal of Clinical Nutrition 1994; 59(5 Suppl): 1103S-1109S.
39. Kafatos A, Kouroumalis I, Vlachonikolis I, Theodorou C, Labadarios D. Coronary-heart-disease risk-factor status of the Cretan urban population in the 1980s. American Journal of Clinical Nutrition 1991; 54(3): 591-598.
40. Sabaté J. Religion, diet and research. British Journal of Nutrition 2004; 92(2): 199-201.
41. Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, Gnardellis C, Lagiou P, Trichopoulos D, et al. Diet and overall survival in elderly people. BMJ 1995; 311(7018): 1457-1460.
42. Heany RP. The bone-remodeling transient: implications for the interpretation of clinical studies of bone mass change. Journal of Bone and Mineral Research 1994; 9: 1515-1523.
43. Kohlenberg-Mueller K. and Raschka L. Calcium balance in young adults on a vegan and lactovegetarian diet. Journal of Bone and Mineral Metabolism 2003; 21(1): 28-33.
44. Ho-Pham LT, Nguyen UD, Pham HN, Nguyen ND, Nguyen TV. Veganism, bone mineral density, and body composition: a study in Buddhist nuns. Osteoporosis International 2009; 20: 2087-2093.
45. Budek AZ, Hoppe C, Michaelsen KF, Molgaard C. High intake of milk, but not meat, decreases bone turnover in prepubertal boys after 7 days. European Journal of Clinical Nutrition 2007; 61(8): 957-962.
46. Volek JS, Gomez AL, Scheett TP, Sharman MJ, French DN, Rubin MR, et al. Increasing fluid milk favorably affects BMD responses to resistance training in adolescent boys. Journal of the American Dietetic Association 2003; 103(10): 1353-1356.
47. Jones G, Riley MD, Whiting SJ. Association between urinary potassium, urinary sodium, current diet, and bone density in prepubertal children. American Journal of Clinical Nutrition 2001; 73(4): 839-844.

48. Uusi-Rasi K, Haapasalo H, Kannus P, et al. Determinants of bone mineralization in 8 to 20 year old Finnish females. European Journal of Clinical Nutrition 1997; 51(1): 54-59.
49. Yoshii S, Kamimotono Sh, Sawai Sh, Matsushita MB, Tanaka F, Rujkorakarn D. Cross-sectional survey on the relationship between dairy product intake and bone density among adult women and high school students. Nutrition Research 2007; 27: 618-624.
50. VandenBerg MF, DeMan SA, Witteman JC, Hofman A, Trouerbach WT, Grobbee DE. Physical activity, calcium intake, and BMC in children in the Netherlands. Journal of Epidemiology and Community Health 1995; 49(3): 299-304.
51. Young D, Hopper JL, Nowson CA, et al. Determinants of bone mass in 10- to 26-year-old females: a twin study. Journal of Bone and Mineral Research 1995; 10(4): 558-567.
52. Cheng JC, Maffulli N, Leung SS, Lee WT, Lau JT, Chan KM. Axial and peripheral bone mineral acquisition: a 3-year longitudinal study in Chinese adolescents. European Journal of Pediatrics 1999; 158(6): 506-512.
53. Kristinsson JO, Valdimarsson O, Steingrimsdottir L, Sigurdsson G. Relation between calcium intake, grip strength and BMD in the forearms of girls aged 13 and 15. Journal of Internal Medicine 1994; 236(4): 385-390.
54. Petridou E, Karpathios T, Dessypris N, Simou E, Trichopoulos D. The role of dairy products and non alcoholic beverages in bone fractures among school age children. Scandinavian Journal of Social Medicine 1997; 25(2): 119-125.
55. Fehily AM, Coles RJ, Evans WD, Elwood PC. Factors affecting bone density in young adults. American Journal of Clinical Nutrition 1992; 56(3): 579-586.
56. Lehtonen-Veromaa MK, Möttönen TT, Nuotio IO, Irjala KM, Leino AE, Viikari JS. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. American Journal of Clinical Nutrition 2002; 76(6): 1446-1453.
57. Welten DC, Kemper HC, Post GB, et al. Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. Journal of Bone and Mineral Research 1994; 9: 1089-1096.
58. Chan GM, Hoffman K, McMurry M. Effects of dairy products on bone and body composition in pubertal girls. Journal of Pediatrics 1995; 126(4): 551-556.

59. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomized, controlled intervention trial. BMJ 1997; 315(7118): 1255-1260.
60. Chryssochoou E, Linardakis M, Chatziagorou E, Tsanakas I, Kafatos AG. The effect of Christian Orthdox Church fasting on the health and growth of children and adolescents [Article in Greek]. Paediatriki 2010; 73(2): 121-129.
61. McDonald JT, Margen S. Wine versus ethanol in human nutrition. III. Calcium, phosphorus, and magnesium balance. American Journal of Clinical Nutrition 1979; 32(4): 823-833.
62. Gallagher JC, Riggs BL. Action of 1.25-dihydroxyvitamin D3 on calcium balance and bone turnover and its effect on vertebral fracture rate. Metabolism 1990; 39(4 Suppl 1): S30-S34.
63. Spencer H, Norris C, Derler J, Osis D. Effect of oat bran muffins on calcium absorption and calcium, phosphorus, magnesium and zinc balance in men. Journal of Nutrition 1991; 121(12): 1976-1983.
64. Hunt JR, Gallagher SK, Johnson LK, Lykken GI. High-versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. American Journal of Clinical Nutrition 1995; 62(3): 621-632.
65. Haack VS, Chesters JG, Vollendorf NW, Story JA, Marlett JA. Increasing amounts of dietary fiber provided by foods normalizes physiologic response of the large bowel without altering calcium balance or fecal steroid excretion. American Journal of Clinical Nutrition 1998; 68(3): 615-622.
66. Ho-Pham LT, Vu BQ, Lai TQ, Nguyen ND, Nguyen TV. Vegetarianism, bone loss, fracture and vitamin D: a longitudinal study in Asian vegans and non-vegans. European Journal of Clinical Nutrition 2012; 66(1): 75-82.
67. de Jonge EA, Kiefte-de Jong JC, Hofman A, Uitterlinden AG, Rivadeneira F, et al. Dietary patterns explaining differences in bone mineral density and hip structure in the elderly: the Rotterdam Study. American Journal of Clinical Nutrition 2017; 105(1): 203-211.
68. Appleby P, Roddam A, Allen N, Key T. Comparative fracture risk in vegetarians and nonvegetarians in EPIC-Oxford. European Journal of Clinical Nutrition 2007; 61(12): 1400-1406.

69. New S. Do vegetarians have a normal bone mass? Osteoporosis International 2004; 15: 679-688.
70. Prentice A. Diet, nutrition and the prevention of osteoporosis. Public Health Nutrition 2004; 7(1A): 227-243.
71. Katzman DK, Bachrach LK, Carter DR, Marcus R. Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. Journal of Clinical Endocrinology & Metabolism 1991; 73(6): 1332-1339.
72. Lloyd T, Andon MB, Rollings N, et al. Calcium supplementation and BMD in adolescent girls. JAMA 1993; 270(7): 841-844.
73. Lloyd T, Martel JK, Rollings N, et al. The effect of calcium supplementation and Tanner stage on bone density, content and area in teenage women. Osteoporosis International 1996; 6(4): 276-283.
74. Winzenberg T, Shaw K, Fryer J, Jones G. Effects of calcium supplementation on bone density in healthy children: meta-analysis of randomized controlled trials. BMJ 2006; 333(7572): 775-780.
75. Taha W, Chin D, Silverberg AI, Lashiker L, Khateeb N, Anhalt H. Reduced spinal BMD in adolescents of an Ultra-Orthodox Jewish community in Brooklyn. Journal of Pediatrics 2001; 107(5): 79.
76. Dibba B, Prentice A, Ceesay M, Stirling DM, Cole TJ, Poskitt EM. Effect of calcium supplementation on bone mineral accretion in Gambian children accustomed to a low-calcium diet. American Journal of Clinical Nutrition 2000; 71(2): 544-549.
77. Nieves JW, Golden AL, Siris E, Kelsey JL, Lindsay R. Teenage and current calcium intake are related to bone mineral density of the hip and forearm in women aged 30–39 years. American Journal of Epidemiology 1995; 141(4): 342-351.
78. Moro M, Van der Meulen MC, Kiratli BJ, Marcus R, Bachrach LK, Carter DR. Body mass is the primary determinant of midfemoral bone acquisition during adolescent growth. Bone 1996; 19(5): 519-526.
79. Maggiolini M, Bonofiglio D, Giorno A, et al. The effect of dietary calcium intake on BMD in healthy adolescent girls and young women in southern Italy. International Journal of Epidemiology 1999; 28(3): 479-484.
80. Bonofiglio D, Maggiolini M, Marsico S, et al. Critical years and stages of puberty for radial bone mass apposition during adolescence. Hormone and Metabolic Research 1999; 31(8): 478-482.

81. Bonofiglio D, Maggiolini M, Catalano S, et al. Parathyroid hormone is elevated but bone markers and density are normal in young female subjects who consume inadequate dietary calcium. British Journal of Nutrition 2000; 84(1): 111-116.
82. Matkovic V, Fontana D, Tominac C, Goel P, Chesnut CH. III. Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. American Journal of Clinical Nutrition 1990; 52(5): 878-879.
83. Bonjour JP, Carrie AL, Ferrari S, et al. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. Journal of Clinical Investigation 1997; 99(6): 1287-1294.
84. Johnston CC Jr, Miller JZ, Slemenda CW, et al. Calcium supplementation and increases in BMD in children. New England Journal of Medicine 1992; 327(2): 82-87.
85. Lee WT, Leung SS, Wang SH, et al. Double-blind, controlled calcium supplementation and bone mineral accretion in children accustomed to a low-calcium diet. American Journal of Clinical Nutrition 1994; 60(5): 744-750.
86. Lee WT, Leung SS, Leung DM, Tsang HS, Lau J, Cheng JC. A randomized double-blind controlled calcium supplementation trial, and bone and height acquisition in children. British Journal of Nutrition 1995; 74(1): 125-139.
87. Stear SJ, Prentice A, Jones SC, Cole TJ. Effect of a calcium and exercise intervention on the bone mineral status of 16–18-y-old adolescent girls. American Journal of Clinical Nutrition 2003; 77(4): 985-992.
88. Yano K, Heilbrun LK, Wasnich RD, Hankin JH, Vogel JM. The relationship between diet and BMC of multiple skeletal sites in elderly Japanese-American men and women living in Hawaii. American Journal of Clinical Nutrition 1985; 42(5): 877-888.
89. Fujiwara S, Kasagi F, Yamada M, Kodama K. Risk factors for hip fracture in a Japanese cohort. Journal of Bone and Mineral Research 1997; 12(7): 998-1004.
90. Nowson CA, Green RM, Hopper JL, et al. A co-twin study of the effect of calcium supplementation on bone density during adolescence. Osteoporosis International 1997; 7(3): 219-225.
91. Kalkwarf HJ, Khoury JC, Lanphear BP. Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. American Journal of Clinical Nutrition 2003; 77: 257-265.

92. Whiting SJ, Lemke B. Excess retinal intake may explain the high incidence of osteoporosis in northern Europe. Nutrition Reviews 1999; 57(6): 192-195.
93. De Fu Ma, Wei Zheng, Ming Ding, Yu Mei Zhang, Pei Yu Wang. Milk intake increases BMC through inhibiting bone resorption: Meta-analysis of randomized controlled trials. e-SPEN 2013; Journal 8: 1-7. Crown Copyright 2012 Published by Elsevier Ltd on behalf of European Society for Clinical Nutrition and Metabolism.
94. Wosje KS, Binkley TL, Fahrenwald NL, Specker BL. High bone mass in a female Hutterite population. Journal of Bone and Mineral Research 2000; 15(8): 1429-1436.
95. Teegarden D, Lyle RM, Proulx WR, Johnston CC, Weaver CM. Previous milk consumption is associated with greater bone density in young women. American Journal of Clinical Nutrition 1999; 69(5): 1014-1017.
96. Sentipal JM, Wardlaw GM, Mahan J, Matkovic V. Influence of calcium intake and growth indexes on vertebral BMD in young females. American Journal of Clinical Nutrition 1991; 54: 425-428.
97. Rubin K, Gendreau P, Sarfarazi M, Mendola R, Dalsky G. Predictors of axial and peripheral BMD in healthy children and adolescents, with special attention to the role of puberty. Journal of Pediatrics 1993; 123(6): 863-870.
98. Gennari C. Calcium and vitamin D nutrition and bone disease of the elderly. Public Health Nutrition 2001; 4(2B): 547-559.
99. Nakamura K, Nashimoto M, Tsuchiya Y, Obata A, Miyanishi K, Yamamoto M. Vitamin D insufficiency in Japanese female college students: a preliminary report. International Journal for Vitamin and Nutrition Research 2001; 71(5): 302-305.
100. Michaëlsson K, Melhus H, Lemming EW, Wolk A, Byberg L. Long term calcium intake and rates of all cause and cardiovascular mortality: community based prospective longitudinal cohort study. BMJ 2013; 346: 228.
101. Michaëlsson K, Wolk A, Langenskiold S., Basu S., Warensjo E, Melhus, Byberg L. Milk intake and risk of mortality and fractures in women and men: cohort study. BMJ 2014; 349: g6015.
102. Giovannucci E, Rimm EB, Wolk A, Ascherio A, Stampfer MJ, Colditz GA, Willet WC. Calcium and fructose intake in relation to risk of prostate cancer. Cancer Research 1998; 58(3): 442-447.

103. Giovannucci E, Liu Y, Stampfer MJ, Willet WC. A prospective study of calcium intake and incident and fatal prostate cancer. Cancer Epidemiology Biomarkers & Prevention 2006; 15(2): 203-210.
104. Chan JM. Dairy products, calcium, and prostate cancer risk in the Physicians' Health Study. American Journal of Clinical Nutrition 2001; 74(4): 549-554.
105. Van Winckel M, Vande Velde S, De Bruyne R, Van Biervliet S. Vegetarian infant and child nutrition, Clinical practice. European Journal of Pediatrics 2011; 170(12): 1489-1494.
106. Levrat-Verny MA, Coudray C, Bellanger J, Lopez HB, Demigné C, Rayssiguier Y, Rémésy C. Whole wheat flour ensures higher mineral absorption and bioavailability than white wheat flour in rats. British Journal of Nutrition 1999; 82(1): 17-21.
107. Weaver CM, Proulx WR, Heaney R. Choices for achieving adequate dietary calcium with a vegetarian diet. American Journal of Clinical Nutrition 1999; 70(3 Suppl): 543S–548S.
108. Cheryan M. Phytic acid interactions in food systems. Critical Reviews in Food Science and Nutrition 1980; 13(4): 297-335.
109. Freeland-Graves J. Mineral adequacy of vegetarian diets. American Journal of Clinical Nutrition 1988; 48(3 Suppl): 859-862.
110. Heaney RP, Weaver CM, Recker RR. Calcium absorbability from spinach. American Journal of Clinical Nutrition 1988; 47(4): 707-709.
111. Weaver CM, Heaney RP, Nickel KP, Packard PI. Calcium bioavailability from high oxalate vegetables: Chinese vegetables, sweet potatoes, and rhubarb. Journal of Food Science 1997; 62(3): 524-525.
112. Heaney RP, Weaver CM. Calcium absorption from kale. American Journal of Clinical Nutrition 1990; 51(4): 656-657.
113. Weaver CM, Heaney RP, Proulx WR, Hinders SM, Packard PT. Absorbability of calcium from common beans. Journal of Food Science 1993; 58(6): 1401-1403.
114. Heaney RP, Weaver CM, Hinders SM, Martin B, Packard PT. Absorbability of calcium from brassica vegetables: broccoli, bok choy, and kale. Journal of Food Science 1993; 58(6): 1378-1380.
115. Maierhofer WJ, Lemann J, Gray RW, Cheung HS. Dietary calcium and serum 1,25-(OH)2-vitamin D concentrations as determinants of calcium balance in healthy men. Kidney International 1984; 26(5): 752-759.

116. Greger JL, Baligar P, Abernathy RP, Bennett OA, Peterson T. Calcium, magnesium, phosphorus, copper, and manganese balance in adolescent females. American Journal of Clinical Nutrition 1978; 31(1): 117-121.
117. Wisker E, Nagel R, Tanudjaja TK, Feldheim W. Calcium, magnesium, zinc, and iron balances in young women: effects of a low-phytate barley-fiber concentrate. American Journal of Clinical Nutrition 1991; 54(3): 553-559.
118. Begum A, Pereira SM. Calcium balance studies on children accustomed to low calcium intakes. British Journal of Nutrition 1969; 23(4): 905-911.
119. Wynckel A, Hanrotel C, Wuillai A, Chanard J. Intestinal calcium absorption from mineral water. Mineral and Electrolyte Metabolism 1997; 23(2): 88-92.
120. Heaney RP. Absorbability and utility of calcium in mineral waters. American Journal of Clinical Nutrition 2006; 84(2): 371-374.
121. Guillemant J, Le HT, Accarie C, Du Montcel ST, Delabroise AM, Arnaud MJ, Guillemant S. Mineral water as a source of dietary calcium: acute effects on parathyroid function and bone resorption in young men. American Journal of Clinical Nutrition 2000; 71(4): 999-1002.
122. Weaver CM. Calcium bioavailability and its relation to osteoporosis. Proceedings of the Society for Experimental Biology and Medicine 1992; 200(2): 157-160.
123. Craig WJ, Mangels AR. American Dietetic Association. Position of the American Dietetic Association: vegetarian diets. Journal of the American Dietetic Association 2009; 109(7): 1266-1282.
124. http://en.wikipedia.org/wiki/Vegetarianism_by_country # Ευρώπης.
125. White RF, Seymour J, Frank E. Vegetarianism among US women physicians. Journal of the American Dietetic Association 1999; 99(5): 595-598.
126. Lea E, Worsley A. The cognitive contexts of beliefs about the healthiness of meat. Public Health Nutrition 2002; 5(1): 37-45.
127. American Dietetic Association, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada. Vegetarian diets. Canadian Journal of Dietetic Practice and Research 2003; 64(2): 62-81.
128. Sabaté J, Lindsted K, Harris RD, Johnston PK. Anthropometric parameters of schoolchildren with different life-styles. American Journal of Diseases of Children 1990; 144(10): 1159-1163.

129. Papadaki A, Valsta LM, Lampi AM, Peñalvo J, Adlercreutz H, Vardavas C, Kafatos A. Differences in nutrient intake during a Greek Orthodox Christian fasting and non-fasting week, as assessed by a food composition database and chemical analyses of 7-day weighed food samples. Journal of Food Composition and Analysis 2011; 24(1): 22-28.

Table 1. Fasting of the Christian Orthodox Church (COC) (28-30).

FASTING	***DAYS***	***PERIODS OF FASTING***
LENT	**41**	From a Clean Monday to Saturday of Lazarus
EASTER WEEK	**6**	From Holy Monday to Saturday
CHRISTMAS	**40**	From November 15 through December 24
ASSUMPTION	**14**	From 1 to August 15
HOLY APOSTLES*	**0 – 30****	On the next day Sunday of All Saints up to June 28, the eve of the feast of Saints Peter and Paul
DAILY	**3**	January 5 (Eve of Epiphany) August 29 (the decapitation of John the Baptist) September 14 (Exaltation of the Holy Cross)
EVERY WEDNESDAY AND FRIDAY	**55 – 63**	All Wednesdays and Fridays of the year, apart from those already in the periods of fasting and absolute or free periods
DAY TOTAL	**159 – 197** **SD:178 ± 19**	

* So named because it precedes the two apostolic feasts: the Apostles Peter and Paul, June 29 and "Synaxis (Bevy) of the 12 Apostles," June 30.
** The duration of this fasting period is undefined, because the launch depends on the moveable feast of Easter.

Table 2. Characteristics of the 22 selected randomized controlled trials of milk and calcium intake for children and adolescents.

Author / year (Study)	No of patients	Ages (years)	Duration	Calcium Intake, mg/day	Milk and Dairy, g/day	Groups	Type of Research	Indices
Kardinaal 1999 (15)	1116 girls and young women / 6 Europe countries	11 – 15	-	609±282 (Italy) 831±363 (Poland 881±335 (France) 1134±462 (Netherlands) 1258±594 (Denmark) 1267±550 (Finland)	Not representative for the respective countries.	750 girls (11 – 15 years of age) 375young women (20 – 23 years of age) from Finland, Italy, Denmark, Poland, Netherlands, France	Cross-sectional study	BMC, BMD
Kröger 1993 (19)	65 children and adolescents / Finland	7 – 20	1 year	<800 - >1200	-	-	Prospective study	BMD
Lloyd 2000 (20)	81 white teenager girls / US	12 – 18	6 years	500 – 1500	-	Sports – exercise No sports	Longitudinal study	BMD
Lloyd 2002 (22)	75 white female adolescents / US	12 – 20	8 years	486 - 1958	-	Exercise, fitness	Longitudinal study	BMD

Kohlenderg-Mueller 2003 (43)	16 young adults / Germany	19 – 24	10 days	843 ± 140 1322 ± 302	Milk 32%	8 Vegan 8 Lactovegeterian	Cross-sectional study	Urinary calcium excretion
Ho-Pham 2009 (44)	210 women / Vietnam	50 - 85	10-72 years vegan diet	375 (330 ± 205) 683 omnivores	-	105 Vegans Buddhist nuns 105 omnivores women	Cross-sectional study	BMD
Budek 2007 (45)	24 boys / Denmark	8	7 days	1000± 300 2900±200	1.5 L/day skimmed milk	12 boys, milk group 12 boys, meat group	Cross-sectional study	Bone turnover markers (s-Osteocalcin, s-CTX, s-BAP)
Volek 2003 (46)	28 boys / US	13 – 17	3 months	1723±274 979±286	236 mL/ serving/d of 1% fluid milk (3 daily servings)	14 boys, milk group 14 boys, juice group	Cross-sectional study	BMC, BMD
Jones 2001 (47)	330 children (215 boys, 115 girls) / Tasmania, Australia	8	5 years	1336±541	590 mL/d	215 boys 115 girls	Cross-sectional study	BMD, Urinary Potassium and Sodium

Uusi-Rasi 1997 (48)	176 girls / Finland	8 – 20	7 days	1018±361 (T1) 1059±460 (T2 & T3) 1231±565 (T4 & T5)	7d calcium intake diary (CaD)	Tanner stage 1(T1) pre-pubertal: 9.2±1.1 age Tanner stage 2 & 3 (T2 & T3) per pubertal: 11.8±1.3 age Tanner stage 4 & 5 (T4 & T5) post-pubertal: 15.9±1.8 age	Cross-sectional study	BMC, BMD, BMAD
Yoshii 2007 (49)	4761 children / Japan	15-18	3 years	-	Dairy (milk, cheese, yogurt, cream): daily, 4-5 times, 2-3 times, ≤ 1 time a week	2651 high school girls 2110 high school boys 1252 women	Cross-sectional study	Bone status (*Z-score*)
VandenBergh 1995 (50)	1359 children / Netherlands	7 – 11	28 months	-	Boys: 1.3±0.44 Girls: 1.2±0.43	653 boys 706 girls	Cross-sectional study	BMC
Young 1995 (51)	215 female twin pairs / Australia	10 – 26	-	60% of total dietary calcium is derived from dairy produce and fish.	Premenarchial: 1178±701 Postmenarchial: 1041±674	122 monozygotic (MZ) 93 dizygotic (DZ)	Cross-sectional twin study	BMD, BMC

Cheng 1999 (52)	179 children / China	12 – 16	3 years	Boys: 722 (beginning of the study) – 700.8 (at the last visit) Girls: 560 – 608.8	-	87 girls 92 boys	Longitudinal study	BMC, BMD
Kristinsson 1994 (53)	162 girls / Iceland	13 and 15	-	1000 - 1200	13 years: 1293±452 15 years: 1082±382	80 girls, 13 years 82 girls, 15 years	Cross-sectional study	BMD, BMC
Petridou 1997 (54)	100 children / Greece	7 – 14	2 years	-	Assess consumption of the calcium rich dairy products	74 boys 26 girls	Case control study	Fracture
Felily 1992 (55)	581 children / United Kingdom	7 – 9	2 years (assessed 14 years later)	Men: 1297±397 1323±319 (Control) Women: 933±278 940±317 (Control)	0-568 ml milk. 190 ml milk on each school day for 6 terms	197 boys and girls 174 controls	Randomized, controlled trial (RCT)	BMC, BMD

Lehtonen-Veromaa 2002 (56)	171 girls / Finnland	9 – 15	3 years	1575±637	Supplement 10 μg Vit. D_2 + 500 mg Ca per day	66 gymnasts 65 runners 60 nonathletic	Longitudinal study	BMD or BMAD
Welten 1994 (57)	182 children / Netherlands	13	15 years	Boys: 1100-1435 Girls: 941.4-1204	80% of the calcium intake is supplied by dairy products	84 boys 98 girls	Longitudinal study	BMD
Chan GM 1995 (58)	48 white girls / US	11	1 year	Control: 728±321 Dairy: 1437±366	Dairy products weekly (1200 mg Ca)	24 Control group 24 Dairy group	Randomized, controlled trial (RCT)	BMC, BMD
Cadogan 1997 (59)	82 white girls / United Kingdom	12.2	18 months	Control: 703 Milk: 1125	Milk group: 568 mL/d	38 Control 44 Milk	Open Randomized intervention trial	BMD, BMC
Chryssochoou 2010 (60)	323 boys and 286 girls / Greece	5 – 15 ½	3 ½ years	1134±28 (TF) 1040±28 (PF) 1090±59 (NF)	451±14 (TF) 383±14 (PF) 427±30 (NF)	Total fastening (TF) 12,1% Partial fastening (PF) 43,3% No fastening (NF)	Cross-sectional study	Growth rate

Chaptel 2. Effect of periodic abstinence from dairy products for approximately half of the year on bone health in adults following the Christian Orthodox Church fasting rules for decades.

[Archives of Osteoporosis. 2019; 14(1):68]

Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass and deterioration of bone microarchitecture, with consequent increased bone fragility and susceptibility to fracture (1). Osteoporosis is a major public health concern, affecting 200 million worldwide (2) and causing 9 million fractures per year, which are accompanied by substantial pain and suffering, impaired quality of life, disability, and even death (3). Since it is a disease associated primarily with old age, its prevalence is expected to rise with improvements in life expectancy.

Although genetic and hormonal factors influence skeletal health, proper nutrition is considered essential for the prevention of osteoporosis (4). In this context, dietary lifestyles that are biased toward specific food sources and/or exclude other food sources offer the opportunity to examine the dependence of bone health on nutrition. One such case is the lifelong periodic fasting implemented by the Christian Orthodox Church (COC).

In COC lifestyle, faith is expressed, among other things, through abstinence from certain foods—predominantly of animal origin except for seafood and snails—for certain periods and days during the year. The total days of COC fasting range from 159 to 197 (average, 178) and include five main periods, three important religious days, Wednesdays, and Fridays, as detailed in Supplemental Table 1. However, to facilitate replenishment of nutrients in the body after prolonged fasting, the COC has set two periods, comprising a total of 47 days, of no food restriction (not even on Wednesdays or Fridays).

A fundamental characteristic of COC fasting rules is the avoidance of meat, poultry, eggs, and dairy products. Consumption of olive oil, wine, and fish is generally permitted during fasting, except on Wednesdays and Fridays, unless these fall on the 47 days during which there is no food restriction. Seafood, such as oysters, mussels, shrimp, cuttlefish, octopus, squid, crab, and lobster, as well as snails, are permitted during all fasting days on which oil consumption is allowed. These characteristics of COC fasting are reminiscent of the Mediterranean diet (5).

At the same time, the characteristics of COC fasting raise concern about possible consequences on bone health. In particular, the exclusion of dairy products (the best source of calcium) and eggs (a good source of cholecalciferol) from the diet during approximately half of the year, as well as the abstention from fish (another good source of cholecalciferol) during approximately five months, may compromise calcium status in the body (6). Therefore, the present study sought to investigate the role of COC fasting on bone mass maintenance and prevalence of osteoporosis among men and women above 50 years of age. To the best of our knowledge, there are no other studies assessing the potential impact of so long periodic abstinence from dairy products on bone health or osteoporosis.

Methods

Participants and study period

This study is part of a larger cross-sectional study aimed at investigating the effects of COC fasting on health (including bone health) by comparing fasting to non-fasting individuals (hereafter referred to as fasters and non-fasters). Because, as mentioned, we are unaware of any similar study, we could not perform a power analysis to determine the necessary sample size. Thus, we intuitively chose to recruit at least 400 adults, equally divided between fasters and non-fasters. This number is much higher than the sample sizes of 120 and 59 in two previous studies that examined the association of COC fasting with serum lipids and obesity (7) and with iron status (8), respectively.

We further decided to divide our sample equally between the ages of < 50 and ≥ 50, with at least 100 fasters and 100 non-fasters in each age group. The present study focuses on the older group; the sample sizes comply with the estimate of Ho-Pham and coworkers (9) that a sample size of 91 vegetarians and 91 omnivores was required to have a power of 0.80 in detecting a difference of 0.05 g/cm^2 in bone mineral density (BMD) at the confidence interval of 95%. Although COC fasters are not vegetarians, the aforementioned study was as close to our study as we could possibly find.

Participants were recruited on a voluntary basis in the province of Thessaloniki, Greece, between September 2013 and October 2015 through churches, a monastery, and public centers for the elderly. Of the 218 individuals who consented to participate, 15 were excluded because they did not meet the inclusion criteria, which were the absence of chronic disease, absence of morbid obesity (BMI ≥ 40 kg/m^2), no dietary supplementation, no medication, and (in the case of the fasting group) adherence to COC fasting for at least

10 years. Three more individuals were excluded because they did not complete all measurements. The remaining 200 participants were 100 fasters and 100 non-fasters. The fasters included 68 women and 32 men; the non-fasters included 66 women and 34 men.

To ensure that the data regarding the fasters were as representative of the entire year as possible, we did not perform measurements during periods of fasting or periods of no food restriction. Rather, all measurements and blood sampling were performed on Saturdays during the periods characterized by fasting on Wednesdays and Fridays only.

Anthropometric and cardiac parameters

Body weight was measured on a digital scale to the nearest 0.1 kilogram with the participant wearing light clothing and no shoes. Standing height was measured with a portable stadiometer to the nearest centimeter. Resting heart rate and blood pressure were measured with an electronic blood pressure monitor (Omron, Hoffman Estates, IL) in sitting position after 10 minutes of rest.

Bone health parameters

BMD and bone mineral content (BMC) were evaluated using dual-energy X-ray absorptiometry (Lunar DPX Bravo, GE Healthcare, equipped with the GE Lunar enCORE software, v. 13.5) at the lumbar spine (L2–L4), right hip, left hip, right femoral neck, and left femoral neck. Osteoporosis was diagnosed as BMD of 2.5 standard deviations (T-score) or more below the young adult reference mean, whereas osteopenia was defined as a T-score between –1.0 and –2.5 (10). Participants were also asked about any history of bone fracture.

Biochemical parameters

Six milliliters of blood were drawn from a forearm vein in sitting position between 8 and 10 a.m., after 12 hours of fast. Blood samples were placed in test tubes without anticoagulant, left to clot at room temperature, and centrifuged at 1,500 *g* for 10 minutes. Serum was separated from other blood components, divided into Eppendorf tubes, and stored at –80^{o}C until analyzed for calcium, vitamin D (specifically, 25-hydroxyvitamin D), and urea (as an index of protein intake). Calcium was measured through the arsenazo III method in a Mindray BS-300 Chemistry Analyzer. Vitamin D was measured through Chemiluminescent Microparticle Immunoassay in an Abbott Architect i2000SR analyzer. Urea was measured through the urease – glutamate dehydrogenase method in a Mindray BS-300 Chemistry Analyzer. The coefficients of variation for the three analytes were 5%, 7%, and 3%, respectively, and the laboratory carrying out these measures was involved in a nationwide external quality control program.

Dietary assessment

Dietary calcium, vitamin D, protein, and alcohol intakes were assessed through interviewer-based recalls of food intake over three days, which included a Wednesday or Friday (during which the fasters obeyed fasting), another weekday, and a weekend day. Participants were interviewed about all foods and liquids consumed during those days. Food intake records were analyzed using the Food Processor Nutrition Analysis software (ESHA, Salem, OR).

In addition to the food intake records, a semi-quantitative food frequency questionnaire with 140 questions was used to assess the participants' dietary habits during a period of one month. Frequency of consumption was based on a typical portion of each food and beverage. This questionnaire was then used to assess the consumption of dairy products (milk, yogurt, cheese, butter, and dairy cream) and soy products (soy milk and soy cheese) as good sources of calcium. Soy products were included to take into account 20 participants, 17 fasters and 3 non-fasters, who obtained part of their calcium intake from them. The questionnaire was also used to evaluate adherence to the Mediterranean diet through the MedDietScore (11), an 11-item index that produces a score ranging from 0 to 55 (0 to 5 for each item); a higher score suggests a greater adherence to the traditional Mediterranean diet.

Physical activity and smoking habits

Participants were asked to rank their habitual physical activity as very low, low, moderate, high, or very high on a 5-point scale. Additionally, they were asked what type of physical activity they practiced for distinction of those who practiced high-intensity/impact weight-bearing exercise from those who did not. Finally, they were asked about their smoking habits. Those who smoked one or more cigarettes per day were classified as smokers.

Ethics

The study protocol was approved by the Bioethics Committee of the Alexander Technological Educational Institute of Thessaloniki, and the study was conducted according to the Declaration of the World Medical Association of Helsinki (1989). Each participant was informed about the aims, benefits, and potential risks of the study and signed a consent form before data collection and blood sampling.

Statistical analysis

The Kolmogorov-Smirnov test and histogram charts were used to assess the normality of distribution of continuous variables. The distribution of most variables differed significantly from normal in at least one of the two groups (fasters or non-fasters). Thus, for the sake of uniformity, we report all continuous variables as median (interquartile range) and compared groups by using the non-parametric Mann-Whitney U test throughout. The few variables that warranted a *t* test did not produce a different outcome from the Mann-Whitney U test. Nominal variables are reported as percentages and were tested through the χ^2 test.

Statistical analysis was performed using the SPSS, version 25 (SPSS, Chicago, IL). All statistical tests and corresponding *P* values were two-sided, and the level of statistical significance was set at 0.05.

Results

Demographic Characteristics

Fasters were 58.2 (54.0–62.3) years old (maximum 77.6) and had been observing COC fasting for 31 (10–63) years, starting at the age of 25 (20–46). Fourteen had started fasting before adulthood (between the ages of 10 and 15). Non-fasters were 56.4 (53.2–61.8) years old (maximum 77.3). All women were postmenopausal, with menopause having occurred at the age of 50 (45–52) in fasters and 49 (47–50) in non-fasters. The two groups did not differ in any of the aforementioned parameters ($P > 0.05$).

Anthropometric and cardiac parameters

Body weight, height; BMI, resting heart rate, systolic blood pressure, and diastolic blood pressure of the participants are shown in Table 1. The two groups did not differ in any anthropometric or cardiac parameter ($P > 0.05$) except for diastolic blood pressure ($P = 0.007$), which was thus more favorable in fasters.

Bone health parameters

The two groups did not differ significantly in BMD (Figure 1) or BMC (Figure 2) at the lumbar spine, right hip, left hip, right femoral neck, or left femoral neck. Likewise, the 14 fasters who started fasting before adulthood did not differ significantly from the 86 who started as adults in any of these parameters. The frequency of osteoporosis and osteopenia at these sites (Table2) was independent of group ($P > 0.05$), as shown by χ^2 tests where all expected frequencies were at least 5% to ensure validity. Likewise, the frequency of osteoporosis and osteopenia was independent of sex. The average prevalence of osteoporosis was 5% (ranging from 0% to 16% across sites, groups, and sexes), while the average prevalence of osteopenia was 35% (ranging from 22% to 50%). Eighty-eight fasters and 82 non-fasters were free of osteoporosis at all 5 sites, although this difference was not statistically significant. Twenty participants from each group, all women, had had bone fractures.

Biochemical and dietary parameters

The serum calcium, 25-hydroxyvitamin D, and urea concentrations are shown in Table 3. The two groups did not differ significantly in calcium or vitamin D, whereas the difference in urea was marginally significant ($P = 0.095$). Daily dietary calcium, vitamin D, and protein intakes, based on the 3-day recalls, are also shown in Table 3. The differences in calcium and protein intake were significant ($P = 0.010$ and 0.028, respectively).

The lower calcium intake of fasters compared to non-fasters was corroborated by the analysis of the food frequency questionnaire: Fasters consumed 10.3 (7.6–16.3) servings of dairy and soy products per week, whereas non-fasters consumed 15.3 (10.6–21.0) servings ($P < 0.001$). Finally, the corresponding MedDietScore was the same for the two groups: 30 (27–34) and 30 (27–33), respectively. Daily alcohol consumption was 0 (0–0) g in both groups, since most participants (89 fasters and 76 non-fasters) reported none.

Physical activity and smoking habits

Fasters did not differ from non-fasters in habitual physical activity (Figure 3). Only 7 fasters and 8 non-fasters practiced high-intensity/impact weight-bearing exercises. Fasters smoked considerably less than non-fasters, since only three fasters were smokers, as compared to 36 non-fasters ($P < 0.001$). Fasters smoked 0 (0-0) cigarettes daily, whereas non-fasters smoked 0 (0-5) cigarettes daily.

Discussion

In the present study, we have examined differences in a number of parameters related to bone health (specifically, BMD, BMC, prevalence of osteopenia and osteoporosis, and history of bone fracture) between individuals of both sexes, aged 50 to 78 years, who had been adhering to periodic fasting according to the rules of the COC for at least 10 years and controls who did not restrict their diet. Our main finding is that bone health did not differ between groups even though fasters had lower consumption of dairy and soy products, as well as lower calcium and protein intakes (but not lower food consumption overall, as evidenced by the absence of difference in BMI). Although previous studies (7,8) have addressed the impact of COC fasting on health indices (specifically, the lipidemic profile, BMI, and iron status), this is the first study, to the best of our knowledge, relating COC fasting to the prevalence of chronic diseases such as osteoporosis.

Sufficient consumption of dairy products is generally recommended due their high contents of high-quality protein, minerals, and vitamins, with most countries suggesting 2 to 3 servings per day for the adult population (12). In our study, consumption of dairy and soy products was below these recommendations for most fasters: Division of the weekly intakes by 7 yielded 1.5 (1.1–2.3) servings per day for fasters and 2.2 (1.5–3.0) servings per day for non-fasters. Yet, fasters did not differ from non-fasters in any of the bone health parameters assessed. These findings challenge the view that dairy consumption is beneficial for bone health and constitute a useful addition to recent controversial findings showing an association of higher milk and total dairy consumption with a lower risk of hip fracture in older adults (13), no clear association of milk or total dairy consumption with the risk of hip fracture (14), and an attenuation of age-related cortical bone loss in consumers of fermented dairy products (such as yogurt) but not in consumers of milk or ripened cheese (15). It is thus possible that bone health depends on other factors than the intermittent intake of specific foods.

A recent meta-analysis has concluded that, compared with omnivores, vegetarians and vegans have lower BMD at the femoral neck and lumbar spine, while vegans have higher fracture rates (16). These conclusions, combined with the findings of the present study, suggest that, although abolition of many or all foods of animal origin for life (vegetarianism and veganism, respectively) is detrimental to bone health, periodic abstinence from these foods for about half of the year (COC fasting) does not.

In accordance with the lower consumption of dairy and soy products, calcium intake by fasters was lower than that by non-fasters. Nevertheless, most fasters obtained more calcium (median 532 mg/day) than the recommended minimum of 400–500 mg/day for the prevention of osteoporosis (17). This may explain the fact that fasters did not differ from non-fasters in BMD, BMC or fracture rate. Considering the fact that the DRI for calcium for older adults is 1,000-1,200 mg (18), our findings suggest that not as much calcium is needed to optimize BMD and BMC and prevent osteoporosis.

In addition to calcium, fasters had lower intakes of two more nutrients related to bone health, that is, vitamin D and protein (19–21), although the difference in vitamin D did not reach statistical significance, despite being numerically large (due to the wide dispersion of the individual values), and although sunshine exposure is usually the most important determinant of circulating 25-hydroxyvitamin D levels. These findings can also be explained by the lower consumption of dairy products and, generally, foods of animal origin by fasters. As with calcium, however, these lower intakes were not reflected in did not prove enough to cause a difference between fasters and non-fasters in the bone health parameters assessed in this study.

Despite a significant difference in dietary calcium intake, the serum calcium concentration did not differ between fasters and non-fasters. This may be explained by the presence of a very efficient calcium homeostatic system in the body (22). Likewise, despite a significant difference in dietary protein intake, the serum urea concentration did not differ between fasters and non-fasters. However, differences in both parameters were numerically small (6% to 10%) and in the same direction (that is, both protein intake and serum urea concentration were higher in the non-fasters). Finally, the serum vitamin D concentration and vitamin D intake did not differ significantly between groups and was numerically higher in non-fasters. In all, the biochemical data seem to be in accordance with the dietary ones.

An interesting finding of our study was the lower diastolic blood pressure of fasters compared to non-fasters. This suggests that some aspect of their lifestyle is more favorable. At present, is not clear what that aspect might be. Further analyses are under way, which might shed light on this difference.

A limitation of the present study is the one always associated with studies involving self-reporting of lifestyle parameters. Therefore, the possibility exists of misreporting (either under- or over-reporting) food intakes, physical activity, and smoking habits. However, there is no reason to suspect that fasters misreported these parameters in a way different from non-fasters. Additionally, because of the variation in dietary habits of fasters over the year, the possibility exists that some of the measured endpoints may fluctuate depending on when they are measured (although it is difficult to think that the primary endpoints, such as BMD, BMC, and prevalence of osteopenia and osteoporosis, can fluctuate during a year). Nevertheless, it would be desirable to perform the same measurements in future studies during periods of fasting and periods of no food restriction in addition to the “intermediate” period assessed in the present study.

In conclusion, despite lower calcium intake and lower consumption of dairy and soy products, older men and women adhering to COC fasting did not differ from peers who did not fast in any of the indices of bone health examined. Thus, periodic abstinence from dairy and, in general, animal products does not seem to harm bone health in older individuals. This finding may prove useful in designing healthy diets for people who wish, or need, to have limited intake of such foods.

References

1. Pisani P, Renna MD, Conversano F, Casciaro E, Di Paola M, Quarta E, Muratore M, Casciaro S. Major osteoporotic fragility fractures: Risk factor updates and societal impact. World J Orthop. 2016;7:171–81.
2. Lin JT, Lane JM. Osteoporosis – a review. Clin Orthop Relat Res. 2004;425:126–34.
3. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int. 2006;17:1726–33.
4. Kruger MC, Wolber FM. Osteoporosis: Modern paradigms for last century’s bones. Nutrients. 2016;8:376.
5. Sarri KO, Linardakis MK, Bervanaki FN, Tzanakis NE, Kafatos AG. Greek Orthodox fasting rituals: a hidden characteristic of the Mediterranean diet of Crete. Br J Nutr. Aristotle University of Thessaloniki; 2004;92:277–84.
6. Papadaki A, Vardavas C, Hatzis C, Kafatos A. Calcium, nutrient and food intake of Greek Orthodox Christian monks during a fasting and non-fasting week. Public Health Nutr. 2008;11:1022–9.
7. Sarri KO, Tzanakis NE, Linardakis MK, Mamalakis GD, Kafatos AG. Effects of Greek orthodox christian church fasting on serum lipids and obesity. BMC Public Health. 2003;3:16.
8. Sarri KO, Kafatos AG, Higgins S. Is religious fasting related to iron status in Greek Orthodox Christians? Br J Nutr. 2005;94:198–203.
9. Ho-Pham L, Nguyen P, Le T, Doan T, Tran N, Le T, Nguyen T. Veganism, bone mineral density, and body composition: a study in Buddhist nuns. Osteoporos Int. 2009;20:2087–93.
10. WHO. WHO scientific group on the assessment of osteoporosis at primary health care level. Geneva: WHO; 2004.
11. Panagiotakos DB, Pitsavos C, Stefanidis C. Dietary patterns: A Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis. 2006;16:559–68.
12. Weaver CM. How sound is the science behind the dietary recommendations for dairy? Am J Clin Nutr. 2014;99:1217S–1222S.
13. Feskanich D, Meyer HE, Fung TT, Bischoff-Ferrari HA, Willett W. Milk and other dairy foods and risk of hip fracture in men and women. Osteoporosis International; 2018;29:385-396.

14. Bian S, Hu J, Zhang K, Wang Y, Yu M, Ma J. Dairy product consumption and risk of hip fracture : a systematic review and meta- analysis. BMC Public Health; 2018;18:165.
15. Biver E, Durosier-Izart C, Merminod F, Chevalley T, van Rietbergen B, Ferrari SL, Rizzoli R. Fermented dairy products consumption is associated with attenuated cortical bone loss independently of total calcium , protein , and energy intakes in healthy postmenopausal women. Osteoporosis International; 2018;29:1771-1782.
16. Iguacel I, Miguel-Berges L, Gómez-Bruton A, Moreno LA, Julián C. Veganism, vegetarianism, bone mineral density, and fracture risk: a systematic review and meta-analysis. Nutr Rev. 2018;77:1–18.
17. WHO. Diet, nutrition and the prevention of chronic diseases. WHO Technical Report Series No. 916. Geneva: WHO; 2003.
18. Ross AC, Taylor CL, Yaktine AL, Del Valle HB. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine. 2011. 634 p.
19. Dolan E, Sale C. Protein and bone health across the lifespan. Proc Nutr Soc. 2018;
20. Rizzoli R, Biver E, Bonjour J-P, Coxam V, Goltzman D, Kanis JA, Lappe J, Rejnmark L, Sahni S, Weaver C, et al. Benefits and safety of dietary protein for bone health. Osteoporos Int. Osteoporosis International; 2018;1–16.
21. Durosier-Izart C, Biver E, Merminod F, van Rietbergen B, Chevalley T, Herrmann FR, Ferrari SL, Rizzoli R. Peripheral skeleton bone strength is positively correlated with total and dairy protein intakes in healthy postmenopausal women. Am J Clin Nutr. 2017;105:513–25.
22. Houillier P, Froissart M, Maruani G, Blanchard A. What serum calcium can tell us and what it can’t. Nephrol Dial Transplant. 2006;21:29–32.

Table 1. Demographic, anthropometric, and cardiac parameters of the participants (median and interquartile range)

Parameter	Fasters (*n* = 100)	Non-fasters (*n* = 100)
Gender (female/male)	68/32	66/34
Age (years)	58.2 (54.0–62.3)	56.4 (53.2–61.8)
Body weight (kg)	75.5 (68.8–84.0)	76.7 (66.5–84.3)
Height (m)	1.63 (1.58–1.69)	1.63 (1.59–1.71)
BMI (kg/m^2)	28.0 (25.8–31.4)	28.1 (25.0–30.5)
Resting heart rate (bpm)	69 (62–76)	70 (64–76)
Systolic blood pressure (mmHg)	130 (123–133)	129 (126–133)
Diastolic blood pressure (mmHg)	80 (74–84)	82 (78–85)*

* *P* = 0.007, significantly different from fasters according to Mann-Whitney U test.

Table 2. Prevalence of osteoporosis and osteopenia at five sites

Site	Osteoporosis (%)		Osteopenia (%)		Normal (%)	
	Fasters	Non-fasters	Fasters	Non-fasters	Fasters	Non-fasters
Lumbar spine	6	15	28	31	66	54
Right hip	3	3	38	28	59	69
Left hip	3	2	35	30	62	68
Right femoral neck	7	1	39	33	54	66
Left femoral neck	5	1	47	43	48	56

Table 3. Biochemical and dietary parameters of the participants (median and interquartile range)

Parameter	Fasters (n = 100)	Non-fasters (n = 100)
Serum calcium (mg/dL)	9.7 (9.4–10.1)	9.7 (9.4–9.9)
Serum 25-hydroxyvitamin D (ng/mL)	16.5 (12.7–20.8)	18.2 (12.7–23.2)
Serum urea (mg/dL)	29 (24–36)	32 (25–40)
Daily dietary calcium intake (mg)	532 (347–752)	659 (474–857)*
Daily dietary vitamin D intake (μg)	1.20 (0.41–2.79)	1.69 (0.65–3.30)
Daily dietary protein intake (g)	0.67 (0.46–0.84)	0.71 (0.58–0.93)*

* $P < 0.05$, significantly different from fasters according to Mann-Whitney U test.

Figure 1. Box plots of bone mineral density at the lumbar spine (A), right hip (B), left hip (C), right femoral neck (D), and left femoral neck (E) of fasters (gray boxes) and non-fasters. Each box represents the interquartile range, and the center line represents the median. Whiskers are extended to the most extreme data point that is no more than 1.5 times the interquartile range from the edge of the box (Tukey style). Dots represent outliers. No significant difference between groups was found (Mann-Whitney U test, *P* > 0.05).

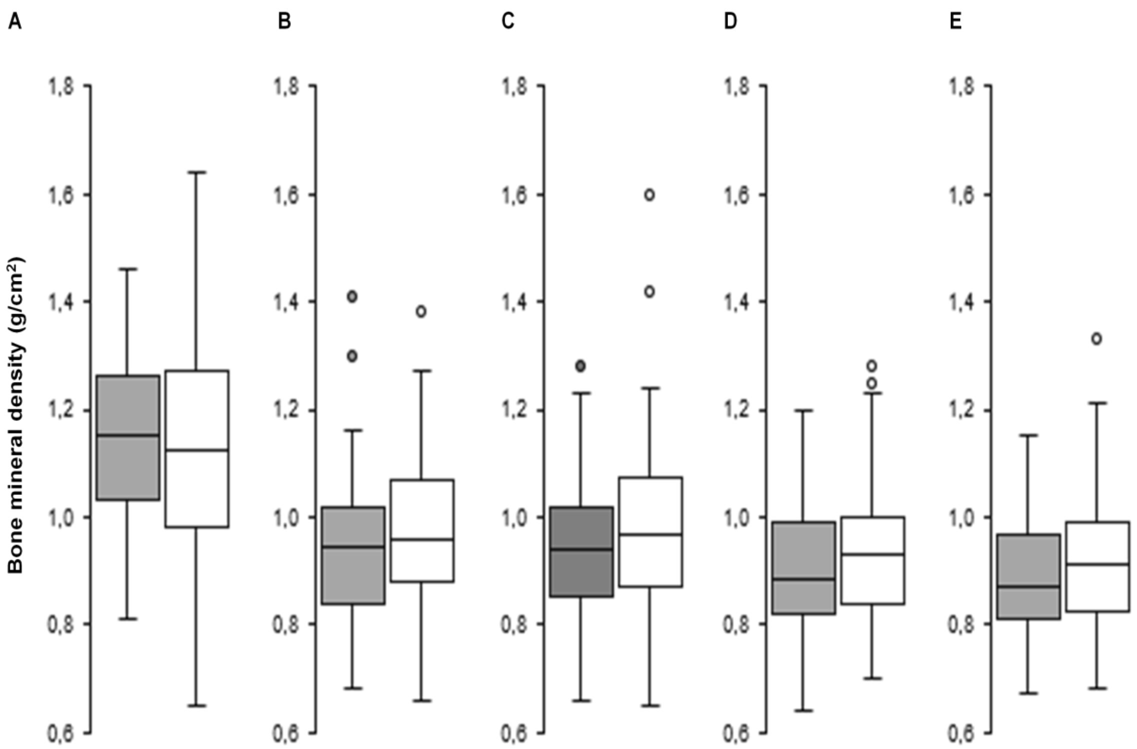


Figure 2. Box plots of bone mineral content at the lumbar spine (A), right hip (B), left hip (C), right femoral neck (D), and left femoral neck (E) of fasters (gray boxes) and non-fasters. See Figure 1 for description of each plot's elements. No significant difference between groups was found (Mann-Whitney U test, $P > 0.05$).

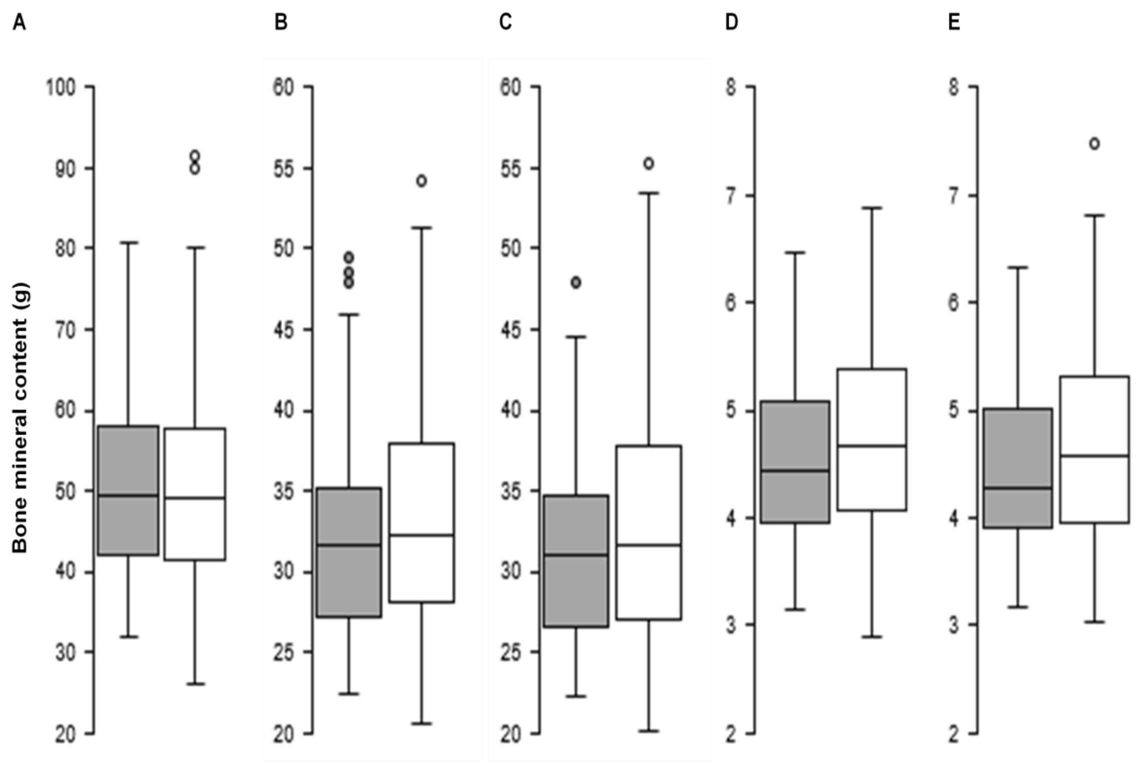
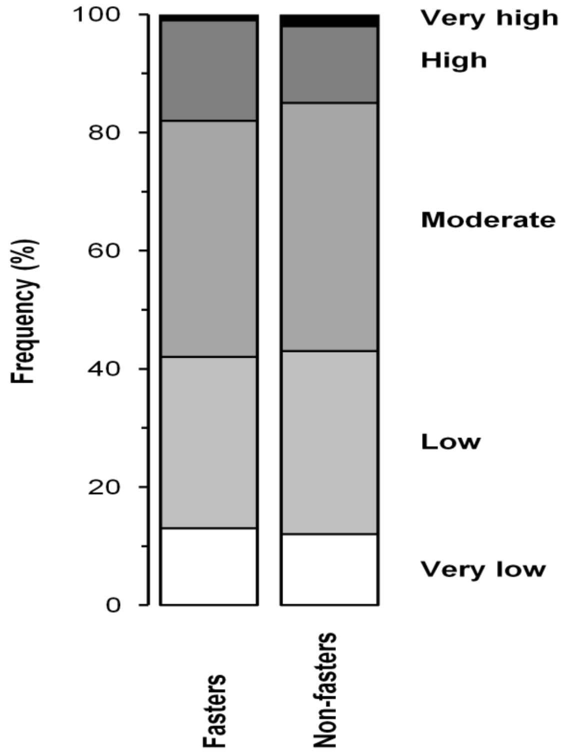


Figure 3. Distribution of physical activity levels in fasters and non-fasters. Groups did not differ significantly (χ^2 test, $P > 0.05$).



Supplemental Table 1. Fasting Periods and Days According to the Rules of the Christian Orthodox Church

Fasting Period/Days	Duration (days/year)	Additional dietary restrictions[1]	Fish consumption
Lent: 6 weeks before the Holy Week of Easter (i.e., Clean Monday to Saturday of Raising of Lazarus)	41	Avoidance of oil and wine on all days except weekends and March 9 (holiday of the Forty Martyrs)	Mandatory on March 25 (Annunciation)
Holy Week: Holy Monday to Holy Saturday	6	Avoidance of oil and wine on all days[2]	
Christmas: November 15 to December 24	40	Avoidance of oil and wine on Wednesdays, Fridays, and Christmas Eve	Mandatory on November 21 (Presentation of Virgin Mary); allowed from November 22 to December 17
Dormition (or Assumption): August 1 to 14	14	Avoidance of oil and wine on all days except weekends	Mandatory on August 6 (Christ's Transfiguration)
Saint Apostles:[3] Monday after All Saints' Sunday Day to June 28	0 to 30[4]	Avoidance of oil and wine on Wednesdays, Fridays, and June 28, unless it falls on a weekend	Mandatory on June 24 (Nativity of John the Baptist)
Important Religious Days: January 5 (Epiphany's Eve), August 29 (Abrupt of John the Baptist's head), September 14 (Exaltation of the Holy Cross)	3	Avoidance of oil and wine, unless they fall on weekends	
Wednesdays and Fridays:[5] All Wednesdays and Fridays except those included in two periods of no food restriction after Easter and Christmas	55 to 63[6]	Avoidance of oil and wine unless they fall on designated holidays	Mandatory on Wednesday of Mid-Pentecost and Wednesday of the sixth week after Easter.
Total	159 to 197		

[1] In addition to avoidance of foods of animal origin.
[2] Consumption of oil and wine can be tolerated at Holy Thursday's lunch (because oil and wine were consumed at the Last Supper.
[3] So called because it proceeds the two apostolic holidays: the feast of Saints Peter and Paul (June 29) and the Council of the Twelve Apostles (June 30).

[4] This varies because its start depends on the mobile feast of Easter. For example, when Easter is celebrated on May 5, the Saint Apostles fasting is not observed.

[5] Strict fasting is preserved as a reminder of the Betrayal (Wednesday) and the Holy Passion of Christ (Friday).

[6] Not counting Wednesdays and Fridays included in all periods described above.

Chaptel 3. The significant effect on musculoskeletal metabolism and bone density of the Eastern Mediterranean Christian Orthodox Church fasting.

[European Journal of Clinical Nutrition. 2020; 74:1736-1742]

Introduction

World's population is steadily aging. By 2050, people over the age of 60 probably will double, reaching 22%, while the elderly will be tripled (1).

After the age of 50, almost 1 in 2 females & 1 in 5 men will suffer fragility fracture (2). In USA, over 2 million osteoporotic fractures per year cost up to $ 17 billion, predicting an increase of over 87% in the 65-74 age group, which is expected to reach 50% of prevalence by 2025 (3), as the age-adjusted incidence of fractures increases exponentially during the seventh decade of life (4).

Aging is accompanied by changes in the dietary status and metabolism of the musculoskeletal system and is clinically manifested either as a reduction in bone density, i.e. osteoporosis, or in muscle mass, i.e. sarcopenia, and/or as muscle-strength impairment, i.e. dynapenia (5). The key point is the inability to maintain the energy balance resulting in frailty (6). In fact, osteoporosis is observed in 20% of malnourished (undernourished) elders (7), while 30% of the frail elder women have severe osteoporosis and sarcopenia (8).

For this reason, a balanced diet with the essential for bone health foods contributes to the prevention of osteoporosis (9). Such a diet is the "Mediterranean" that appears to have beneficial effects on bone health (10). A special variation of this is the diet of the Greek Orthodox Christian Church (M.C.O.C. diet) as it is considered a healthy eating habit (11), where abstinence from dairy and animal proteins lasts for 180 ± 19 days a year.

The purpose of this case-control study is to investigate the effects of the Hellenic (Greek) or Mediterranean C.O.C. fasting on bone density and musculoskeletal metabolism in men and women in menopause, over 50 years of age.

Methods

The permission of the Ethics Committee of the Alexandrian Technological Educational Institute as well the written consent given by the participants made the study feasible.

The study group (M.C.O.C. diet followers) consisted of 100 fasters (68 women and 32 men, mean 59 ± 6.5), with 32 years average fasting time and the control group (typical Mediterranean diet followers) of 100 non-fasters (66 females and 34 males, mean 58.1 ± 6.8). Interviews gathered personal information along with medical and nutritional history.

In addition to the food intake records, a semi-quantitative food frequency questionnaire with 140 questions was used to assess the participants' dietary habits during a period of one month. Frequency of consumption was based on a typical portion of each food and beverage. This questionnaire was also used to evaluate adherence to the Mediterranean diet through the MedDietScore (12), an 11-item index that produces a score ranging from 0 to 55 (0 to 5 for each item); a higher score suggests a greater adherence to the traditional Mediterranean diet.

The post-hoc analysis of the data obtained showed strength of 88.6%. Therefore, the total number of 200 individuals (100 in each group) is considered to meet the requirements for proper statistical processing and reliable results.

Of the 218 people who agreed to participate, 15 were excluded because they did not meet the integration criteria, which were the absence of chronic disease, the absence of morbid obesity (BMI ≥ 40 kg/m^2), no dietary supplement, no medication and adherence to COC fasting for at least 10 years. Three more people were excluded due to lack of complete data.

The participants were recruited on a voluntary basis and came from the province of Thessaloniki, in particular through parishes, two monasteries and public centers for the care of the elders.

The fasting group was formed by people dedicated to fasting for more than 10 years.

Also, no intervention was performed and the statistical processing was based exclusively on measurement data.

Everyone underwent basic physical assessment, height, body weight and BMI measurements. Biochemical blood tests calculated key-metabolic markers and DEXA tests analyzed body composition (fat and lean mass) as well as BMD, BMC, T-score, and Z-score of L2-4 vertebrae and hips.

Nutrition history through a 3-day food record during a non-fasting period provided data of macro-, micronutrient and calorie intakes processed by Food Processor Nutrition Analysis software. Finally, energy intakes calculated on a daily food consumption mode.

To ensure that the data regarding the fasters were as representative of the entire year as possible, we did not perform measurements during periods of fasting or periods of no food restriction. Rather, all measurements and blood sampling were performed on Saturdays during the periods characterized by fasting on Wednesdays and Fridays only.

Including a statement confirming that informed consent was obtained from all subjects.

Results

The IBM SPSS 25.0 software performed statistical process. A comparative analysis conducted between fasters and non-fasters via Chi-square and Student T-test, having previously set an acceptable level of significance of 5%.

In short, fasters showed better T-scores for the lumbar spine, as opposed to the right femoral neck, not only for normal bone density levels, but also for osteoporosis. In terms of statistical significance:

1. Overall lumbar spine osteoporosis incidence was as low as 9% (18 individuals out of 200), namely 4% of fasters and the - more than three times higher - 14% of non-fasters.

2. The exactly opposite was observed for the right hips as 5% of fasters demonstrated femoral neck osteoporosis versus null of the non-fasters. (Figures 1 and 2).

3. Among non-fasters, 35% were smokers instead of 4% of fasters.

4. Non-fasters exceeded in the following intakes: macronutrients (protein, total, monounsaturated & polyunsaturated fat and dietary fibers), vitamins (B3, B12, D3, E, and folic acid), minerals and trace minerals (calcium, ferrum, magnesium, manganese, phosphorus and selenium).

5. As for potassium, an inverse correlation detected for those within the range of sufficiency, ie 1600 - 2000 mg (32.7% of fasters vs 13.2% of non-fasters), despite the fact that daily intake over 2000 mg recorded the 50.9% of non-fasters and the 41.82% of fasters (X^2 & Mann-Whitney). (Table 1).

6. The corresponding MedDietScore was the same for the two groups: 30 (27–34) and 30 (27–33), respectively. The weight in the two groups did not differ, for faster was 75.5 kg (68.8–84.0) and for the non-fasters was 76.7 kg (66.5–84.3).

In contrast to the above, the blood levels of all nutrients did not show statistically significant differences between the two study groups.

Discussion

The present study investigated the possible effects of the M.C.O.C. diet on musculoskeletal metabolism, particularly on bone density in individuals over 50 years and in postmenopausal women. Fasting, for Orthodox Christianity, is the consumption of "dry food" with no meat, fish, eggs, dairy, oil or wine, once a day, and even at 9 o'clock according to the ecclesiastical timetable of the Holy Sacraments (around 3:00 p.m.). In fact, this nutritional habit is a variation of the typical Mediterranean diet with the approved beneficial effects on the human musculoskeletal system (10). It consists of bread, fruit, legumes, nuts, seafood, snails and vegetables during the fasting periods (13), resembling to a vegetarianism variant or Dietary Restriction type.

The existence of cyclic alternations of fasting periods with those of restriction-free on food consumption is the main difference from the classic Mediterranean pattern. Additionally, the same restrictions do not apply to all fasts of the year. For example, during Christmas fasting, fish can be consumed all days except Wednesdays, Fridays and the final week until Christmas Eve.

From a spiritual point of view, fasting in combination with self-restraint (i.e. limitation of quantities consumed) helps believers to appease their passions, strengthen their morale and cleanse their bodies from the toxins, to wit, this behavior is not a mere abstinence from certain types of food, but also a "physical exercise" with mental benefits (14).

The majority of participants were overweight and obese, as the proportion of normal BMI subjects was 16% and 25% for fasters and non-fasters respectively, with more than half being above the upper - normal waist circumference levels (58.2% and 55%). These are in line with the observations of Sarri et al., where no BMI reduction observed in the largest part of the sample (15).

Everyone engaged a sub-caloric diet, as the minimum daily requirement of 1600 Kcal (IOM, 2005) reached by the 67.56% of fasters and the 37.14% of non-fasters. Regarding men, 83.3% and 88.8% respectively, reached the threshold of 2000 Kcal, all consistent with relevant published data (16).

There was evidence of a low-protein intake in about 60% of individuals; 65.4% of the fasters and 58.5% of the non-fasters reported an average daily intake below 0.8 g/kg BW as 0.8-1.5 g/kg BW is the range of sufficiency for adequate BMD (17). These findings confirm previously published data (18). However, calculations in mass units, daily protein intake provided evidence of sufficiency, as over 25 g/24h recorded the 94.5% and 98.1% respectively.

It has been reported that high protein intake is a risk factor for osteoporosis as the increase of the acidic "load" promotes catabolism of calcium phosphate crystals (including those of hydroxyapatite), resulting to increased blood calcium and hypercalciuria (19). On the other hand, reduction of dietary protein can cause decreased calcium absorption, leading to secondary hyperparathyroidism (20). In our study, both blood calcium levels as the relevant biochemical markers were within the normal range.

The Institute of Medicine recommends 800 mg/day calcium as estimated average requirement (EAR) for men of 51 through 71 years old and 1000 mg/day for women over 51 and men aged 71 and older, while RDAs are 1000 and 1200 mg/day respectively (21).

Our study showed that calcium ≤800 mg/24h measured in 81.8% of fasters and in 62.26% of non-fasters. Calcium above 1000 mg/24h recorded by 5.45% and 13.2%, respectively. Similarly, 1.85% and 11.3% noted daily calcium intake above 100% RDA, compatible with those already published (14, 22).

RDA percentage for phosphorus ranged to satisfactory levels, as the 100% achieved the >63% of fasters and the 68% of non-fasters. Indeed, unaltered levels of phosphorus intake have also reported in M.C.O.C. fasters (23).

Despite the differences in macro- and micronutrient intakes and the almost steady small amounts consumed by fasters, the blood tests did not show statistically significant fluctuations. Noteworthy, serum 25 (OH) D3 ranged at low levels as a daily intake of less than 100% RDA noted in 95% of fasters. Indeed, >20 ng/ml found in 29.51% of fasters and in 41% of non-fasters. Concerning B12, low intake levels were found in 40.7% and 54.7% respectively, as for vitamin C in 64.8% and 56.6%, all the above similar to the already known data (15).

Alkaline phosphatase found to be within the normal range in 77.42% of fasters and among non-fasters, 80.64% in males and 18.3% in females. Menopause could explain the difference between women and men. Nevertheless, there was no laboratory evidence of bone catabolic activity.

Besides the M.C.O.C. fasts, relevant published data regarding other religious fastings are in line with our findings. In a comparative study between Catholic nuns and Buddhists following a hybrid vegan/lactovegetarian diet, no differences found between daily intakes and biochemical markers or even somatometric changes. The same work points to the "agreement" between other studies conducted on vegetarians of other "neighboring" doctrines such as Chinese and Korean Taoists and/or Protestants, such as Seventh Day Adventists (24). Table 2 provides comparative data regarding fasting features among various doctrines.

DEXA measurements showed a statistically significant difference in favor of fasters; where the incidence of osteoporosis was 4% versus 14% of the non-fasters, although the overall 9% confirm the already known beneficial effect of the Mediterranean diet on bone metabolism. The statistically significant difference in bone density of the right hips versus the left ones attributed to right-handed participants and, therefore, not taken into account.

As mentioned, few studies have so far focused on the relationship between "religion-related" eating habits and bone density disorders. In one of those conducted in elder vegetarians, vegans, lactovegetarians and omnivores, lower BMD found in femoral neck and Ward's triangle of vegetarians, as compared to the latter, but not in the spine (25).

These are consistent with our findings as well with antecedent studies as Lau at al. have reported. There was also agreement in correlations between daily intakes and somatometric data.

Several studies assess possible BMD changes through the prism of classical dietary patterns. As stated, though there is no statistically significant correlation between the Mediterranean diet and the bone metabolism’s biochemical markers, it appears that adoption of these dietary habits ensures optimum bone density with minimization of the fracture risk (10).

Other studies examine the effect of dietary interventions for specific purposes, e.g. weight loss in obesity. A recent study examining the effect of Calorie Restriction versus Intermittent Fasting and vegetarian/vegan patterns has concluded that BMD - but not bone quality - is negatively affected mainly in vegans since they do not exercise systematically, as compared to omnivores (26). There is no data on the level of physical activity in this document, and this is a limitation.

A similar meta-analysis indicates that in relation to meat-eaters, vegetarians and vegans as well demonstrate a correspondingly lower BMD as well as a fracture risk of 32% (27). Again, the lack of FRAX data did not allow conclusions to be drawn, and this is another one limitation.

On ending up the attempts for comparative evaluation of our study's findings, a mention should be made to the valid CALERIE study. Despite the methodological differences (ie study design, inclusion/exclusion criteria) that exceeded the obvious similarities such as bone health/metabolism (28), this comparison enhanced the importance of our conclusions.

Indeed, while BMD results from CALERIE ranged from non-statistically significant increases in obese young adults (29) to almost unchanged in non-obese young adults and obese elders (30), our study showed a statistically significant increase in BMD in overweight and obese men and postmenopausal women aged 50+ years without the use of dietary supplements and despite low levels of vitamin D3.

According to the anthropology of nutrition, geographic peculiarities determine the availability of raw materials, forming the basic dietary pattern (31). So far, C.O.C. diet in Mediterranean countries seems to be unique among other C.O.C. nations around the world with regard to antioxidant load and detoxification potential of the body especially during the non-fasting periods as there are time-intervals where the antioxidant consumption overrides the low consumption of animal fats. For example, the "north-European" Russians who consume more animal fat than the "southern" Greeks do. However, there is flexibility in choosing through a "wide" group of foods, e.g. of vegetable/animal origin with some exceptions (fish, oil, wine). The latter becomes important, as these foods are beneficial for the prevention or treatment of osteoporosis, as modern therapies with fewer drugs propose (32). Incidentally, the lack of data on oxidative stress is another limitation, as low protein intake does not exclusively represent the total oxidative potential.

The strength of this study is that it contemplates a less-studied eating habit in the light of the musculoskeletal metabolism and osteoporosis in a crucial age of over 50, and especially in menopausal women. This is, as it seems, a novelty in the field of Nutrition and Orthopedics.

Conclusion

The M.C.O.C. diet based on the Mediterranean pattern has a favorable effect bone metabolism and bone density. The - overall low - incidence of osteoporosis in the Mediterranean diet was over the threefold that of the M.C.O.C. diet. It turns out that abstinence from dairy products and meat does not affect bone density or iron levels and generally does not adversely affect musculoskeletal metabolism.

There were also significant differences in daily consumption - excluding potassium - of proteins, fats (including polyunsaturated), dietary fibers, vitamins (B3, B12, folate, D3,

E) and minerals (calcium, manganese selenium, iron, phosphorus) in favor of traditional Mediterranean diet. However, the “periodic” M.C.O.C. diet, which helps in cell detoxification and - potentially - in "hormonal" balance, seems to be a healthy lifestyle habit, along with the low incidence of smoking in fasters.

In conclusion, the periodic restriction of dietary intake of animal origin into a slightly hypocaloric diet, combined with increased consumption of plant foods of all "colors", seems to affect beneficially the musculoskeletal metabolism, perhaps better than the exclusive consumption of dietary supplements. Future research expected to enrich our knowledge in this unique nutritional model.

References

1. WHO (2014) Interesting facts about ageing. https://www.who.int/ageing/about/facts/en/ (cited 30.09.14).
2. Sambrook P, Cooper C. Osteoporosis, Lancet. 2006; 367:2010-2018.
3. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and Economic Burden of Osteoporosis-Related Fractures in the United States, 2005–2025. J Bone Miner Res. 2007; 22:465-475.
4. Kanis JA, McCloskey EV, Johansson H, Cooper C, Rizzoli R, Reginster J Y. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Inter. 2013; 24(1):23–57.
5. Lang PO, Michel JP, Zekry D. Frailty syndrome: A transitional state in a dynamic process. Gerontology. 2009; 55(5):539-549.
6. Rockwood K, Mitnitski A. Frailty in relation to the accumulation of deficits. Journals of Gerontology. 2007; 62A(7):722-727.
7. Van Bokhorst-de van der Schueren MAE, Lonterman-Monasch S, de Vries OJ, Danner SA, Kramer MHH, Muller M. Prevalence and determinants for malnutrition in geriatric outpatients, Clin Nutr. 2013; 32(6):1007-1011.
8. Frisoli A, Chaves PH, Ingham SJM, Fried LP. Severe osteopenia and osteoporosis, sarcopenia, and frailty status in community-dwelling older women: results from the Women's Health and Aging Study (WHAS) II. Bone. 2011; 48:952-957.
9. Higgs J, Derbyshire E, Styles K. Nutrition and osteoporosis prevention for the orthopaedic surgeon: A wholefoods approach. EFORT open reviews. 2017; 2(6):300–308.
10. Romero Pérez A, Rivas Velasco A. Adherence to Mediterranean diet and bone health. Nutr Hosp. 2014; 29(5):989–996.
11. Chliaoutakis JE, Drakou I, Gnardellis C, Galariotou S, Carra H, Chliaoutaki M. Greek Christian orthodox ecclesiastical lifestyle: Could it become a pattern of health-related behavior. Preventive Medicine. 2002; 34(4):428–435.
12. Panagiotakos DB, Pitsavos C, Stefanidis C. Dietary patterns: A Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis. 2006; 16:559–68.

13. Sarri KO, Tzanakis NE, Linardakis MK, Mamalakis GD, Kafatos AG. Effects of Greek orthodox christian church fasting on serum lipids and obesity. BMC Public Health. 2003; 3:1–8.
14. Papadaki A, Vardavas C, Hatzis C, Kafatos A. Calcium, nutrient and food intake of Greek Orthodox Christian monks during a fasting and non-fasting week. Public Health Nutrition. 2008; 11(10):1022–1029.
15. Sarri KO, Linardakis MK, Bervanaki FN, Tzanakis NE, Kafatos AG. Greek Orthodox fasting rituals: a hidden characteristic of the Mediterranean diet of Crete. BJN. 2004; 92(2):277–284.
16. Sarri KO, Kafatos AG, Higgins S. Is religious fasting related to iron status in Greek Orthodox Christians? BJN. 2005; 9:198–203.
17. Sarri K, Bertsias G, Linardakis M, Tsibinos G, Tzanakis N, Kafatos A. The effect of periodic vegetarianism on serum retinol and alpha-tocopherol levels. Int J Vitam Nutr Res. 2009; 79(5–6):271–280.
18. Darling AL, Millward DJ, Torgerson DJ, Hewitt CE, Lanham-New SA. Dietary protein and bone health: A systematic review and meta-analysis. Am J Clin Nutr. 2009; 90(6):1674-1692.
19. Kerstetter JE, O'Brien KO, Insogna KL. Dietary protein, calcium metabolism, and skeletal homeostasis revisited. Am J Clin Nutr. 2003; 78(3 Suppl):584S-592S.
20. Kerstetter JE, O'Brien KO, Caseria DM, Wall DE, Insogna KL. The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. J Clin Endocrinol Metab. 2005; 90(1):26–31.
21. Ross AC, Manson JAE, Abrams SA et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know. J Clin Endocrinol Metab. 2011; 96(1):53-58.
22. Rizzoli R, Abraham C, Brandi M-L. Nutrition and bone health: turning knowledge and beliefs into healthy behaviour. Current Medical Research and Opinion. 2014; 30(1):131–141.
23. Sarri K, Linardakis M, Codrington C, Kafatos A. Does the periodic vegetarianism of Greek Orthodox Christians benefit blood pressure? Preventive Medicine. 2007; 44(4):341–348.
24. Lee Y, Krawinkel M. Body composition and nutrient intake of Buddhist vegetarians. Asia Pac J Clin Nutr. 2009; 18(2):265–271.

25. Lau EMC, Kwok T, Woo J, Ho SC. Bone mineral density in Chinese elderly female vegetarians, vegans, lacto-vegetarians and omnivores. Eur J Clin Nutr. 1998; 52(1):60–64.
26. Veronese N, Reginster J-Y. The effects of calorie restriction, intermittent fasting and vegetarian diets on bone health. Aging Clin Exper Res. 2019; 31(11):1-6.
27. Iguacel I, Miguel-Berges ML, Gómez-Bruton A, Moreno LA, Julián C. Veganism, vegetarianism, bone mineral density, and fracture risk: A systematic review and meta-analysis. Nutr Rev. 2019; 77(1):1–18.
28. Rickman AD, Williamson DA, Martin CK, Gilhooly CH, Stein RI, Bales CW, Roberts S, Das SK. The CALERIE study: design and methods of an innovative 25% caloric restriction intervention Contemp Clin Trials, 2011; 32, pp. 874-881.
29. Redman LM, Rood J, Anton SD, Champagne C, Smith SR, Ravussin E; Pennington Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) Research Team. Calorie restriction and bone health in young, overweight individuals. Arch Intern Med. 2008; 168(17):1859-1866.
30. Villareal DT, Fontana L, Das SK, Redman L, Smith SR, Saltzman E, Bales C, Rochon J, Pieper C, Huang M, Lewis M, Schwartz AV. Effect of Two-Year Caloric Restriction on Bone Metabolism and Bone Mineral Density in Non-Obese Younger Adults: A Randomized Clinical Trial. J Bone Miner Res. 2016; 31:40-51.
31. Mintz SW, Du Bois CM. The Anthropology of Food and Eating. Ann Rev Anthropol. 2002; 31(1):99–119.
32. Liu H, Bian W, Liu S, Huang K. Selenium protects bone marrow stromal cells against hydrogen peroxide-induced inhibition of osteoblastic differentiation by suppressing oxidative stress and ERK signaling pathway. Biol Trace Elem Res. 2012; 150(1–3):441–450.
33. Sarkic N, López JH, López-costas O, Anglade AG. Eating in silence: isotopic approaches to nuns ' diet at the convent of Santa Catalina de Siena (Belmonte, Spain) from the sixteenth to the twentieth century. Archaeol Anthropol Sci. 2019; 3895–3911.
34. Bahijri SM, Ajabnoor GM, Borai A, Al-aama JY, Chrousos GP. Effect of Ramadan fasting in Saudi Arabia on serum bone profile and immunoglobulins. Ther Adv Endocrinol Metab. 2015; 6(5):223–232.
35. El-Desouki MI, Sulimani RA. High prevalence of osteoporosis in Saudi men. Saudi Med J. 2007; 28; 774-777.

https://www.researchgate.net/profile/Riad_Sulimani/publication/6372636_High_prevalence_of_Osteoporosis_in_Saudi_men/links/00b49529ad7fb9b766000000.pdf

36. Le LT, Sabaté J. Beyond Meatless, the Health Effects of Vegan Diets: Findings from the Adventist Cohorts. 2014; 2131–2147.
37. Zhonghua XUE. Explore the Role and Function of Bigu on Taoism Health Preservation. Croos-Cultural Communication. 2015; 11(10):90–93.
38. Banta JE, Lee JW, Hodgkin G, Yi Z, Fanica A., Sabate J. The Global Influence of the Seventh-Day Adventist Church on Diet. Religions. 2018; 9(9):251.
39. Lousuebsakul-Matthews V, Thorpe D, Knutsen R, Beeson WL, Fraser GE, Knutsen SF. Impact of Walking and Running on the Heel bone: the Adventist Health Study-2. J Sci Res Rep. 2015; 7(3):165-177.
40. Thorpe DL, Knutsen SF, Beeson WL, Rajaram S, Fraser GE. Effects of meat consumption and vegetarian diet on risk of wrist fracture over 25 years in a cohort of peri- and postmenopausal women. Public Health Nutr. 2008; 11(6):564–572.
41. Sadat-Ali M, Al-Habdan IM, Al-Turki HA, Azam MQ. An epidemiological analysis of the incidence of osteoporosis and osteoporosis-related fractures among the Saudi Arabian population. Ann Saudi Med. 2012; 32:637-641.
42. Chiu J, Lan S, Yang C, Wang P, Yao W, Su I, Hsieh C. Long-Term Vegetarian Diet and Bone Mineral Density in Postmenopausal Taiwanese Women. Calcif Tissue Int. 1997; 60(3):245–249.

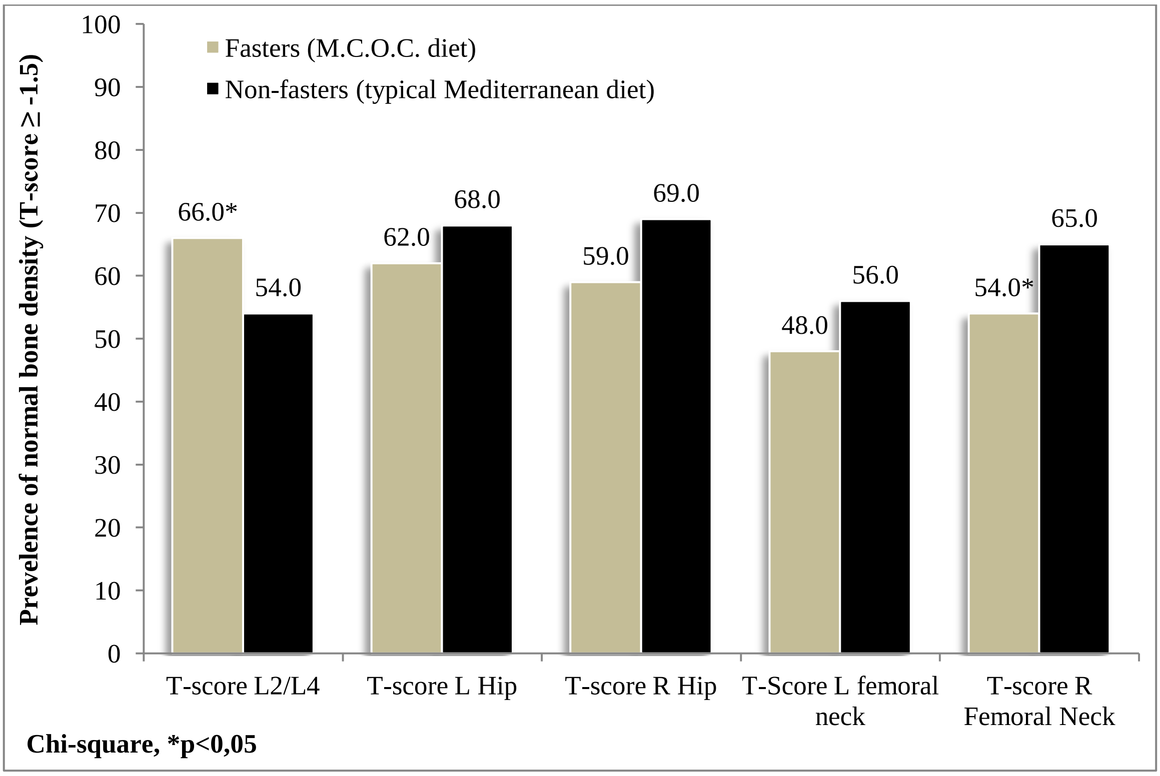
Table 1. Nutritional intakes.

Macro- & micro-nutrient Intakes					
	Fasters (C.O.C. diet) (n=53)		**Non-fasters** (Mediterranean diet) (n=55)		
	Mean	**Median**	**Mean**	**Median**	**p-value**
Proteins (gr)	51.0	49.0	59.5	57.1	.040
Total fat (gr)	79.5	73.5	90.9	89.5	.005
Polysaturated fat (gr)	8.9	8.5	10.8	9.7	.033
Dietary fibers (gr)	18.0	17.0	25.0	22.0	.005
B3 vitamin (% RDA)	56.5	42.0	74.8	64.0	.004
B12 vitamin (mcg)	2.7	1.9	5.3	2.5	.007
B12 vitamin (% RDA)	114.7	79.5	219.7	105.0	.010
D3 vitamin (mcg)	1.9	0.9	5.3	2.3	.001
D3 vitamin (% RDA)	20.1	9.5	36.8	21.0	.002
E vitamin (% RDA)	46.0	40.5	65.3	48.5	.029
Folic acid (%RDA)	44.5	40.0	57.5	48.0	.001
Calcium (%RDA)	565	511	763	686	.014
Calcium (mg)	48.2	42.5	64.4	57.0	.016
Ferrum (mg)	8.5	7.9	11.0	9.4	.021
Magnesium (mg)	163.3	159.0	216.2	186.7	.024
Manganese (%RDA)	53.5	42.0	71.3	59.0	.013
Manganese (mg)	1.1	0.9	1.5	1.2	.024
Phosphorus (%RDA)	110.6	105.0	138.3	126.0	.027
Phosphorus (mg)	785.0	735.0	971.0	880.0	.026
Potassium (1.6-2.0 g)	32.7%		13.2%		.016
Selenium (%RDA)	72.2	65.0	99.5	78.0	.035
Selenium (mg)	40.6	34.4	55.9	43.0	.027

Table 2. Concise features of the fasts of various religious doctrines.

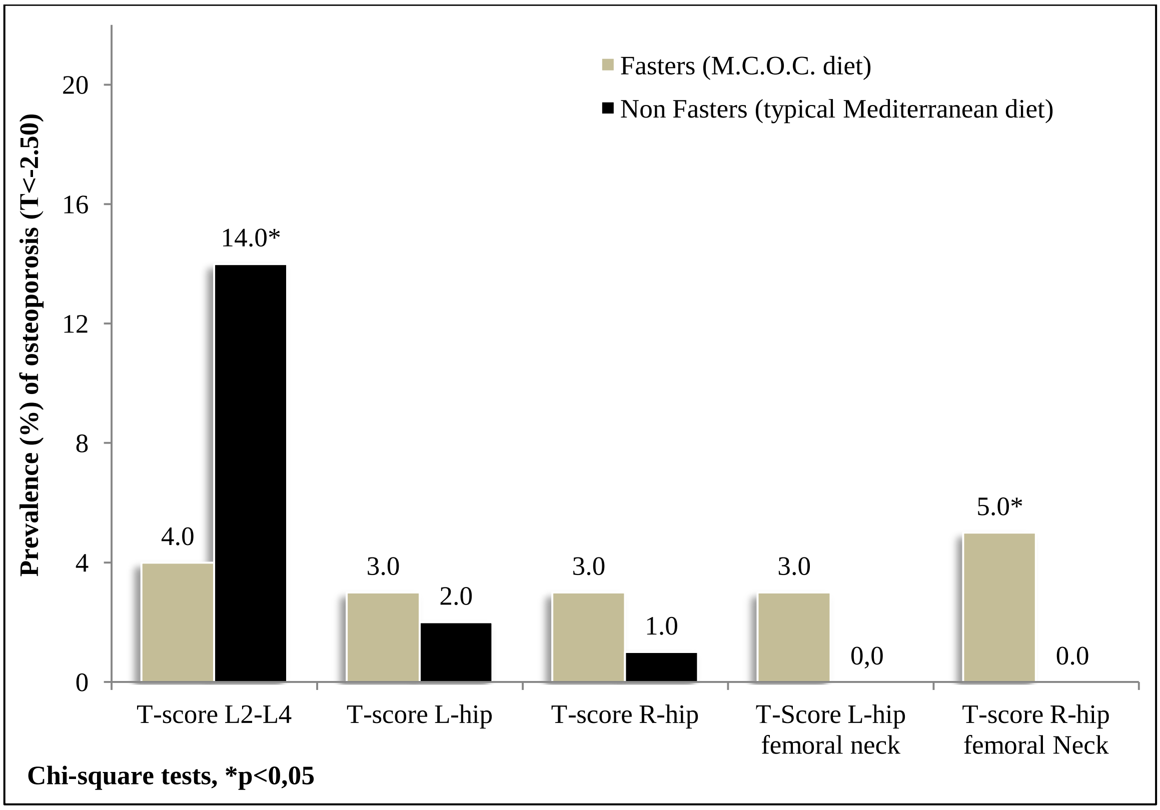
	Eastern Christian Orthodoxy (C.O.C. fasting) (11-17, 23)	Christian Catholicism (33)	Seventh Day Adventism (protestantism)	Islam (34)	Buddhism/Taoism
Fasting type	Periodic fasting (abstinence of certain foods) on Mediterranean pattern, slightly CR	Hybrid (Calorie Restriction/ vegetarianism), not necessarily CR	Biblical diet & Various types of veganism/ vegetarianism/ semi-vegetarianism (pesco-) /CR (24, 36)	Intermittent Fasting (Time-restricted feeding)	Various types Buddhists' Temple food: vegetarianism/ semi-vegetarianism (lacto- /ovo-), not necessarily CR (24) Taoists' bigu diet: IF-to-CR (37)
Annual duration	180 +/- 19 days	40 days	Throughout the year (36)	One month	Throughout the year[178]
Abstinence of	meat, fish, eggs, dairies, oil, wine	Meat	Pigs, shellfish, vultures/meat (38)	Food and water	Meat (Taoists & Buddhists), five pungent vegetables, alcohol, high amounts of processed food (Buddhists), (24) grain (Taoists) (37)
Physical activity	Regular (no restriction)	Regular (no restriction)	Sabbatarianism (24h drawback) (38, 39)	Regular (no restriction)	Reduced (prayer)-to-regular
BMI	Increased	Normal-to-high	Low-to-normal (36, 38)	Normal (34)- to-high (35)	Normal (37)-to-high, higher than omnivores (i.e. Koreans) (24)
Bone Health	Positive impact	No negative impact	Positive impact (38, 40)	Controversial [beneficial on PTH and Calcium (34)/early osteoporosis (41)]	Controversial (no negative impact/ osteoporosis) (25, 42)

Figure 1. Prevalence of normal bone density.



Columns of prevalence of normal bone density (T-score ≥ 1.5) at the lumbar spine (L2/L4), left hip (L Hip), right hip (R Hip), left femoral neck (L femoral neck), and right femoral neck (R Femoral Neck) of fasters (gray boxes) and non-fasters (black boxes). A comparative analysis conducted between fasters and non-fasters via Chi-square & Student T-test, having previously set an acceptable level of significance of 5%. Fasters showed better T-scores for the lumbar spine (Chi-square, $p < 0.05$), as opposed to the right femoral neck, not only for normal bone density levels.

Figure 2. Prevalence of Osteoporosis.



Columns of prevalence (%) of osteoporosis at the lumbar spine (L2/L4), left hip (L Hip), right hip (R Hip), left femoral neck (L femoral neck), and right femoral neck (R Femoral Neck) of fasters (gray columns) and non-fasters (black columns). A comparative analysis conducted between fasters and non-fasters via Chi-square & Student T-test, having previously set an acceptable level of significance of 5%. Overall lumbar spine osteoporosis incidence was as low as 9%, namely 4% of fasters and the - more than three times higher - 14% of non-fasters (Chi-square, $p < 0.05$). The exactly opposite was observed for the right hips as 5% of fasters demonstrated femoral neck osteoporosis versus null of the non-fasters.

Chaptel 4. Bone status of young adults with periodic avoidance of dairy products since childhood.

[European Journal of Pediatrics. 2020; 179(4):645-651]

Introduction

Bone health is an important determinant of overall health and longevity. Maximizing peak bone mass by young adulthood is considered crucial for reducing the risk of bone fracture and osteoporosis in later life (1). In fact, longitudinal studies show that measures of bone health, such as bone mineral density (BMD) and bone mineral content (BMC), track strongly from childhood through adolescence and young adulthood (2-4). This stresses the importance of ensuring optimal bone development during childhood and adolescence.

Among the many factors determining bone mass and strength development in youth, nutrition (in the sense of both individual nutrient intake and food patterns) plays a pivotal role (5). This is why unusual food patterns, emphasizing specific foods and/or excluding others, during childhood and adolescence can provide valuable insight into the dependence of bone development on nutrition. A case in point is the periodic fasting dictated by the Christian Orthodox Church (COC), which consists in abstention from certain foods—mostly of animal origin notwithstanding seafood and snails—for specific periods and days during the year (6) and has many characteristics of the Mediterranean diet (7).

Because COC bars dairy products (the best dietary source of calcium) and eggs (a good source of cholecalciferol) for approximately half of the year, as well as fish (another good source of cholecalciferol) for approximately 5 months, it may lower calcium status in the body, with consequences on bone health, especially during development. We recently showed that older adults who had been fasting for decades did not differ in BMD, BMC, or prevalence of osteoporosis from non-fasting peers despite lower calcium intake (6).

However, most (86%) had started fasting as adults. To address the question whether fasting in childhood and adolescence affects bone mass in adulthood, we investigated the effect of COC fasting since childhood on stature and bone health of young men and women.

Material and methods

Participants and study design

This is part of a larger cross-sectional study, conducted in the province of Thessaloniki, Greece, between September 2013 and October 2015 and aimed at examining the effects of COC fasting on health (including bone health) by comparing fasting to non-fasting individuals (hereafter referred to as fasters and non-fasters) of both sexes. We chose to include at least 400 participants, equally divided between fasters and non-fasters, as well as between the ages of 18-35 and ≥ 50, with at least 100 subjects in each subgroup. The present study refers to the younger group, while findings on the older group have been published (6). The study protocol was approved by the Bioethics Committee of the Alexander Technological Educational Institute of Thessaloniki.

Participants were recruited on a voluntary basis from the Aristotle University of Thessaloniki, the (then) Alexander Technological Educational Institution of Thessaloniki (presently International Hellenic University), churches, and two monasteries. Of the 236 persons who agreed to participate, 25 were excluded because, at their first visit, they were found not to meet all the inclusion criteria, which were the absence of chronic disease, absence of morbid obesity (BMI ≥ 40 kg/m^2), no dietary supplementation, no medication, and (in the case of the fasters) observance of COC fasting for at least 10 years or from the age of 10. Eleven more individuals were excluded because they did not complete all measurements. That left 200 subjects (100 fasters and 100 non-fasters).

To make certain that data regarding fasters were as representative of the whole year as possible, no measurements were done during periods of fasting or no food restriction. Instead, measurements and blood sampling were performed on Saturdays during periods characterized by fasting on Wednesdays and Fridays only.

Anthropometric and cardiac parameters

Body weight was measured on a digital scale and height was measured to the nearest centimeter with a stadiometer. Resting heart rate and blood pressure were measured with an electronic blood pressure monitor (Omron, Hoffman Estates, IL).

Bone status parameters

BMD and BMC were measured through dual-energy X-ray absorptiometry at five sites, as described (6). BMD was considered low if it was 2.0 standard deviations (Z-score) or more below the mean of a healthy population of the same age and sex; BMD was considered normal if it was above that limit (8). Subjects were asked whether they had had any bone fracture.

Biochemical parameters

Participants provided 6 mL of venous blood after 12 hours of fast while seated. Serum, produced after clotting, was analyzed for calcium, vitamin D (specifically, 25-hydroxyvitamin D), and urea (as an index of protein intake), as described (6).

Dietary assessment

Dietary calcium, vitamin D, protein, and alcohol intakes were assessed through interviewer-based recalls of food intake, as described (6). Moreover, the subjects' dietary habits during a period of one month were assessed through a food frequency questionnaire to estimate the consumption of dairy and soy products as good sources of calcium, as well as adherence to the Mediterranean diet through the MedDietScore (9).

Physical activity and smoking habits

Subjects were asked about the amount and type of physical activity to help us pinpoint high-impact weight-bearing exercises that better promote BMD (10). Finally, they were interviewed about their smoking habits, and those smoking at least one cigarette daily were considered smokers.

Statistical analysis

Continuous variables were examined for normality of distribution with the Kolmogorov-Smirnov test and histogram charts. Because the distribution differed significantly from normal in most cases and to facilitate comparisons across parameters, we report all continuous variables as median (interquartile range) and compared groups by the non-parametric Mann-Whitney *U* test. (None of the few variables that warranted a *t* test produced a different outcome.) Nominal variables are reported as percentages and were tested through the χ^2 test.

The SPSS, version 25 (SPSS, Chicago, IL), was used in all statistical analyses. All statistical tests and corresponding *P* values were two-sided, and statistical significance was declared at $P < 0.05$.

Results

Demographic Characteristics

Fasters were 26.7 (22.2–33.4) years old and had been observing COC fasting for 14 (10–21) years, starting at the age of 10 (10–12). Non-fasters were 24.0 (21.0–28.9) years old. Although significant (P = 0.004), this difference of 2.7 years between groups is not expected to have any bearing on the results.

Anthropometric and cardiac parameters

Distribution of sexes, body weight, height, BMI, resting heart rate, systolic blood pressure, and diastolic blood pressure did not differ between groups (P > 0.05, Table 1).

Bone status parameters

No significant difference between groups was found in BMD (Figure 1) or BMC (Figure 2) at the lumbar spine, right hip, left hip, right femoral neck, or left femoral neck.

The frequency of low and normal BMD at these sites (Table 2) was independent of group (P > 0.05), as shown by the χ^2 test. The average prevalence of low BMD was 4% (ranging from 2% to 9% across sites and groups). Eighty-nine fasters and 88 non-fasters had normal BMD at all 5 sites (non-significant difference). Fifteen fasters and 13 non-fasters, all women, reported that they had had bone fractures (non-significant difference).

These had occurred in the upper limbs (8 and 6, respectively), lower limbs (6 in each group), and shoulder (one in each group).

Biochemical and dietary parameters

Table 3 presents the serum calcium, 25-hydroxyvitamin D, and urea concentrations of fasters and non-fasters. The differences in calcium or 25-hydroxyvitamin D were not significant, whereas the difference in urea was (P = 0.004). Table 3 also presents the daily calcium, vitamin D, protein, and alcohol intakes, based on the 3-day recalls. The differences in calcium, protein, and alcohol intakes were significant (P = 0.010, 0.022, and 0.005, respectively), whereas the difference in vitamin D intake was marginally significant (P = 0.095). In all cases, values were higher in non-fasters.

Analysis of the food frequency questionnaires showed that fasters consumed 11.5 (7.5–17.0) servings of dairy products per week, whereas non-fasters consumed 14.0 (9.0–22.4) servings (P = 0.039). When the consumption of soy products by 33 participants (32 fasters and one non-faster) was added, the difference between groups was dampened: fasters consumed 12.0 (7.5–18.5) servings of dairy and soy products per week, whereas non-fasters still consumed 14.0 (9.0–22.4) servings (P = 0.103). Weekly alcohol consumption was 2 (0–4) drinks in fasters and 4 (2–6) in non-fasters (P < 0.001). Finally, the MedDietScore was 30 (26–33) and 29 (26–32), respectively (P = 0.643). These values indicate moderate adherence to the traditional Mediterranean diet, since the MedDietScore ranges from 0 (no adherence) to 55 (full adherence) (9).

Physical activity and smoking habits

Habitual physical activity did not differ between fasters and non-fasters (Figure 3).

Twenty-nine fasters and 32 non-fasters practiced high- impact weight-bearing exercises (non-significant difference). Only seven fasters were smokers, compared to 22 non-fasters ($P = 0.003$). Smokers did not differ significantly from non-smokers in any bone status parameter.

Discussion

We have investigated differences in indices of bone status (specifically, stature, BMD, BMC, prevalence of low BMD, and history of bone fracture) between young adults of both sexes, who had been practicing periodic religious fasting for at least 10 years or from the age of 10, and non-fasting controls. We found that none of these indices differed between groups despite fasters having lower dairy consumption and calcium intake. We are unaware of other research on the potential impact of prolonged periodic abstinence from dairy products since childhood on bone status. Longitudinal studies for up to 17 years have found that bone mass tracks strongly from childhood all the way to young adulthood, with tracking correlation coefficients ranging from 0.6 to 0.9 (2-4). These findings indicate that early levels of bone mass are important for later bone health and reinforce the relevance of the present study.

A recent position statement and systematic review on peak bone mass development and lifestyle factors has concluded that the best evidence (grade A) is available for positive effects of calcium intake and physical activity (5). In our study, the latter did not differ between groups, whereas the former was lower in fasters. Nevertheless, this did not seem to affect bone development or, in general, bone health. It appears that this lower calcium intake was still sufficient for good bone status.

In accordance with the lower calcium intake, consumption of dairy products was lower by fasters compared to non-fasters. Evidence for a positive effect of dairy consumption on bone mass is considered good (5), and most countries suggest 2 to 3 servings per day (11). In the present study, dairy consumption was below these recommendations for most fasters, as can be seen when one divides the weekly intakes reported under Results by 7, which yields 1.6 (1.1–2.4) servings per day. By comparison, non-fasters consumed 2.0 (1.3–3.2) servings per day; thus, half of them met the minimum recommendation. The lack of differences between groups in the bone status parameters assessed questions the importance of dairy products for bone health. Thus, our findings add to recent controversial findings ranging from a positive to no association of dairy consumption with indices of bone health (12-14).

Fasters had lower intakes of two more nutrients that have been linked to bone status, namely, vitamin D and protein (5, 15). These differences are apparently due to the lower consumption of dairy products and, in general, foods of animal origin by fasters. As with calcium, however, these differences were not reflected in a difference between groups regarding bone status. Of the biochemical parameters measured, the serum calcium concentration did not differ between fasters and non-fasters (despite the significant difference in calcium intake), indicative of the strong calcium homeostasis in the body (16). On the other hand, the serum urea concentration was lower in fasters, in accordance with their lower protein intake.

Among the lifestyle factors differing between fasters and non-fasters were alcohol consumption and smoking. Despite drinking more alcohol, non-fasters, as well as fasters, had all light to moderate consumption, not exceeding two drinks per day. Because such consumption is generally reported to be beneficial for bone health, unlike heavy consumption (17), one cannot argue that the higher alcohol consumption by non-fasters had a detrimental effect on bone status, one that could have counterbalanced a detrimental effect of lower calcium and dairy consumption in fasters, resulting in no difference between groups. Likewise, it seems unlikely that the higher prevalence of smoking among non-fasters could have counterbalanced the lower calcium and dairy consumption by fasters: first, because the evidence for a detriment of smoking on bone health is limited and inconsistent (5, 18); and second, because smokers did not differ from non-smokers in any of the bone status parameters measured.

The findings of the present study are in accordance with those of our previous study (6), which showed that older men and women (50 years and over) observing religious fasting for a median of 31 years did not differ in BMD, BMC, prevalence of osteoporosis, or history of bone fracture from non-fasting controls despite having lower dairy consumption and calcium intake. At the same time, it has the same limitations, that is, (i) the possibility of participants misreporting (either under- or over-reporting) food intakes, physical activity, and smoking habits (although it seems unlikely that one group misreported differently from the other) and (ii) the possible fluctuation of some parameters during periods of fasting and periods of no food restriction in fasters, which could be remedied by performing multiple measurements over the year in future studies.

In conclusion, young men and women adhering to COC fasting since childhood did not differ from peers who did not fast in stature or any of the indices of bone health examined, despite lower calcium intake and lower consumption of dairy products. Taken together with those of our recent study in older individuals, our findings show that periodic abstinence from dairy and, in general, animal products over decades, even starting in childhood, does not compromise bone health. This conclusion may be exploited for the formulation of healthy dietary plans for persons who desire, or need, to have low intakes of such foods.

References

1. Golden NH, Abrams SA, Committee on Nutrition. Optimizing bone health in children and adolescents. Pediatrics, 2014; 134(4):e1229-e1243.
2. Rønne M, Heidemann M, Schou A, Lauren JO, Bojesen AB, et al. Tracking of bone mass from childhood to puberty: a 7-year follow-up. The CHAMPS study DK. Osteoporos Int. 2018; 29(8):1843-1852.
3. Wren TAL, Kalkwarf HJ, Zemel BS, et al. Longitudinal tracking of dual-energy X-ray absorptiometry bone measures over 6 years in children and adolescents: Persistence of low bone mass to maturity. J Pediatr. 2014; 164(6):1280-1285.e2.
4. Yang Y, Wu F, Winzenberg T, Jones G. Tracking of areal bone mineral density from age eight to young adulthood and factors associated with deviation from tracking: a 17-year prospective cohort study. J Bone Miner Res. 2018; 33(5):832-839.
5. Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, et al. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporos Int. 2016; 27(4):1281-1386.
6. Rodopaios NE, Mougios V, Konstantinidou A, Iosifidis S, Koulouri AA, et al. Effect of periodic abstinence from dairy products for approximately half of the year on bone health in adults following the Christian Orthodox Church fasting rules for decades. Arch Osteoporos. 2019; 14(1):68.
7. Sarri KO, Linardakis MK, Bervanaki FN, Tzanakis NE, Kafatos AG. Greek Orthodox fasting rituals: a hidden characteristic of the Mediterranean diet of Crete. Br J Nutr. 2004; 92;277-284.
8. Lewiecki EM, Gordon CM, Baim S, Leonard MB, Bishop NJ, et al. International Society for Clinical Densitometry 2007 Adult and Pediatric Official Positions. Bone. 2008; 43(6):1115-1121.
9. Panagiotakos DB, Pitsavos C, Stefanidis C. Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis. 2006; 16:559-568.
10. Xu J, Lombardi G, Jiao W, Banfi G. Effects of exercise on bone status in female subjects, from young girls to postmenopausal women: an overview of systematic reviews and meta-analyses. Sport Med. 2016; 46(8):1165-1182.
11. Weaver CM. How sound is the science behind the dietary recommendations for dairy?

Am J Clin Nutr. 2014; 99(5):1217S-1222S.
12. Bian S, Hu J, Zhang K, Wang Y, Yu M, Ma J. Dairy product consumption and risk of hip fracture: a systematic review and meta-analysis. BMC Public Health. 2018; 18:165.
13. Biver E, Durosier-Izart C, Merminod F, Chevalley T, van Rietbergen, et al. Fermented dairy products consumption is associated with attenuated cortical bone loss independently of total calcium, protein, and energy intakes in healthy postmenopausal women. Osteoporos Int. 2018; 29:1771-1782.
14. Feskanich D, Meyer HE, Fung TT, A B-FH, C WW. Milk and other dairy foods and risk of hip fracture in men and women. Osteoporos Int. 2018; 29:385-396.
15. Dolan E, Sale C. Protein and bone health across the lifespan. Proc Nutr Soc. 2019; 78:45-55.
16. Houillier P, Froissart M, Maruani G, Blanchard A. What serum calcium can tell us and what it can’t. Nephrol Dial Transplant. 2006; 21(1):29-32.
17. Gaddini GW, Turner RT, Grant KA, Iwaniec UT. Alcohol: a simple nutrient with complex actions on bone in the adult skeleton. Alcohol Clin Exp Res. 2016; 40(4):657-671.
18. Strozyk D, Gress TM, Breitling LP. Smoking and bone mineral density: comprehensive analyses of the third National Health and Nutrition Examination Survey (NHANES III). Arch Osteoporos. 2018; 13(1):16.

Table 1. Demographic, anthropometric and cardiac parameters of the participants (median and interquartile range)

Parameter	Fasters (*n* = 100)	Non-fasters (*n* = 100)
Sex (female/male)	63/37	60/40
Body weight (kg)	67.5 (57.2–80.1)	66.9 (58.0–81.7)
Height (m)	1.68 (1.62–1.75)	1.69 (1.63–1.78)
BMI (kg/m^2)	23.9 (21.3–27.5)	23.5 (20.8–26.2)
Resting heart rate (bpm)	69 (63–77)	72 (65–82)
Systolic blood pressure (mmHg)	120 (113–130)	123 (114–130)
Diastolic blood pressure (mmHg)	76 (68–81)	76 (71–87)

Table 2. Prevalence of low and normal bone mineral density values at five sites

Site	Low (%)		Normal (%)	
	Fasters	Non-fasters	Fasters	Non-fasters
Lumbar spine	7	9	93	91
Right hip	2	4	98	96
Left hip	4	3	96	97
Right femoral neck	3	3	97	97
Left femoral neck	2	3	98	97

Table 3. Biochemical and dietary parameters of the participants (median and interquartile range)

Parameter	Fasters (*n* = 100)	Non-fasters (*n* = 100)
Serum calcium (mg/dL)	9.7 (9.4–10.1)	9.7 (9.4–9.9)
Serum 25-hydroxyvitamin D (ng/mL)	16.5 (12.7–20.8)	18.2 (12.7–23.2)
Serum urea (mg/dL)	29 (24–36)	32 (25–40)
Daily dietary calcium intake (mg)	532 (347–752)	659 (474–857)*
Daily dietary vitamin D intake (μg)	1.20 (0.41–2.79)	1.69 (0.65–3.30)
Daily dietary protein intake (g)	0.67 (0.46–0.84)	0.71 (0.58–0.93)*
Daily alcohol intake (g)	0.0 (0.0–0.0)	0.0 (0.0–7.2)*

* $P < 0.05$, significantly different from fasters according to Mann-Whitney *U* test.

Figure 1. Box plots of bone mineral density at the lumbar spine (A), right hip (B), left hip (C), right femoral neck (D), and left femoral neck (E) of fasters (gray boxes) and non-fasters. Each box represents the interquartile range, and the center line represents the median. Whiskers are extended to the most extreme data point that is no more than 1.5 times the interquartile range from the edge of the box (Tukey style). Dots represent outliers. No significant difference between groups was found (Mann-Whitney *U* test, $P > 0.05$).

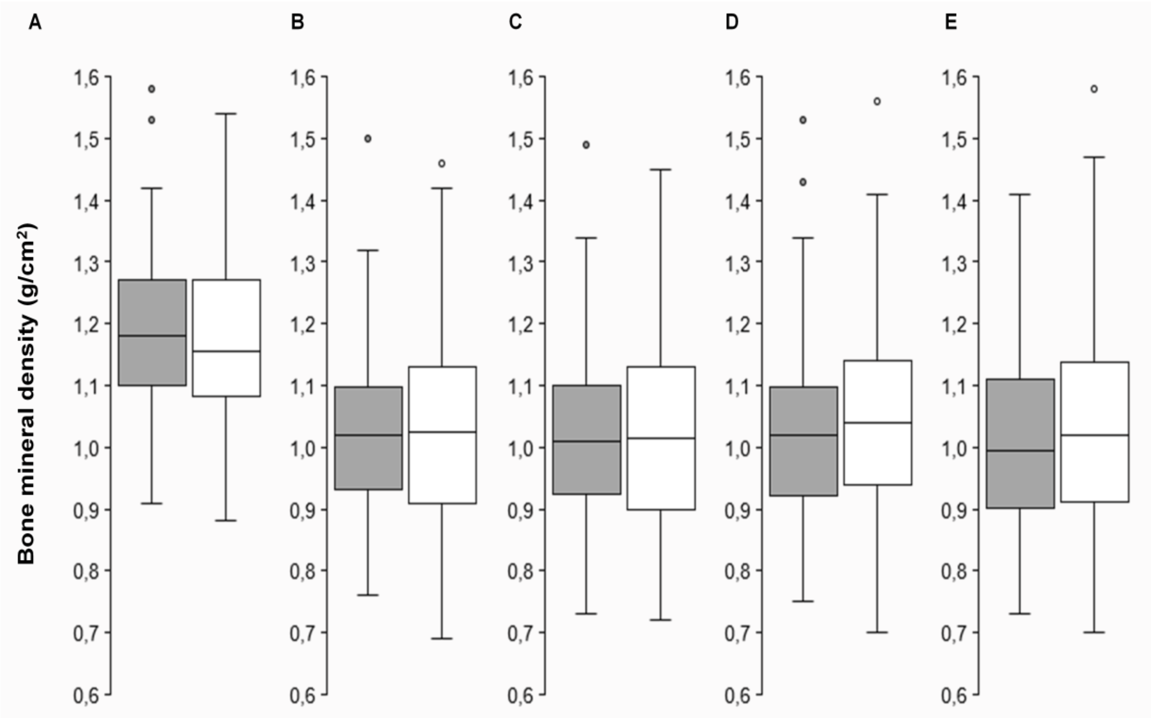
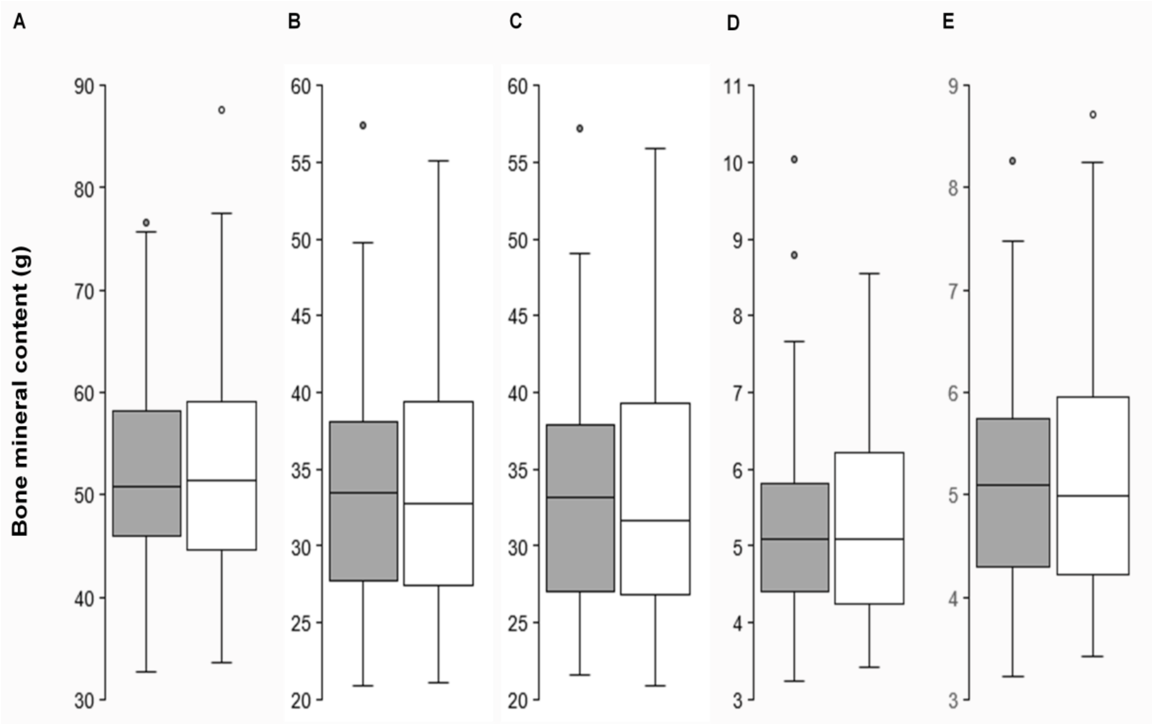
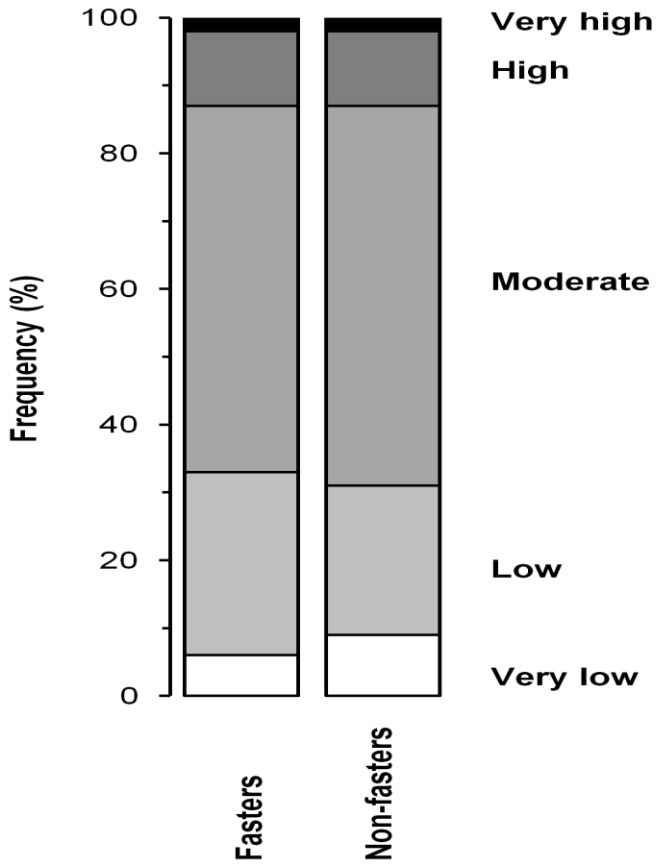


Figure 2. Box plots of bone mineral content at the lumbar spine (A), right hip (B), left hip (C), right femoral neck (D), and left femoral neck (E) of fasters (gray boxes) and non-fasters. See Figure 1 for description of each plot's elements. No significant difference between groups was found (Mann-Whitney *U* test, *P* > 0.05).



-

Figure 3. Distribution of physical activity levels in fasters and non-fasters. Groups did not differ significantly (χ^2 test, $P > 0.05$).



Chaptel 5. Dietary protein intake from different animal and plant sources plays a minor role in the bone health of adults with or without intermittent fasting for decades.

[International Journal of Food Sciences and Nutrition. Publiced on line: 07 Dec 2020]

Introduction

The integrity and health of osseous tissue are important for the overall health, well-being, quality of life, and longevity of an individual. Osteoporosis, on the other hand, by augmenting bone fragility and susceptibility to fracture, is associated with considerable pain, impaired quality of life, disability, and even death (1, 2). Justifiably then, numerous studies have sought to elucidate how bone status can be improved.

Among the factors that may have an impact on skeletal health and integrity, dietary protein intake remains one of the most controversial. This is partly due to the presence of several conflicting factors that, at least in theory, may lead to negative or positive effects of dietary protein on bone status (3). On one hand, a high protein intake may increase the renal acid load, which induces calcium loss from bone to neutralize pH and promotes bone demineralization (4). On the other hand, dietary protein is needed for bone formation, since protein constitutes approximately one quarter of human bone mass (5), upregulates anabolic hormones and growth factors with osteogenic action (6), and promotes muscle growth (7), thus indirectly benefiting bone by placing increased mechanical load on it.

Moreover, dietary protein increases dietary calcium absorption (8), thus counteracting the potentially negative effect of acidification.

The aforementioned conflicting factors may explain the variable findings of studies that have examined the association of protein intake with bone health and integrity. As an example, a recent meta-analysis has found a small positive effect of high protein intake on bone mineral density (BMD) at one skeletal site but not at other sites (9). Analysis of the association between dietary patterns characterized by consumption of different protein sources (such as meat vs fish) has also produced variable findings (as detailed in the Discussion) (10).

One such dietary pattern is the periodic fasting advocated by the Christian Orthodox Church (COC), which consists in avoidance of certain foods (mainly of animal origin except seafood and snails) for certain periods and days (totaling approximately half of the year) and shares many characteristics with the Mediterranean diet (11). As such, COC fasting is low in red meat, poultry, eggs, and dairy products, while being high in seafood.

Foods predominating in the fasters' diet during fasting days include cereals, pulses, vegetables, and fruits. Foods predominating in the fasters' diet during non-fasting days and in the non-fasters' diet at all times include milk, cheese, and dishes of all sorts based on red meat, whereas consumption of vegetables and fruits is lower.

Because of its particularly demanding character, full adherence to COC fasting rules is nowadays rare. We have recently shown that men and women fully adhering to COC fasting for decades, whether being older (11) or younger (12), did not differ in indices of bone integrity, such as BMD, bone mineral content (BMC), and prevalence of osteopenia, osteoporosis, and bone fracture, from non-fasting counterparts.

The aim of the present work was to contribute to the clarification of the role of dietary protein in bone integrity and health. To this end, we took advantage of the aforementioned lack of differences in bone status between fasters and non-fasters to investigate whether protein intake or source differed or not between groups in the total sample of our two previous studies (11, 12). The former possibility (differences in protein intake or source despite no differences in bone status) would not favor a role of protein in bone integrity or health, whereas the latter possibility (no differences in protein intake or source, paralleled by no differences in bone status) would leave such a role open.

Materials and Methods

Participants and study period

This cross-sectional study included 400 adults, equally divided between fasters and non-fasters. The study was conducted between September 2013 and October 2015, with each participant being assessed once. Participants were recruited on a voluntary basis from the Aristotle University of Thessaloniki, the (then) Alexander Technological Educational Institution of Thessaloniki (presently International Hellenic University), churches, monasteries, and public centers for the elderly, all located in the province of Thessaloniki, Greece. Of the 454 individuals who consented to participate, 40 were excluded because, at their first visit, they were found not to meet the inclusion criteria, which were the absence of chronic disease, absence of morbid obesity (BMI $\geq$ 40 kg/m^2), no dietary supplementation, and no medication. Additionally, to qualify as fasters, they were needed to declare full adherence to COC fasting without interruption for at least the past 10 years or (for those who were 18 or 19 years old) from the age of 10; to qualify as non-fasters, they were needed to declare no avoidance of any food.

Fourteen more individuals were excluded because they did not complete all the measurements described below, thus leaving 400 participants (200 fasters and 200 non-fasters). The fasters included 131 women and 69 men; the non-fasters included 126 women and 74 men. The participant flow diagram is shown in Figure 1.

Full adherence to COC fasting requires avoidance of foods of animal origin (except seafood and snails) during five periods of the year (totaling 101-131 days, depending on when Easter falls) and allows unrestricted eating during two other periods (totaling 47 days), as described in detail elsewhere (11). The remainder of the year consists in "moderate" fasting, that is, avoidance of animal source foods on Wednesdays and Fridays only. The total days of fasting range from 159 to 197 (average, 178). To ensure that the data regarding the fasters were as representative of the entire year as possible, no measurements were performed during the five periods of fasting or the two periods of no food restriction. Rather, all measurements, interviews, and blood sampling were performed during periods of moderate fasting.

Primary and secondary outcome measures

The predeclared primary outcome measures of our research were BMD and BMC at five sites; prevalence of bone fracture; frequency of consumption of protein sources; and protein intake from these sources. The predeclared secondary outcome measures were anthropometric parameters and the serum concentrations of urea, creatinine, and uric acid.

Anthropometric parameters

Body weight was measured on a digital scale to the nearest 0.1 kilogram with the participant wearing light clothing and no shoes. Standing height was measured with a portable stadiometer to the nearest centimeter.

Bone status parameters

BMD and BMC were evaluated using dual-energy X-ray absorptiometry (Lunar DPX Bravo, GE Healthcare, equipped with the GE Lunar enCORE software, v. 13.5) at the lumbar spine (L2-L4), right hip, left hip, right femoral neck, and left femoral neck. Subjects were also asked about any history of bone fracture.

Dietary assessment

The participants' dietary habits were assessed through a validated semi-quantitative food frequency questionnaire comprising 14 food groups (red meat; poultry and eggs;

seafood; dairy products; pulses; grains and cereals; nuts and seeds; vegetables; fruits; oils and fats; sweets; beverages; alcoholic drinks; and composite dishes) (13), which was administered by a certified dietitian. Participants were asked to report the frequency of consumption of foods in portions per week on average over the past month. Questionnaire data were then used to calculate the consumption of the following protein sources: red meat; poultry and eggs; seafood; dairy products; pulses; and grains and cereals. Portion sizes were set according to the National Nutrition Guide for Greek Adults (14) and are presented in Table 1. Protein intake was assessed on the basis of the protein content of these foods (15, 16). It should be noted that grains and cereals were included in the list despite their rather low protein contents because they made a considerable quantitative contribution to the total protein intake of the participants, as will be shown under Results.

The food frequency questionnaire was also used to assess alcohol intake in drinks per week.

Biochemical parameters

Blood samples were drawn into test tubes without anticoagulant from a forearm vein in sitting position between 8 and 10 a.m., after 12 hours of fast. Samples were left to clot at room temperature and centrifuged at 1,500 *g* for 10 minutes. Serum was removed and analyzed for urea, creatinine, and uric acid through the urease - glutamate dehydrogenase, sarcosine oxidase, and uricase-peroxidase methods, respectively, in a Mindray BS-300 Chemistry Analyzer. The coefficients of variation for the three analytes were 3%, 3%, and 1%, respectively, and the laboratory carrying out the measures participated in a nationwide external quality control program.

Physical activity and smoking habits

Subjects were asked to rank their habitual physical activity as very low, low, moderate, high, or very high on a 5-point scale. Additionally, they were asked what type of physical activity they practiced to let us identify high-intensity/impact weight-bearing exercises that better promote BMD (17). Finally, they were interviewed about their smoking habits; those smoking one or more cigarettes daily were considered smokers.

Ethics

The study protocol was approved by the Bioethics Committee of the Alexander Technological Educational Institute of Thessaloniki, and the study was conducted according to the Declaration of the World Medical Association of Helsinki (1989). Each participant was informed about the aims, benefits, and potential risks of the study and signed a consent form before data collection and blood sampling.

Statistical analysis

The Kolmogorov-Smirnov test and histogram charts were used to assess the normality of distribution of continuous variables. The distribution of most variables differed significantly from normal in at least one of the two groups (fasters or non-fasters). Thus, for the sake of uniformity, we report all continuous variables as median (interquartile range) and compared groups by using the non-parametric Mann-Whitney *U* test throughout. The few variables that warranted a *t* test did not produce a different outcome from the Mann-Whitney *U* test. Nominal variables were tested through the χ^2 test. Correlation analysis was performed by determining Spearman's ρ correlation coefficient.

Statistical analysis was performed using the SPSS, version 25 (SPSS, Chicago, IL).

All statistical tests and corresponding *P* values were two-sided, and statistical significance was declared at $P < 0.05$.

Results

Demographic Characteristics

Fasters were 43.0 (26.7–58.2) years old (ranging from 18.2 to 77.6). They had been observing COC fasting for 15 (10–32) years, starting at the age of 15 (10–26). Non-fasters were 43.0 (24.0–56.5) years old (ranging from 18.0 to 77.3). Sixty-eight women in the fasting group and 66 in the non-fasting group were postmenopausal, with menopause having occurred at the ages of 50 (45–52) and 49 (47–50), respectively. The two groups did not differ in any of the aforementioned parameters ($P > 0.05$).

Anthropometric parameters

Body weight of fasters and non-fasters was 72.9 (62.9–81.4) kg and 71.2 (60.5–84.0) kg, respectively. The corresponding values were 1.66 (1.60–1.73) m and 1.66 (1.61–1.74) m for height; and 26.6 (23.0–29.5) kg/m^2 and 25.6 (22.7–29.1) kg/m^2 for BMI. The two groups did not differ in any anthropometric parameter ($P > 0.05$).

Bone health parameters

The two groups did not differ significantly in BMD (Figure 2) or BMC (Figure 3) at the lumbar spine, right hip, left hip, right femoral neck, or left femoral neck. Thirty-five fasters (17.5%) and 33 non-fasters (16.5%), all women, reported that they had had bone fractures (non-significant difference). These had occurred in the upper limbs (17 in each group), lower limbs (17 and 13, respectively), and shoulder (one and three, respectively).

Dietary parameters

Table 2 presents the weekly frequency of consumption of protein sources by fasters and non-fasters. The latter had significantly higher consumption of red meat, poultry-eggs, dairy products, and grains-cereals, whereas the former had higher consumption of seafood. The same differences between groups were found in daily protein intake (Table 3). In addition, the sum of protein intake from these sources was higher in non-fasters. In both groups, dairy and grains-cereals were the major protein sources, followed, in sequence, by red meat, poultry-eggs, seafood, and pulses.

Weekly alcohol consumption was 0 (0–2) drinks in fasters and 2 (0–6) drinks in non-fasters ($P < 0.001$).

Biochemical parameters

In agreement with the higher meat and protein intakes by non-fasters, their serum urea, creatinine, and uric acid concentrations were numerically higher compared to fasters (Table 4), although only the difference in urea reached statistical significance.

Physical activity and smoking habits

Fasters did not differ from non-fasters in habitual physical activity level or percentage of subjects practicing high-intensity/impact weight-bearing exercises ($P > 0.05$). Fasters smoked considerably less than non-fasters, since only 10 fasters were smokers, as compared to 58 non-fasters ($P < 0.001$). Fasters did not differ from non-fasters in any bone status parameter.

Correlation between bone status and protein source

We examined whether BMD or BMC correlated with protein source independent of group (to increase the power of the analysis). Results are shown in Tables 5 and 6. Both BMD and BMC were positively correlated with red meat and poultry-egg consumption. Seafood consumption exhibited either negative or no significant correlation with the two bone parameters. Dairy consumption displayed only two positive correlations, whereas pulse and grain-cereal consumption was not correlated with any bone parameter. All significant correlations were weak, explaining only 1.0% to 2.8% of the variation in bone parameters. Because of this and because of the lack of normal distribution in most of the bone and dietary parameters, we did not proceed to multiple regression analysis.

Discussion

The present study examined the relationship of dietary protein intake and source with a number of parameters related to bone integrity and health (namely, BMD, BMC, and prevalence of bone fracture) of 400 men and women by taking advantage of a rare, a priori defined, dietary pattern (full adherence to religious fasting), characterized by periodic avoidance of foods from animal sources. Movassagh and Vatanparast, in their scoping review (18), stress that dietary-pattern approaches in the study of bone health take into account contributions from various aspects of the diet and can thus complement single-nutrient and single-food studies.

Fasters did not differ from non-fasters in demographic characteristics or anthropometric measures, thus providing a sound basis for comparing the main study outcomes between groups. The dietary analysis performed showed distinct differences between fasters and non-fasters in the consumption of the major protein sources, i.e., dairy products, grains-cereals, red meat, and poultry-eggs, all in favor of non-fasters.

Conversely, fasters had higher consumption of seafood, a minor protein source in both groups. As a result of these differences, daily protein intake was higher in non-fasters. Nevertheless, groups did not differ in BMD, BMC, or incidence of bone fracture.

The connection of dietary protein intake with bone integrity and bone health is not clear. Dolan and Sale (3), summarizing systematic reviews and meta-analyses by Darling et al. (19) and Shams-White et al. (9), conclude that higher protein intakes have a small positive impact on BMD. In particular, Darling et al. (19) found a weak positive relationship of dietary protein intake with BMD and BMC, only explaining 1-2 % of the variation in BMD. Likewise, the meta-analysis of randomized controlled trials and prospective cohort studies by Shams-White et al. (9) reported a small positive effect (0.52%) of higher, compared to lower, protein intakes (defined differently in each analyzed study) on BMD at the lumbar spine but no effect at other sites (that is, total hip, femoral neck or total body). Similarly, Rizzoli et al. (20) summarizing systematic reviews and meta-analyses on the benefits and risks of dietary protein intakes for bone health in adults, concluded that high protein intakes may be beneficial in reducing bone loss and hip fracture risk, provided calcium intakes are adequate. Variation in protein intakes within the "normal" range accounted for 2-4% of BMD variance. Such small differences can also be seen in most of the panels of Figures 2 and 3 in the present paper, where the median BMD and BMC of non-fasters was 1% to 3% higher than that of fasters. Although these differences did not reach statistical significance in the present single study, our data can make a useful contribution to future meta-analyses examining the connection between dietary protein intake and bone health.

To further examine the possible connection of protein source with bone status, correlation analysis was performed, which produced weak (explaining just 1% to 3% of the variation in BMD or BMC) positive results for red meat, poultry-eggs, and (partly) dairy products, weak negative results for seafood, as well as no significant correlation for pulses and grains-cereals. Again, there are controversies in the literature regarding the connection of specific protein sources with bone status. In agreement with the present findings, Durosier-Izart et al. (21) reported that bone strength was positively associated with total, animal, and dairy protein intakes but not with vegetable protein intake in healthy postmenopausal women. Nevertheless, Wallace and Frankenfeld, (22) in their systematic review and meta-analysis, conclude that, when it comes to the beneficial effect of high protein intake on BMD, there are no differences between animal and plant proteins, although data in this area are scarce.

Regarding seafood consumption, the present findings differ from those of Li et al. (23), who reported that Chinese men with high fish consumption (without consideration for portion size) had a lower prevalence of osteoporosis. Of interest is the study of Choi and Park (24), who found a weak positive association (with correlation coefficients ranging from 0.047 to 0.181) with BMD in Koreans (who consumed seafood 23 times per month on average) but not in Americans (who consumed seafood only 5 times per month on average). The authors suggest that a minimum seafood intake might be necessary for a substantial effect on bone status. Although comparison of their findings with ours is hampered by the fact that they used times of consumption, not portions, a median of 1.5 portions per week (for the entire sample in the present study) places the Greek participants closer to Americans than to Koreans.

Also of interest is the systematic review by Perna et al. (10) on the association between dietary patterns of meat (including processed meat and poultry) and fish consumption with BMD or fracture risk. The review in question included 37 studies with a total of 432,924 subjects. The meat patterns were positively associated with bone health in the minority of studies (encompassing 4% of subjects), whereas the remainder of the studies showed either no association (48% of subjects) or negative association (48% of subjects). The fish patterns were positively associated with bone health in the minority of studies again (encompassing 13% of subjects), whereas the remaining studies showed either no association (84% of subjects) or negative association (3% of subjects). The authors note, however, that negative effects of meat diets on bone were seen in the setting of a Western diet, not in Mediterranean or Asian diets. These conclusions highlight how complex the interrelationship of the different food components is in determining bone health.

The biochemical measures employed in the present study corroborate the dietary data. Specifically, serum urea, an accepted index of dietary protein intake (25), was significantly higher in non-fasters than fasters, as was protein intake. Serum creatinine and uric acid, known indices of meat consumption (25, 26), were also higher, although not significantly, in non-fasters. Biochemical data do not usually accompany dietary data in similar studies; thus, we consider them an important part of the present study.

Alcohol consumption and smoking were two lifestyle factors differing between fasters and non-fasters, both being higher in the latter. Despite drinking more alcohol, non-fasters (as well as fasters) had all light to moderate consumption, that is, less than two drinks per day. Because such consumption is thought to be beneficial for bone health (as opposed to heavy consumption) (27), it seems unlikely that the higher alcohol consumption by non-fasters had a detrimental effect on bone status, such that could counterbalance a detrimental effect of lower protein intake by fasters, resulting in no differences between groups. Likewise, it seems unlikely that the higher prevalence of smoking among non-fasters could have counterbalanced the lower protein intake by fasters. This is because the evidence for a detriment of smoking on bone health is limited and inconsistent (28, 29) and because smokers did not differ from non-smokers in any of the bone status parameters measured.

The main strength of the present study is the examination of a relatively large group of individuals who followed a distinct, rare dietary pattern (COC fasting). Their marked differences in food intake from non-fasting controls enabled a robust testing of the relation between bone health and protein intake from different animal and plant sources. On the other hand, as with all studies based on self-reported lifestyle parameters, the present study is limited by the likelihood of misreporting food intakes. However, given the similarity of the two groups in demographic and anthropometric characteristics, it seems improbable that fasters misreported differently from non-fasters. In addition, the variation in the fasters' diet over the year may lead to fluctuations in some endpoints, making their values dependent on when they are measured (although it is difficult to think that BMD and BMC can fluctuate during a year).

Conclusions

Despite having lower protein intakes, men and women who had been periodically avoiding animal source foods for a median of 15 years did not differ in BMD, BMC, or prevalence of bone fracture from controls with no food restrictions. BMD and BMC exhibited only weak (if any) correlations with consumption of protein-rich foods. Thus, protein intake seems to play a minor (if any) role in bone health. These findings may aid in delineating the role of dietary protein in bone health and producing practical recommendations on the matter. Future studies could employ assessment of fasters during periods of fasting and periods of no food restriction (in addition to the moderate-fasting period chosen in the present study) for a more accurate characterization of the relation between nutrient intake and bone health.

References

1. Pisani P, Renna MD, Conversano F, et al. Major osteoporotic fragility fractures: Risk factor updates and societal impact. World J Orthop. 2016; 7(3):171-181.
2. Tarrant SM, Balogh ZJ. The global burden of surgical management of osteoporotic fractures. World J Surg. 2020; 44(4):1009-1019.
3. Dolan E, Sale C. Protein and bone health across the lifespan. Proc Nutr Soc. 2019; 78:45-55.
4. Carnauba RA, Baptistella AB, Paschoal V, Hübscher GH. Diet-induced low-grade metabolic acidosis and clinical outcomes: A review. Nutrients. 2017; 9(6):e538.
5. Boskey AL. Bone composition: relationship to bone fragility and antiosteoporotic drug effects. Bonekey Rep. 2013; 2(September):e447.
6. Bonjour J-P. The dietary protein, IGF-I, skeletal health axis. Horm Mol Biol Clin Investig. 2016; 28(1):39-53.
7. Witard OC, Wardle SL, Macnaughton LS, Hodgson AB, Tipton KD. Protein considerations for optimising skeletal muscle mass in healthy young and older adults. Nutrients. 2016; 8(4):e181.
8. Calvez J, Poupin N, Chesneau C, Lassale C, Tomé D. Protein intake, calcium balance and health consequences. Eur J Clin Nutr. 2012; 66(3):281-295.
9. Shams-White MM, Chung M, Du M, et al. Dietary protein and bone health: a systematic review and meta-analysis from the National Osteoporosis Foundation. Am J Clin Nutr. 2017; 105:1528-1543.
10. Perna S, Avanzato I, Nichetti M, D’Antona G, Negro M, Rondanelli M. Association between dietary patterns of meat and fish consumption with bone mineral density or fracture risk: A systematic literature. Nutrients. 2017; 9:1029.
11. Rodopaios NE, Mougios V, Konstantinidou A, et al. Effect of periodic abstinence from dairy products for approximately half of the year on bone health in adults following the Christian Orthodox Church fasting rules for decades. Arch Osteoporos. 2019; 14(1):e68.
12. Rodopaios NE, Mougios V, Koulouri AA, et al. Bone status of young adults with periodic avoidance of dairy products since childhood. Eur J Pediatr. 2019; 179:645-651.
13. Papadopoulou SK, Barboukis V, Dalkiranis A, Hassapidou M, Petridou A, Mougios V. Validation of a questionnaire assessing food frequency and nutritional intake in Greek adolescents. Int J Food Sci Nutr. 2008; 59(2).
14. Institute of Preventive Medicine Environmental and Occupational Health. Εθνικός Διατροφικός Οδηγός Για Ενήλικες, Greek (National Nutrition Guide for Greek Adults).

Athens, Greece: Labrakis Press Organization; 2014.

15. Food Standards Agency. McCance & Widdowson's the Composition of Foods. Cambridge, UK: Royal Society of Chemistry; 2002.
16. Trichopoulou A. Πίνακες Σύνθεσης Τροφίμων Και Ελληνικών Φαγητών, Greek (Composition Tables of Foods and Greek Dishes). Athens, Greece: Parisianou; 2004.
17. Xu J, Lombardi G, Jiao W, Banfi G. Effects of exercise on bone status in female subjects, from young girls to postmenopausal women: an overview of systematic reviews and meta-analyses. Sport Med. 2016; 46(8):1165-1182.
18. Movassagh EZ, Vatanparast H. Current Evidence on the Association of Dietary Patterns and Bone Health: A Scoping Review. Adv Nutr. 2017; 8(1):1-16.
19. Darling AL, Millward DJ, Torgerson DJ, Hewitt CE, Lanham-New SA. Dietary protein and bone health: a systematic review and meta-analysis. Am J Clin Nutr. 2009; 90(3):1674-1692.
20. Rizzoli R, Biver E, Bonjour J-P, et al. Benefits and safety of dietary protein for bone health. Osteoporos Int. 2018; 29(9):1933-1948.
21. Durosier-Izart C, Biver E, Merminod F, et al. Peripheral skeleton bone strength is positively correlated with total and dairy protein intakes in healthy postmenopausal women. Am J Clin Nutr. 2017; 105(2):513-525.
22. Wallace TC, Frankenfeld CL, C FT, Frankenfeld CL. Dietary protein intake above the current RDA and bone health : A systematic review and meta-analysis. J Am Coll Nutr. 2017; 36(6):481-496.
23. Li X, Lei T, Tang Z, Dong J. Analyzing the association between fish consumption and osteoporosis in a sample of Chinese men. J Health Popul Nutr. 2017; 36:13.
24. Choi E, Park Y. The association between the consumption of fish/shellfish and the risk of osteoporosis in men and postmenopausal women aged 50 years or older. Nutrients. 2016; 8:113.
25. Wu A. Tietz Clinical Guide to Laboratory Tests. Philadelphia: Saunders; 2006.
26. Choi HK, Liu S, Curhan G. Intake of purine-rich foods, protein, and dairy products and relationship to serum levels of uric acid: The third National Health and Nutrition Examination Survey. Arthritis Rheum. 2005; 52(1):283-289.
27. Gaddini GW, Turner RT, Grant KA, Iwaniec UT. Alcohol: a simple nutrient with complex actions on bone in the adult skeleton. Alcohol Clin Exp Res. 2016; 40(4):657-671.
28. Weaver CM, Gordon CM, Janz KF, et al. The National Osteoporosis Foundation's position

statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporos Int. 2016; 27(4):1281-1386.

29. Strozyk D, Gress TM, Breitling LP. Smoking and bone mineral density: comprehensive analyses of the third National Health and Nutrition Examination Survey (NHANES III). Arch Osteoporos. 2018; 13(1):16.

Table 1. Portion sizes and protein content of the foods whose intake was assessed in the study

Food group	Foods	Portion size (g)	Protein content (g/portion)
Red meat	Beef, pork, lamb, goat, rabbit, game (cooked)	140	35.0
	Cold cuts	30	5.2
Poultry and eggs	Chicken, turkey (cooked)	140	36.0
	Eggs	60	7.4
Seafood	Fish, mollusks, shellfish (cooked)	140	21.0
Dairy	Milk, yogurt	240	11.3
	cheese	30	5.2
	dairy cream	120	3.8
Pulses	Beans, lentils, soybeans (cooked)	180	24.5
Grains and cereals	Bread	40	3.7
	Rusks	60	6.1
	Bagels, breadsticks, pita bread	30	2.7
	Pasta, rice, frumenty (cooked)	220	7.3
	Sweet corn (cooked)	50	1.4
	Breakfast cereals	35	3.2

Table 2. Frequency of consumption of food groups that are good protein sources by the participants of the study (portions/week)

Food group	Fasters (*n* = 200)	Non-fasters (*n* = 200)	*P*
Red meat	2.0 (1.0-3.5)	3.0 (1.5-4.5)	< 0.001
Poultry and eggs	3.5 (2.5-5.5)	4.5 (3.0-7.0)	0.001
Seafood	2.0 (1.0-2.5)	1.5 (1.0-2.0)	0.010
Dairy	11.5 (7.5-17.9)	15.0 (11.5-21.5)	< 0.001
Legumes	1.5 (1.5-1.5)	1.5 (1.5-1.5)	0.050
Grains and cereals	19.0 (15.0-27.0)	21.8 (15.5-30.4)	0.017

Table 3. Daily protein intake from food groups that are good protein sources by the participants of the study (g/kg body weight)

Food group	Fasters (*n* = 200)	Non-fasters (*n* = 200)	*P*
Red meat	0.12 (0.07-0.20)	0.16 (0.10-0.23)	< 0.001
Poultry and eggs	0.11 (0.06-0.13)	0.13 (0.10-0.16)	< 0.001
Seafood	0.09 (0.05-0.12)	0.08 (0.04-0.12)	0.031
Dairy	0.17 (0.10-0.27)	0.22 (0.14-0.32)	< 0.001
Legumes	0.07 (0.06-0.10)	0.07 (0.06-0.09)	0.121
Grains and cereals	0.18 (0.12-0.26)	0.20 (0.14-0.29)	0.049
Sum	0.78 (0.63-1.02)	0.91 (0.72-1.18)	< 0.001

Table 4. Serum concentrations of metabolites related to protein and meat intake in the participants of the study

Metabolite	Fasters (*n* = 200)	Non-fasters (*n* = 200)	*P*
Urea (mg/dL)	27.0 (21.0-33.0)	30.0 (24.0-36.8)	0.001
Creatinine (mg/dL)	0.90 (0.83-1.03)	0.94 (0.83-1.06)	0.102
Uric acid (mg/dL)	4.3 (3.5-5.3)	4.5 (3.7-5.5)	0.343

Table 5. Correlation between frequency of consumption of food groups that are good protein sources and bone mineral density at five sites in the participants of the study (*n* = 400).

	Lumbar	Right hip	Left hip	Right femoral neck	Left femoral neck
Red meat	ns	0.149 (0.003)	0.150 (0.003)	0.136 (0.006)	0.168 (0.001)
Poultry and eggs	ns	0.142 (0.004)	0.131 (0.009)	0.161 (0.001)	0.139 (0.005)
Seafood	ns	-0.106 (0.034)	-0.122 (0.015)	-0.132 (0.008)	-0.160 (0.001)
Dairy	ns	ns	ns	ns	ns
Legumes	ns	ns	ns	ns	ns
Grains and cereals	ns	ns	ns	ns	ns

Values are Spearman's ρ, followed by *P* value in parentheses. ns, not significant ($P > 0.05$).

Table 6. Correlation between frequency of consumption of food groups that are good protein sources and bone mineral content at five sites in the participants of the study (n = 400).

	Lumbar	Right hip	Left hip	Right femoral neck	Left femoral neck
Red meat	0.136 (0.006)	0.156 (0.002)	0.150 (0.003)	0.159 (0.001)	0.152 (0.002)
Poultry and eggs	0.109 (0.029)	0.110 (0.028)	0.100 (0.046)	0.162 (0.001)	0.143 (0.004)
Seafood	ns	ns	ns	ns	ns
Dairy	ns	ns	ns	0.115 (0.021)	0.101 (0.044)
Legumes	ns	ns	ns	ns	ns
Grains and cereals	ns	ns	ns	ns	ns

Values are Spearman's ρ, followed by P value in parentheses. ns, not significant ($P > 0.05$).

Figure 1. Participant flow diagram of the study.

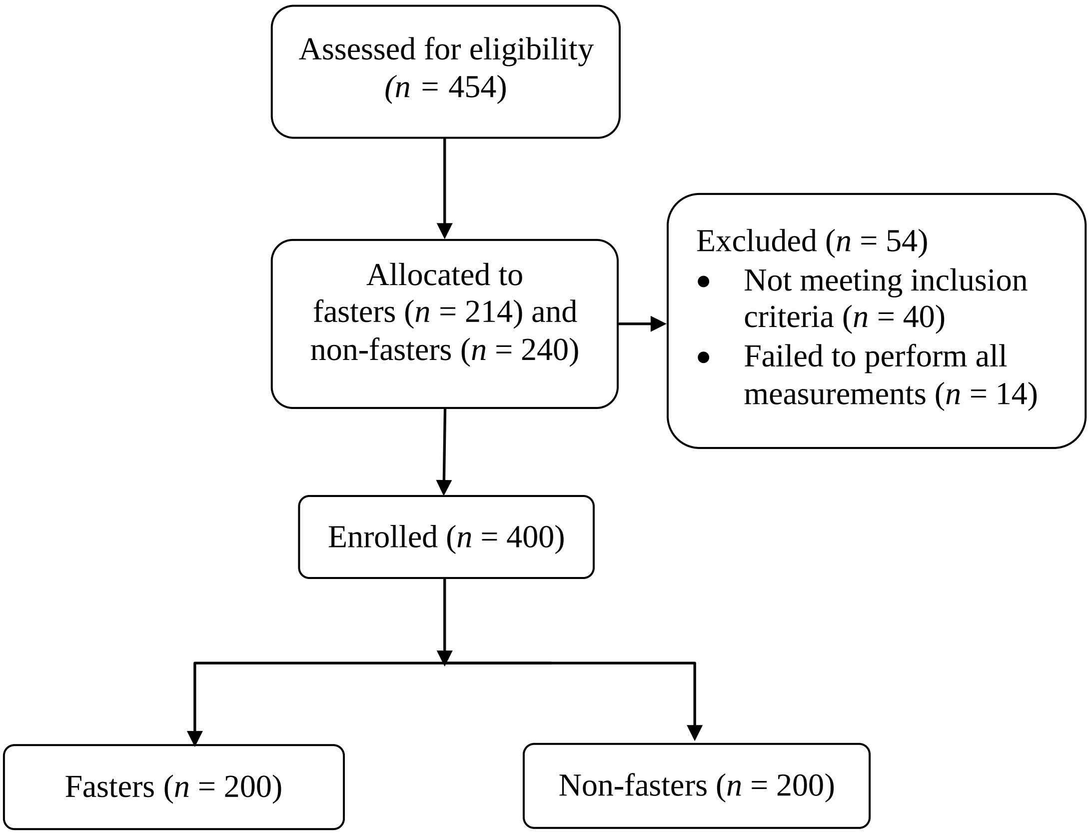


Figure 2. Box plots of bone mineral density at the lumbar spine (A), right hip (B), left hip (C), right femoral neck (D), and left femoral neck (E) of fasters (gray boxes) and non-fasters. See Figure 1 for description of the graph elements. No significant difference between groups was found (Mann-Whitney U test, *P* > 0.05).

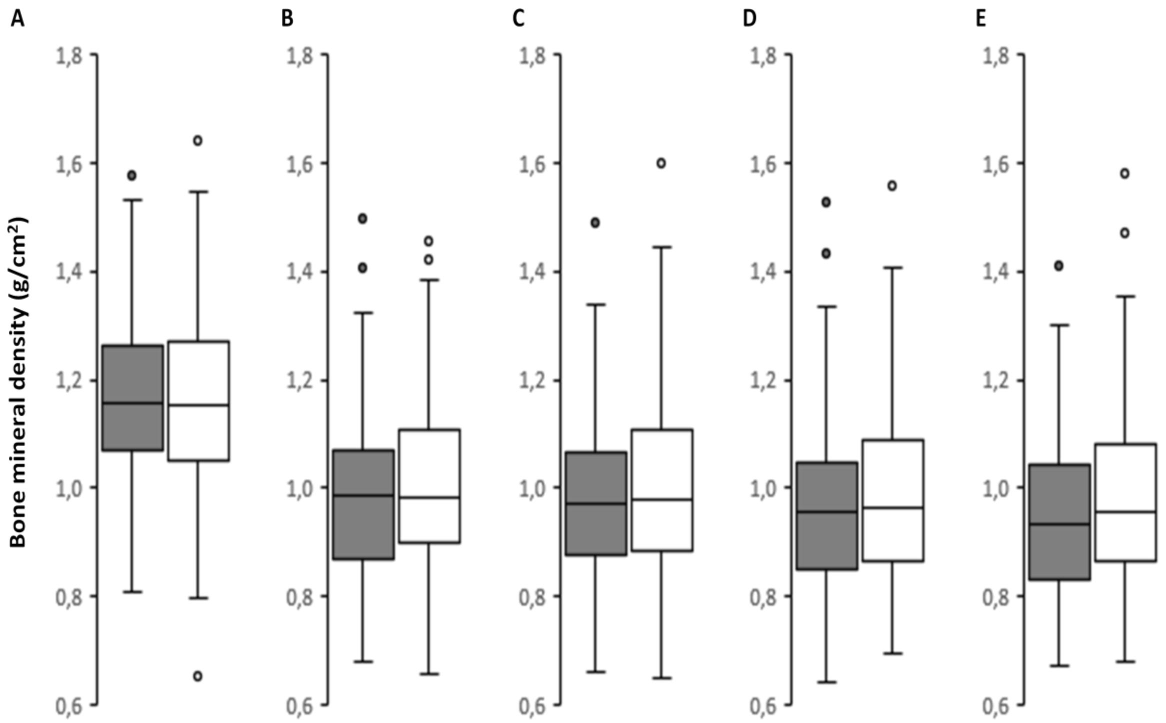
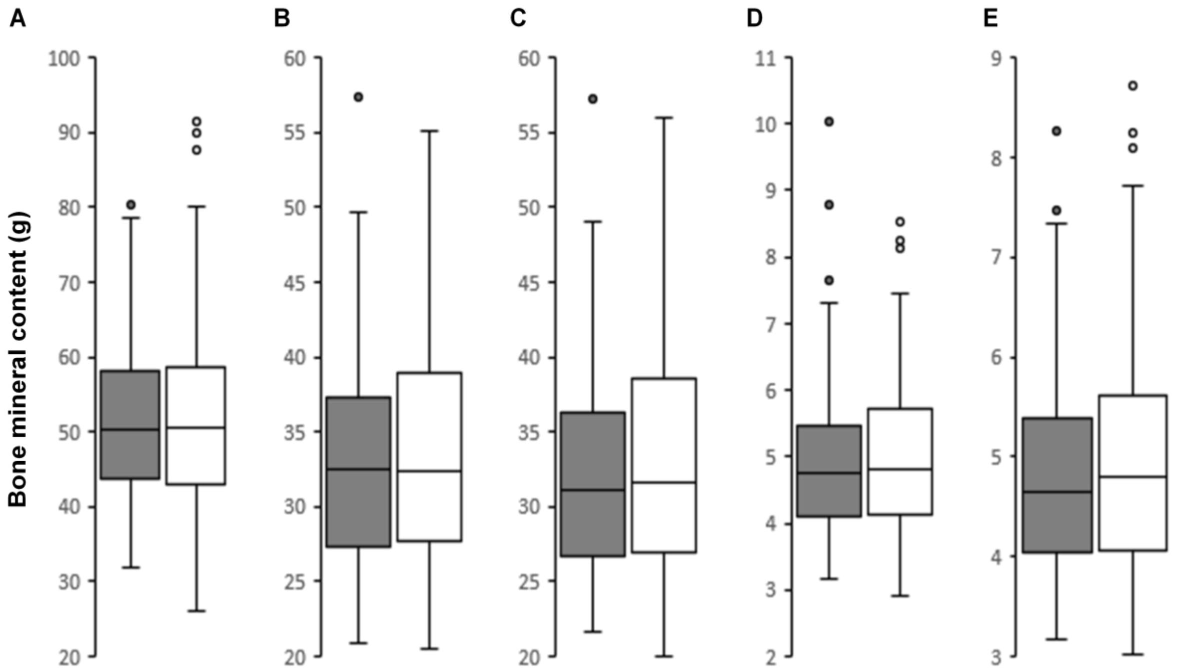


Figure 3. Box plots of bone mineral content at the lumbar spine (A), right hip (B), left hip (C), right femoral neck (D), and left femoral neck (E) of fasters (gray boxes) and non-fasters. See Figure 1 for description of the graph elements. No significant difference between groups was found (Mann-Whitney U test, $P > 0.05$).



Supplemental Table 1. Periods of assessment of fasters in relation to fasting rules of the Christian Orthodox Church

Period	Duration (days)	Dietary restrictions	Seafood consumption	Individuals assessed
September 17 (onset of study) to November 14, 2013: Moderate fasting	59	Avoidance of animal source foods (except seafood), oil, and wine on Wednesdays and Fridays	Allowed except on Wednesdays and Fridays	58
November 15 to December 24, 2013: Nativity fast	40	Avoidance of animal source foods (except seafood, see right); avoidance of oil and wine on Wednesdays, Fridays, and Christmas Eve	Mandatory on November 21 (Presentation of Virgin Mary); allowed from November 22 to December 17	
December 25, 2013, to January 6, 2014: Unrestricted diet	12[a]			
January 7 to February 9, 2014: Moderate fasting	34	Avoidance of animal source foods (except seafood), oil, and wine on Wednesdays and Fridays	Allowed except on Wednesdays and Fridays	11
February 10 to March 2, 2014: Unrestricted diet	21			
March 3 to April 13, 2014: Lent	42	Avoidance of animal source foods (with two exceptions, see right); avoidance of oil and wine on all days except weekends and March 9 (holiday of the Forty Martyrs)	Mandatory on March 25 (Annunciation); optional on April 13 (Palm Sunday)	
April 14 to 19, 2014: Holy Week	6	Avoidance of animal source foods, oil, and wine		
April 20 to 27, 2014: Unrestricted diet	8			
April 28 to June 8, 2014: Moderate fasting	42	Avoidance of animal source foods (except seafood), oil, and wine on Wednesdays and Fridays	Allowed except on Wednesdays and Fridays; mandatory on May 14 (Wednesday of Mid-Pentecost) and May 28 (Wednesday of the sixth week after Easter)	21
June 9 to 15, 2014: Unrestricted diet	7			

June 16 to 28, 2014: Saint Apostles' fast	13	Avoidance of animal source foods (with one exception, see right); avoidance of oil and wine on Wednesdays, Fridays, and June 28, unless it falls on a weekend	Mandatory on June 24 (Nativity of John the Baptist)	
June 29 to July 31, 2014: Moderate fasting	33	Avoidance of animal source foods (except seafood), oil, and wine on Wednesdays and Fridays	Allowed except on Wednesdays and Fridays	17
August 1 to 14, 2014: Dormition (or Assumption) fast	14	Avoidance of animal source foods (with one exception, see right); avoidance of oil and wine on all days except weekends	Mandatory on August 6 (Christ's Transfiguration)	
August 15, 2014: Unrestricted diet	1			
August 16 to November 14, 2014: Moderate fasting	91	Avoidance of animal source foods (except seafood), oil, and wine on Wednesdays and Fridays	Allowed except on Wednesdays and Fridays	21
November 15 to December 24, 2014: Nativity fast	40	Avoidance of animal source foods (except seafood, see right); avoidance of oil and wine on Wednesdays, Fridays, and Christmas Eve	Mandatory on November 21 (Presentation of Virgin Mary); allowed from November 22 to December 17	
December 25, 2014, to January 6, 2015: Unrestricted diet	12[a]			
January 7 to February 1, 2015: Moderate fasting	26	Avoidance of animal source foods (except seafood), oil, and wine on Wednesdays and Fridays	Allowed except on Wednesdays and Fridays	21
February 2 to 22, 2015: Unrestricted diet	21			
February 23 to April 5, 2015: Lent	42	Avoidance of animal source foods (with two exceptions, see right); avoidance of oil and wine on all days except weekends and March 9 (holiday of the Forty Martyrs)	Mandatory on March 25 (Annunciation); optional on April 5 (Palm Sunday)	
April 6 to 11, 2015: Holy Week	6	Avoidance of animal source foods, oil, and wine		
April 12 to 19, 2015: Unrestricted diet	8			

April 20 to May 31, 2015: Moderate fasting	42	Avoidance of animal source foods (except seafood), oil, and wine on Wednesdays and Fridays	Allowed except on Wednesdays and Fridays; mandatory on May 6 (Wednesday of Mid-Pentecost) and May 20 (Wednesday of the sixth week after Easter)	24
June 1 to 7, 2015: Unrestricted diet	7			
June 8 to 28, 2015: Saint Apostles' fast	21	Avoidance of animal source foods (with one exception, see right); avoidance of oil and wine on Wednesdays, Fridays, and June 28, unless it falls on a weekend	Mandatory on June 24 (Nativity of John the Baptist)	
June 29 to July 31, 2015: Moderate fasting	33	Avoidance of animal source foods (except seafood), oil, and wine on Wednesdays and Fridays	Allowed except on Wednesdays and Fridays	22
August 1 to 14, 2015: Dormition (or Assumption) fast	14	Avoidance of animal source foods (with one exception, see right); avoidance of oil and wine on all days except weekends	Mandatory on August 6 (Christ's Transfiguration)	
August 15, 2015: Unrestricted diet	1			
August 16 to October 10, 2015 (end of study): Moderate fasting	56	Avoidance of animal source foods (except seafood), oil, and wine on Wednesdays and Fridays	Allowed except on Wednesdays and Fridays	5

Shown are five periods of fasting (orange), five periods of unrestricted eating (green), and four periods of moderate fasting (yellow) per year. Assessment of fasters took place only during the latter periods.

[a] Not counting January 5 (Epiphany's Eve), which is a day of fasting.

Chaptel 6. Vitamin D status, vitamin D intake, and sunlight exposure in adults adhering or not to intermittent religious fasting for decades.

[International Journal of Food Sciences and Nutrition. 2020. Draft]

Introduction

Vitamin D refers to a group of fat-soluble steroids, the most important of which are vitamin D_2, or ergocalciferol, and vitamin D_3, or cholecalciferol (1). The major natural source of vitamin D in humans is the biosynthesis of cholecalciferol in the skin through a process that depends on sunlight exposure (specifically ultraviolet B radiation) (2), whereas the dietary contribution to vitamin D status is small. Vitamin D originating from the skin or the diet enters the circulation and is metabolized to 25-hydroxyvitamin D in the liver, which reenters the circulation and is converted to 1,25-dihydroxyvitamin D, the biologically active form of vitamin D, in the kidneys. 1,25-dihydroxyvitamin D is important for the absorption of calcium and phosphorus from the intestine and kidneys; skin development; and, possibly, muscle function and performance (3).

Vitamin D status in the body is assessed through the serum 25-hydroxyvitamin D concentration. According to the Endocrine Society's clinical practice guideline, vitamin D deficiency is defined as a 25-hydroxyvitamin D below 20 ng/mL, vitamin D insufficiency between 20 and below 30 ng/mL, and sufficiency as at least 30 ng/mL for achieving a healthy lifestyle (4–6), although the Institute of Medicine has concluded that 20 ng/mL is the top end of the requirement for a serum vitamin D level in almost all of the general population (7).

In recent years a vitamin D deficiency pandemic has been described, since more and more people worldwide have serum 25-hydroxyvitamin D concentration below 20 ng/mL (6). This has been attributed to avoidance of sunlight and/or use of sunscreens due to greater concern for skin cancer, inadequate time spent in outdoor activities due to a more sedentary way of life in indoor environments, and inadequate dietary intake of vitamin D due to decreased consumption of good sources such as oily fish (despite the fact that many foods, mainly dairy and cereals, are fortified with vitamin D, although not in Greece) (6). An additional cause of vitamin D deficiency might be the increased incidence of obesity, which is inversely associated with serum 25-hydroxyvitamin D concentration (7–9). Consequences of vitamin D deficiency are primarily reflected on bone health and relate to rickets, osteomalacia, and increased risk of fracture (4). In addition, vitamin D sufficiency has been linked, albeit with less certainty, to a number of health benefits such as reduced risks of cardiovascular disease, cancer, diabetes, autoimmune diseases, and respiratory diseases (10), including current coronavirus disease 2019 (COVID-19) pandemic (11).

Faithful followers of the Christian Orthodox Church (COC) embrace lifelong intermittent fasting (for almost half of the year) characterized by abstinence from certain foods, predominantly of animal origin, such as dairy products and meat (12). Additionally, they usually choose a rather conservative lifestyle that includes covering large parts of the body with clothes and preferring indoor, rather than outdoor, activities. These habits raise concern about possible consequences on their vitamin D status due to the exclusion of dairy products (which are the foods most frequently fortified with vitamin D) and eggs (a good source of cholecalciferol) when they fast, as well as due to possible low sunlight exposure. Nevertheless, as we have recently shown, men and women fully adhering to COC fasting for decades, whether being older (12) or younger (13), did not differ in indices of bone integrity, such as bone mineral density, bone mineral content, and prevalence of osteopenia, osteoporosis, and bone fracture, from non-fasting counterparts. Therefore, we wondered whether vitamin D status, dietary vitamin D intake, and sunlight exposure differed between fasters and non-fasters. The presence of differences would question the role of vitamin D in the bone status of these individuals.

Methods

Study design

The present study is part of a larger cross-sectional study aimed at investigating possible consequences of lifelong intermittent abstinence from animal source products on various health indicators. The study lasted from September 2013 to October 2015 and, of the 454 individuals who volunteered to participate, 400 healthy adults, equally divided between fasters and non-fasters, met the inclusion criteria and completed all measurements. The distribution of participants between genders was 131 women and 69 men in the fasters and 126 women and 74 men in the non-fasters. The participant flow chart is shown in Supplemental Figure 1. The fasters had been adhering to religious fasting for a median of 15 years. Details of the study design and the participants have been presented (12,13).

Anthropometric characteristics

Body weight and height were measured on a digital scale with a built-in stadiometer to the nearest 0.1 kg and cm, respectively, and body mass index (BMI) was calculated from these measures. Percentage body fat and fat mass were estimated through bioelectrical impedance using a Tanita BC-532 analyser (Tanita, Yiewsley, UK). Waist-to-hip ratio was measured using a stretch-resistant tape.

Vitamin D status

We assessed vitamin D status of the participants through serum 25-hydroxyvitamin D concentration. Fasting venous blood samples were drawn and treated as described (12). Serum 25-hydroxyvitamin D was measured through chemiluminescent microparticle immunoassay in an Abbott Architect i2000SR analyzer. The coefficient of variation was 5% and the laboratory carrying out the assay was involved in a nationwide external quality control program.

Dietary vitamin D intake

Dietary vitamin D intake was assessed through interviewer-based recalls of food intake over 3 days, which included a Wednesday or Friday (during which the fasters obeyed fasting), another weekday, and a weekend day. Participants were interviewed about all foods and liquids consumed during those days. None of the participants in the study received any dietary supplement. Food intake records were analyzed using the Food Processor Nutrition Analysis software (ESHA, Salem, OR).

Sunlight exposure

The estimation of the participants' sunlight exposure was based on the questions proposed by Macdonald (14) and included additional ones, as presented in Table 1. The questions were answered during a direct interview with the same researcher (NER).

Bone status

Bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry (Lunar DPX Bravo, GE Healthcare, equipped with the GE Lunar enCORE software, v. 13.5) at the lumbar spine (L2–L4), right hip, left hip, right femoral neck, and left femoral neck.

Ethical approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Bioethics Committee of the then Alexander Technological Educational Institute of Thessaloniki, presently International Hellenic University; approval number 31.5/5679/17-12-2013. Each participant was informed about the aims, benefits, and potential risks of the study and written informed consent was obtained from all subjects before data collection and blood sampling.

Statistical analyses

The Kolmogorov-Smirnov test and histogram charts were used to assess the normality of distribution of continuous variables. The distribution of most variables differed significantly from normal in at least one of the two groups (fasters or non-fasters). Thus, for the sake of uniformity, we report all continuous variables as median (interquartile range) and compared groups by using the non-parametric Mann-Whitney *U* test throughout. The few variables that warranted a *t* test did not produce a different outcome from the Mann-Whitney *U* test. Vitamin D concentration across seasons was compared through the Kruskal-Wallis test. Nominal variables were tested through the χ^2 test. Correlation analysis was performed by determining Spearman's ρ correlation coefficient. Statistical analysis was performed using the SPSS, version 25 (SPSS, Chicago, IL). All statistical tests and corresponding *P* values were two-sided, and statistical significance was set at $P < 0.05$.

Results

Characteristics of participants

Participants' age, body weight, height, BMI, percentage body fat, fat mass, and waist-to-hip ratio are presented in Table 2. Fasters did not differ significantly from non-fasters in any of these characteristics ($P > 0.05$). Over half of the participants were overweight or obese (BMI > 25 kg/m^2).

Vitamin D concentration and intake

Groups did not differ in serum 25-hydroxyvitamin D concentration significantly [fasters, 16.8 (12.7–22.5) ng/mL; non-fasters, 18.7 (14.5–23.5) ng/mL; $P = 0.105$]. Of the 400 participants, only 22 (5.5%) had values ≥ 30 ng/mL; 131 (32.8%) had values ≥ 20 and < 30 ng/mL; and 247 (61.8%) had values < 20 ng/mL. According to the 3-day recalls, non-fasters had significantly higher daily vitamin D intake [1.74 (0.59–3.18) μg] compared to fasters [1.25 (0.45–2.73) μg; $P = 0.026$]. Vitamin D intake was significantly correlated to serum 25-hydroxyvitamin D concentration ($\rho = 0.150$, $P = 0.003$, $n = 400$), although only 2% (that is, ρ^2) of the variation in the former explained the variation in the latter. Serum

25-hydroxyvitamin D was negatively correlated to age (ρ = –0.125, P = 0.012), BMI (ρ = –0.111, P = 0.026), percentage body fat (ρ = –0.184, P < 0.001), and fat mass (ρ = –0.182, P < 0.001).

Vitamin D concentration and season of blood sampling

We examined serum 25-hydroxyvitamin D concentration as a function of the meteorological season of blood sampling. There were significant differences between seasons (P < 0.001), with the highest concentration seen in autumn [20.4 (15.6–24.3) ng/mL], followed by summer [(17.7 (13.3–23.8) ng/mL], winter [16.8 (12.8–21.5) ng/mL], and spring [15.9 (12.1–21.0) ng/mL]. Non-fasters had significantly higher concentration in winter (P = 0.005) and spring (P = 0.001), compared to fasters (Figure 1).

Sunlight exposure

Fasters did not differ significantly from non-fasters in most of the responses to the questions related to sunlight exposure. Data on the questions in which significant differences were detected are shown in Table 3. Non-fasters spent more time outside during spring (P = 0.002), exposed larger parts of the body/skin (specifically, hands and face) during winter (P = 0.003), and applied less protection against sunlight on a sunny day, as more fasters applied combined protection (P < 0.001).

Bone status

BMD at the lumbar spine, right hip, left hip, right femoral neck, and left femoral neck were not correlated to serum 25-hydroxyvitamin D concentration. BMD at these sites did not differ significantly depending on the season of measurement, either overall or by group.

Discussion

The aim of the present study was to examine whether adhering to intermittent fasting and to habits related to sunshine exposure for decades in the context of religious beliefs affect vitamin D status, which plays important roles in bone health and, possibly, conditions related to immune health, including COVID-19 (4,10,11). Fasters and their non-fasting counterparts did not differ in age or anthropometric characteristics related to fat content, which protects our findings from these potential confounders. Indeed, we found serum 25-hydroxyvitamin D to be negatively correlated with age, which can be attributed to decreased capacity of the skin to produce vitamin D_3 with aging (15). The literature is not clear on this issue. For example, in their review, Manios et al. (16) conclude that the degree of vitamin D deficiency and/or insufficiency is higher in infants and adolescents than in adults and elderly, stating however that such comparisons need to be interpreted cautiously because of differences in latitude, ethnic mix, and season of blood sampling. Concerning the impact of body composition on circulating vitamin D, obese individuals tend to have lower serum 25-hydroxyvitamin D compared to normal-weight individuals (7,9,17). It has even been recommended that obese adults require 2–3 times the RDA of vitamin D to both treat and prevent vitamin D deficiency, because much of vitamin D is stored in adipose tissue, where it is not bioavailable (5,6). Indeed, we found serum 25-hydroxyvitamin D to be negatively correlated to BMI, percentage body fat, and fat mass in the participants of the present study.

Overall, the two groups did not differ significantly in vitamin D concentration, although non-fasters had numerically higher values than fasters. This absence of significant difference, despite the significantly higher daily vitamin D intake by the non-fasters, could be attributed to the small impact of the diet on vitamin D status, since solar radiation is the major contributor to circulating vitamin D. The minor impact of dietary vitamin D intake was confirmed by the finding that it explained only 2% of the variation in the serum 25-hydroxyvitamin D concentration through correlation analysis. It is noteworthy that only 5.5% of the participants had vitamin D sufficiency, whereas 61.8% had vitamin D deficiency, without, nevertheless, considerable impact on bone health, as found in our previous studies (12,13). The absence of correlation between BMD and circulating vitamin D concentration found in the present study is in line with the low impact of vitamin D status on bone health, as also suggested by Taylor et al. (7).

The high rates of vitamin D insufficiency and deficiency are even more remarkable and worrying, given the fact that the mean monthly modeled ultraviolet B doses in Greece, including Thessaloniki (where the present study was conducted), are much higher than those in any of the other European countries included in the analysis of O’Neill et al. (18). In addition, Manios et al. (16) point out that, contrary to expectations, there is a high prevalence of low vitamin D status in Southern Europe and Eastern Mediterranean, despite abundant sunshine, although there are not enough data for safe conclusions so far. According to van Schoor et al. (19), vitamin D deficiency has a high prevalence all over the world; the authors attribute the low serum 25-hydroxyvitamin D in Greece to more skin pigmentation and sunshine avoiding behavior, compared to Northern Europe.

In addition to higher vitamin D intake, non-fasters exceeded fasters in sunlight exposure, as judged by their responses to three relevant questions. This may be due to a more relaxed lifestyle, with no commitments to religious customs and traditions. It has been reported that the vitamin D deficiency observed in recent years is more prevalent among individuals who avoid sun exposure by wearing clothes covering most of their body due to religious and cultural beliefs irrespective of latitude (20). In the study of Lee et al. (21), personal sunlight exposure, measured using the ambulatory lux meter, was not significantly related with serum vitamin D level change and the sunlight exposure score measured by sunlight exposure questionnaire, although several limitations in the sunlight measurement existed.

When the serum vitamin D concentration was compared between the two groups depending on the season, the non-fasters exhibited significantly higher values than fasters in winter and spring. These differences are in line with the differences concerning sunlight exposure, as two of the three significantly different responses were related to indices of sunlight exposure during the same seasons. In particular, non-fasters spent more time outside during spring and exposed larger parts of the body/skin during winter, compared to fasters. Additionally, non-fasters applied less protection against sunlight on a sunny day, as more fasters applied combined protection, which probably indicates more relaxed attitude of non-fasters toward sunlight exposure.

Our findings on the seasonal variation of the serum vitamin D concentration are in accordance with findings showing highest concentrations in the summer and autumn (22). In addition, the finding that the highest concentration was seen in autumn, while the lowest in spring, is in agreement with Kroll et al. (23), who found that the concentration peaked in September and troughed in March. It seems that high exposure to sunlight in spring and summer increases vitamin D concentration in summer and autumn, suggesting a delayed effect of sunlight exposure on circulating vitamin D.

A limitation of the present study, as with the majority of studies based on self-reported lifestyle parameters, is the likelihood of misreporting food intakes and sunlight exposure. However, given the similarity of the two groups in demographic and anthropometric characteristics, it seems improbable that fasters misreported differently from non-fasters. Additionally, an objective measure of sunlight exposure (through meters) would be desirable, although this was not possible to apply to the 400 participants of the present study due to financial constraints.

In conclusion, in this study examining the vitamin D status in 400 individuals of both sexes, half of whom followed religious customs and traditions that included many years of intermittent abstinence from foods of animal origin and half did not, we found no differences in serum vitamin D concentration between groups overall, although when groups were compared by season, non-fasters had higher concentrations in winter and spring. Additionally, non-fasters had higher dietary vitamin D intake and partly higher sunlight exposure. These differences were not reflected in bone status and health in the present and our two previous studies (12,13), suggesting a not so determinant role of vitamin D in the bone status of these individuals. The low serum 25-hydroxyvitamin D concentrations in the 378 out of the 400 participants adds to the evidence for prevalent vitamin D deficiency. Randomized studies are needed to provide safe conclusions on the effect of vitamin D sufficiency on human health.

References

1. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr. 2004; 80:1678–88.
2. Holick M, MacLaughlin J, Clark M, Holick S, Potts J, Anderson R, Blank I, Parrish J, Elias P. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science (80-). 1980; 210:203–5.
3. Mougios V. Exercise biochemistry. Human Kinetics Publishers Inc.; 2019. 105-119 p.
4. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011; 96:1911–30.
5. Holick MF. Biological effects of sunlight, ultraviolet radiation, visible light, infrared radiation and Vitamin D for health. Anticancer Res. 2016; 36:1345–56.
6. Holick MF. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. Rev Endocr Metab Disord. Reviews in Endocrine and Metabolic Disorders; 2017; 18:153–65.
7. Taylor CL, Thomas PR, Aloia JF, Millard PS, Rosen CJ. Questions about Vitamin D for Primary Care Practice: Input from an NIH Conference. American Journal of Medicine. Elsevier Inc; 2015. p. 1167–70.
8. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000; 72:690–3.
9. Valtueña J, González-Gross M, Huybrechts I, Breidenassel C, Ferrari M, Mouratidou T, Gottrand F, Dallongeville J, Azzini E, Sioen I, et al. Factors associated with vitamin D deficiency in European adolescents: The HELENA study. J Nutr Sci Vitaminol (Tokyo). 2013; 59:161–71.
10. Holick MF. Vitamin D: A D-Lightful health perspective. Nutr Rev. 2008; 66:182–94.
11. Meltzer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of Vitamin D Status and Other Clinical Characteristics With COVID-19 Test Results. JAMA Netw open [Internet]. 2020; 3:e2019722. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32880651
12. Rodopaios NE, Mougios V, Konstantinidou A, Iosifidis S, Koulouri AA, Vasara E, Papadopoulou SK, Skepastianos P, Dermitzakis E, Hassapidou M, et al. Effect of periodic abstinence from dairy products for approximately half of the year on bone

health in adults following the Christian Orthodox Church fasting rules for decades. Arch Osteoporos. 2019; 14:1–8.

13. Rodopaios NE, Mougios V, Koulouri AA, Vasara E, Papadopoulou SK, Skepastianos P, Dermitzakis E, Hassapidou M, Kafatos AG. Bone status of young adults with periodic avoidance of dairy products since childhood. Eur J Pediatr. 2020; 179:645–51.
14. Macdonald HM. Contributions of Sunlight and Diet to Vitamin D Status. Calcif Tissue Int. 2013; 92:163–76.
15. Holick MF. Vitamin D Deficiency. N Engl J Med [Internet]. 2007; 357:266–81. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMra070553
16. Manios Y, Moschonis G, Lambrinou C-P, Tsoutsoulopoulou K, Binou P, Karachaliou A, Breidenassel C, Gonzalez-Gross M, Kiely M, Cashman KD. A systematic review of vitamin D status in southern European countries. Eur J Nutr [Internet]. Springer Berlin Heidelberg; 2018; 57:2001–36. http://dx.doi.org/10.1007/s00394-017-1564-2
17. Leiu KH, Chin YS, Shariff ZM, Arumugam M, Chan YM. High body fat percentage and low consumption of dairy products were associated with Vitamin D inadequacy among older women in Malaysia. PLoS One. 2020; 15:1–13.
18. O'Neill CM, Kazantzidis A, Ryan MJ, Barber N, Sempos CT, Durazo-Arvizu RA, Jorde R, Grimnes G, Eiriksdottir G, Gudnason V, et al. Seasonal changes in vitamin D-effective UVB availability in Europe and associations with population serum 25-hydroxyvitamin D. Nutrients. 2016; 8(9):533.
19. Van Schoor NM, Lips P. Worldwide vitamin D status. Best Pract Res Clin Endocrinol Metab. Elsevier Ltd; 2011; 25:671–80.
20. Saraff V, Shaw N. Sunshine and Vitamin D. Arch Dis Child. 2016; 101:190–2.
21. Lee SH, Park SJ, Kim KM, Lee DJ, Kim WJ, Park RW, Joo NS. Effect of sunlight exposure on serum 25-hydroxyvitamin d concentration in women with vitamin d deficiency: Using ambulatory lux meter and sunlight exposure questionnaire. Korean J Fam Med. 2012; 33:381–9.
22. Holick MF. Ultraviolet B Radiation: The Vitamin D Connection. In: S. Ahmad, editor. Ultraviolet Light in Human Health, Diseases and Environment, Advances in Experimental Medicine and Biology 996. Springer International Publishing AG 2017; 2017. p. 137–54.
23. Kroll MH, Bi C, Garber CC, Kaufman HW, Liu D, Caston-balderrama A, Zhang K,

Clarke N, Xie M, Reitz RE, et al. Temporal Relationship between Vitamin D Status and Parathyroid Hormone in the United States. PLoS One. 2015; 25:1–13.

Table 1. Questions and answers to estimate sunlight exposure

Question	Answer
Do you go outside during the day?	Yes No
On a weekly basis, how often do you go outside during the day?	Rarely Moderately often Often
How long do you stay outside during autumn?	Less than 15 min Between 15 and 30 min Between 30 min and 2 h More than 2 h
How long do you stay outside during winter?	Less than 15 min Between 15 and 30 min Between 30 min and 2 h More than 2 h
How long do you stay outside during spring?	Less than 15 min Between 15 and 30 min Between 30 min and 2 h More than 2 h
How long do you stay outside during summer?	Less than 15 min Between 15 and 30 min Between 30 min and 2 h More than 2 h
Which parts of the body/skin have been exposed during autumn?	Face only Hands and face Hands and face plus arms and/or legs Hands and face plus arms and/or legs and some/all of trunk
Which parts of the body/skin have been exposed during winter?	Face only Hands and face Hands and face plus arms and/or legs

	Hands and face plus arms and/or legs and some/all of trunk
Which parts of the body/skin have been exposed during spring?	Face only Hands and face Hands and face plus arms and/or legs Hands and face plus arms and/or legs and some/all of trunk
Which parts of the body/skin have been exposed during summer?	Face only Hands and face Hands and face plus arms and/or legs Hands and face plus arms and/or legs and some/all of trunk
What kind of protection against sunlight do you apply on a sunny day?	No protection Hat Sunscreen Stay in the shade Clothes with long sleeves Combined protection (at least two of the above)
Do you avoid the sun during summer?	Yes No
Do you sometimes stay in the sun during summer?	Yes No
Do you prefer to stay in the sun during summer?	Yes No

Table 2. Characteristics of participants in the two groups (median and interquartile range)

Characteristic	Fasters (*n* = 200)	Non-fasters (*n* = 200)
Age (years)	45 (27–58)	46 (24–57)
Body weight (kg)	72.9 (62.9–81.4)	71.3 (60.5–84.0)
Height (m)	1.66 (1.60–1.72)	1.66 (1.61–1.74)
BMI (kg/m^2)	26.6 (23.0–29.5)	25.6 (22.7–29.1)
Body fat (%)	31.1 (24.0–38.9)	30.3 (24.1–36.3)
Fat mass (kg)	22.5 (14.7–30.2)	21.5 (15.9–28.6)
Waist-to-hip ratio	0.89 (0.82–0.97)	0.89 (0.81–0.98)

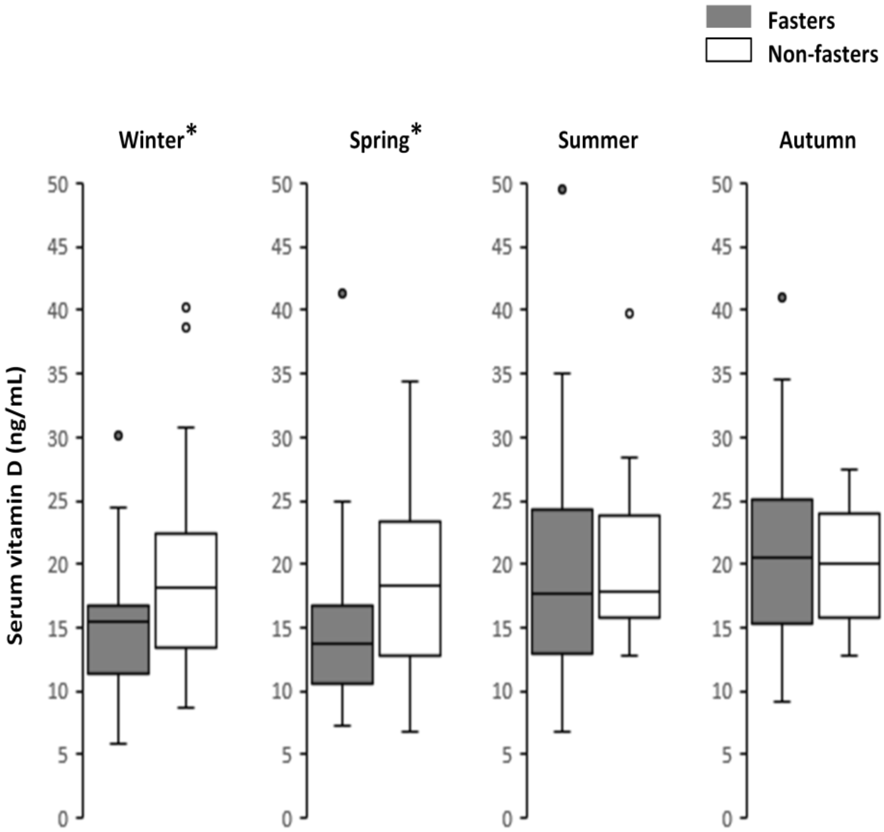
Table 3. Significantly different answers to the sunlight exposure questions between groups (%, $P < 0.05$)

Question	**Answer**		
		Fasters	**Non-fasters**
How long do you stay outside during spring?	Less than 15 min	9.5	7
	Between 15 and 30 min	7	3.5
	Between 30 min and 2 h	59.5	44.5
	More than 2 h	24	**42***
Which parts of the body/skin have been exposed during winter?	Face only	73	59.5
	Hands and face	26	**40**
	Hands and face plus arms and/or legs	0.5	0.5
	Hands and face plus arms and/or legs and	0.5	0

	some/all of trunk		
What kind of protection against sunlight do you apply on a sunny day?	No protection	0	2.5
	Hat	3.5	10.5
	Sunscreen	8.5	17.5
	Stay in the shade	38.5	44
	Clothes with long sleeves	8	1.5
	Combined protection (at least two of the above)	41.5	**24**

*Numbers in bold indicate superior sunlight exposure.

Figure 1. Serum vitamin D concentration in fasters and non-fasters according to meteorological season of blood sampling in the Northern Hemisphere. Winter, December to February; spring, March to May; summer, June to August; autumn, September to November. *Significantly different between groups ($P < 0.01$)



Annex

Published Articles in Scientific Journals

Austin Journal of Nutrition and Food Sciences

Open Access

Austin Publishing Group

Review Article

Is Periodic Abstinence from Dairy Products for Half a Year, Detrimental to Bone Health of Children and Adolescents?

Rodopaios NE[1]*, Manolarakis GE[1], Peppas C[2] and Kafatos A[1]

[1]Department of Preventive Medicine and Nutrition Unit, School of Medicine, University of Crete, Greece

[2]Department of Surgeon, Metropolitan Hospital, Athens

***Corresponding author:** Nikolaos E. Rodopaios, Preventive Medicine and Nutrition Unit, School of Medicine, University of Crete, Greece

Received: December 01, 2017; **Accepted:** December 22, 2017; **Published:** December 29, 2017

Abstract

The question of "What's the best diet for children and adolescents which ensures their optimum bone health?" has triggered this review study of all nutritional habits including the Christian Orthodox Church's diet with its particular periods of fasting. In order to evaluate the abstinence from dairy products in childhood and adolescence for more than 180 days per year, we assessed the relative knowledge on vegans and vegetarians in relation to calcium intake. We researched the world-wide published experience as well as studies focused on Christian Orthodox Church's fasting and its contribution to bone health status in children and teenagers with at least one year follow-up randomized controlled trials. During the last 30 years, more than 120 published studies all over the world dispute the generalization that milk consumption during childhood and adolescence relates to "strong bones". World-wide research experience on vegans and vegetarians reveals that non-diary food resources of calcium fortify the bones, ensure their integrity and development and that calcium daily intake, essential for the bone health, and can be gained from foods other than dairy. Abstinence from dairy products in childhood and adolescence for about 180 days per year –as proposed by the Christian Orthodox Church- seems to act as a means to bone integrity and optimal development.

Keywords: Milk; Dairy; Children; Adolescents; Fast

Abbreviations

AF: All Fasting Periods; BMC: Bone Mineral Content; BMD: Bone Mineral Density; CaD: Calcium Intake Diary; COC: Christian Orthodox Church; EAR: Estimated Average Requirement; IOM: Institute of Medicine; NF: Never Fasted; PF: Partially Fasted; RDI: Recommended Daily Intake; s-BAP: Alkaline Phosphatase; s-CTX: C-Terminal Telopeptides Of Type I Collagen; s-OC: Serum Osteocalcin; WHO: World Health Organization.

Introduction

Proper nutrition is important for the development and conservation of the bone mass. 80-90% of the Bone Mineral Content (BMC) is formed by calcium and phosphorus [1], along with other important molecules and elements (proteins, magnesium, copper, iron) and vitamins (A, C, D, K) [2].

Milk is broadly consumed because of its high nutritional value. It contains various elements such as bioactive peptides, calcium, and growth factors that interfere to the bone metabolism stages, namely bone turnover [3].

The Estimated Calcium Average Requirement (EAR) for the adolescent ranges between 800 to 1050 mg/day while the Recommended Daily Intake (RDI) is 1000-1300 mg/day; it's also well understood that EAR as well RDI vary under certain conditions as in pregnancy, lactation, menopause and aging [4].

For more than two decades, USA recommends to children and adults the intake of calcium for prevention of osteoporosis [1,5-8]. They suggest the dairy servings as the best way for that, depending on age, for 4-8 years old is 3 servings and 9-18 years old, 4 servings daily. Three servings of dairy provide 828 mg calcium and 4 servings 1104 mg Ca [9].

In the USA over 120 food products are calcium-fortified. Therefore taking the recommended portions of diary daily plus the calcium from non dairy products far exceeds the RDI intake of calcium. It is also amazing that although the dietary intake of calcium ranks first around the world, representing the 72% of global dietary calcium intake [10], osteoporosis and bone fractures rates are high as they range between 700 and 1000 incidents per 100000 population annually [11,12]. Excessive calcium intake has been questioned on its efficacy in preventing osteoporosis and its potentially negative effects in health [13-17].

Recent epidemiological studies in women [18], children and adolescents [19-22], have questioned the efficacy of dairy products and other calcium-containing products for the good health of the bones [23].

World Health Organization (WHO) recommendations for the prevention of osteoporosis highlight the "calcium paradox" and suggest a minimum intake of 400-500 mg/day of calcium from all sources for individuals above 50 who live in countries with high rate of bone fractures [24]. Furthermore, WHO concludes that "there is no chance to globally approximate the calcium intake for the general population". This statement also includes children and adolescents.

Citation: Rodopaios NE, Manolarakis GE, Peppas C and Kafatos A. Is Periodic Abstinence from Dairy Products for Half a Year, Detrimental to Bone Health of Children and Adolescents?. Austin J Nutri Food Sci. 2017; 5(3): 1094.

Table 1: Fasting of the Christian Orthodox Church (COC) [28-30].

FASTING	DAYS	PERIODS OF FASTING
LENT	41	From a Clean Monday to Saturday of Lazarus
EASTER WEEK	6	From Holy Monday to Saturday
CHRISTMAS	40	From November 15 through December 24
ASSUMPTION	14	From 1 to August 15
HOLY APOSTLES*	0 – 30**	On the next day Sunday of All Saints up to June 28, the eve of the feast of Saints Peter and Paul
DAILY	3	January 5 (Eve of Epiphany) August 29 (the decapitation of John the Baptist) September 14 (Exaltation of the Holy Cross)
EVERY WEDNESDAY AND FRIDAY	55 – 63	All Wednesdays and Fridays of the year, apart from those already in the periods of fasting and absolute or free periods
DAY TOTAL	159 – 197 SD:178 ± 19	

* So named because it precedes the two apostolic feasts: the Apostles Peter and Paul, June 29 and "Synaxis (Bevy) of the 12 Apostles," June 30.
** The duration of this fasting period is undefined, because the launch depends on the moveable feast of Easter.

On the other hand, European and UK reports suggest dietary reference intake of calcium through dairy products between 800 and 1300 mg/day for all individuals –USA included- for their whole life [25].

Skeletal health indexes such those of bone growth as well of biological maturity of bony structures can be determined through correlation between Bone Mineral Density (BMD) and/or Bone Mineral Content (BMC) outcomes and calcium intake from milk and dairy products [25]. The Institute of Medicine (IOM) in order to assess the importance of calcium and vitamin D intake determined the optimum Dietary Reference Intake (DRI) [26].

According to Recommended Dietary Allowances (RDA) recommendations, 700 mg of calcium is the optimum daily dosage for children between 1 and 3 years old, whereas 1000mg for those of 4-8 years old. Adolescents need higher calcium levels, no less than 1300mg per day [27].

However, prospective epidemiological studies have raised questions about the effectiveness of the use of dairy products in the promotion of bone health [28]. Over the past two decades an increasing number of studies it has been carried out on religious fasts and their impact on human health [27]. The Christian Orthodox Church (COC) diet proposes entirely different dietary habits that are worth to be mentioned because through alternating time-fixed fasting periods they achieve the optimal balance in an admittedly healthy nutritional pattern: the Mediterranean diet.

COC recommends abstaining from dairy, meat, and eggs for about 180 days per year (159-197, SD: 178±19) and instead of 155 days/year from fish for adults and children (Table 1).

For that reason, it may be considered as periodic vegetarianism although seafood allowed in all fasting periods [31-35].

Low consumption of dairy, meat and egg products leads to reduced intake of saturated fats, while the use of olive oil in large quantities has shown positive effects on the prevention of chronic diseases [36]. In addition, COC fasts as nutritional health-promotive habits [37,38], are still the main characteristics of Mediterranean diet in Greece [39-41]. The objective of this study is to review the existing literature on the effects of consumption of dairy products and total dietary calcium intake on bone integrity in children and adolescents, primarily to assess whether the evidence supports the following:

(1) Which one of the two nutritional habits promotes the optimum bone growth during childhood and adolescence: a daily consumption of 3-4 servings of dairy products, or a periodic fasting that includes calcium from non-dairy foods?

(2) Does the statement "the recommended servings of dairy products is the optimal choice for promoting bone health and integrity in contrast to other sources of food or supplements containing calcium" seem to be valid?

(3) Has the 180-day abstinence from dairy products per year, as suggested by the COC, negative effects on bone growth in children and adolescents?

Methods

The MEDLINE, COCHRANE, EMBASE and PUBMED databases were searched. Articles not included in those databases and studies on vegetarian diets as well as studies from the Christian Orthodox Church on fasting were also searched.

Keywords used were: dairy, dairies, dairy products, vegan, fast, fasting, plus bones, Bone Mineral Density (BMD), Bone Mineral Content (BMC), osteoporosis, osteopenia, including only studies and reports to humans, children and teenagers, aging above 3 years-old, published in English language, from 1990 to January 2017.

The research focused on age, sex, and race of the participants and also on the activity level and the pubertal status. Other factors such as socioeconomic status, exposure to the sun or caffeine consumption didn't be taken into consideration.

As changes in BMD develop slowly [42] we focused on trials and studies having at least one-year follow-up.

Finally, our search yielded to 12 cross-sectional studies; 7 longitudinal prospective studies, 3 randomized and one case-control study.

Results

We studied 22 selected randomized controlled trials of milk and calcium intake for children and adolescents (Table 2).

Kardinaal et al. in cross-sectional study investigated the

Table 2: Characteristics of the 22 selected randomized controlled trials of milk and calcium intake for children and adolescents.

Author / year (Study)	No of patients	Ages (years)	Duration	Calcium Intake, mg/day	Milk and Dairy, g/day	Groups	Type of Research	Indices
Kardinaal 1999 [15]	1116 girls and young women / 6 Europe countries	11-15	-	609±282 (Italy) 831±363 (Poland 881±335 (France) 1134±462 (Netherlands) 1258±594 (Denmark) 1267±550 (Finland)	Not representative for the respective countries.	750 girls (11-15 years of age) 375 young women (20-23 years of age) from Finland, Italy, Denmark, Poland, Netherlands, France	Cross-sectional study	BMC, BMD
Kroger 1993 [19]	65 children and adolescents / Finland	7-20	1 year	<800 - >1200	-	-	Prospective study	BMD
Lloyd 2000 [20]	81 white teenager girls / US	12-18	6 years	500-1500	-	Sports-exercise No sports	Longitudinal study	BMD
Lloyd 2002 [22]	75 white female adolescents / US	12-20	8 years	486 - 1958	-	Exercise, fitness	Longitudinal study	BMD
Kohlenderg-Mueller 2003 [43]	16 young adults / Germany	19-24	10 days	843±140 1322±302	Milk 32%	8 Vegan 8 Lacto vegeterian	Cross-sectional study	Urinary calcium excretion
Ho-Pham 2009 [44]	210 women / Vietnam	50-85	10-72 years vegan diet	375 (330±205) 683 omnivores	-	105 Vegans Buddhist nuns 105 omnivores women	Cross-sectional study	BMD
Budek 2007 [45]	24 boys / Denmark	8	7 days	1000±300 2900±200	1.5 L/day skimmed milk	12 boys, milk group 12 boys, meat group	Cross-sectional study	Bone turnover markers (s-Osteocalcin, s-CTX, s-BAP)
Volek 2003 [46]	28 boys / US	13-17	3 months	1723±274 979±286	236 mL/ serving/d of 1% fluid milk (3 daily servings)	14 boys, milk group 14 boys, juice group	Cross-sectional study	BMC, BMD
Jones 2001 [47]	330 children (215 boys, 115 girls/ Tasmania, Australia	8	5 years	1336±541	590 mL/d	215 boys 115 girls	Cross-sectional study	BMD, Urinary Potassium and Sodium
Uusi-Rasi 1997 [48]	176 girls / Finland	8-20	7 days	1018±361 (T1) 1059±460 (T2 & T3) 1231±565 (T4 & T5)	7d calcium intake diary (CaD)	Tanner stage 1(T1) pre-pubertal: 9.2±1.1 age Tanner stage 2 & 3 (T2 & T3) per pubertal: 11.8±1.3 age Tanner stage 4 & 5 (T4 & T5) post-pubertal: 15.9±1.8 age	Cross-sectional study	BMC, BMD, BMAD
Yoshii 2007 [49]	4761 children / Japan	15-18	3 years	-	Dairy (milk, cheese, yogurt, cream): daily, 4-5 times, 2-3 times, ≤ 1 time a week	2651 high school girls 2110 high school boys 1252 women	Cross-sectional study	Bone status (*Z-score*)
VandenBergh 1995 [50]	1359 children / Netherlands	7-11	28 months	-	Boys: 1.3±0.44 Girls: 1.2±0.43	653 boys 706 girls	Cross-sectional study	BMC
Young 1995 [51]	215 female twin pairs / Australia	10-26	-	60% of total dietary calcium is derived from dairy produce and fish.	Premenarchial: 1178±701 Postmenarchial: 1041±674	122 monozygotic (MZ) 93 dizygotic (DZ)	Cross-sectional twin study	BMD, BMC
Cheng 1999 [52]	179 children / China	12-16	3 years	Boys: 722 (beginning of the study)-700.8 (at the last visit) Girls: 560-608.8	-	87 girls 92 boys	Longitudinal study	BMC, BMD
Kristinsson 1994 [53]	162 girls / Iceland	13 and 15	-	1000 - 1200	13 years: 1293±452 15 years: 1082±382	80 girls, 13 years 82 girls, 15 years	Cross-sectional study	BMD, BMC
Petridou 1997 [54]	100 children / Greece	7-14	2 years	-	Assess consumption of the calcium rich dairy products	74 boys 26 girls	Case control study	Fracture
Felily 1992 [55]	581 children / United Kingdom	7-9	2 years (assessed 14 years later)	Men: 1297±397 1323±319 (Control) Women: 933±278 940±317 (Control)	0-568 ml milk. 190 ml milk on each school day for 6 terms	197 boys and girls 174 controls	Randomized, controlled trial (RCT)	BMC, BMD
Lehtonen-Veromaa 2002 [56]	171 girls / Finnland	9-15	3 years	1575±637	Supplement 10 μg Vit. D_2 + 500 mg Ca per day	66 gymnasts 65 runners 60 nonathletic	Longitudinal study	BMD or BMAD

Welten 1994 [57]	182 children / Netherlands	13	15 years	Boys: 1100-1435 Girls: 941.4-1204	80% of the calcium intake is supplied by dairy products	84 boys 98 girls	Longitudinal study	BMD
Chan GM 1995 [58]	48 white girls / US	11	1 year	Control: 728±321 Dairy: 1437±366	Dairy products weekly (1200 mg Ca)	24 Control group 24 Dairy group	Randomized, controlled trial (RCT)	BMC, BMD
Cadogan 1997 [59]	82 white girls / United Kingdom	12.2	18 months	Control: 703 Milk: 1125	Milk group: 568 mL/d	38 Control 44 Milk	Open Randomized intervention trial	BMD, BMC
Chryssochoou 2010 [60]	323 boys and 286 girls / Greece	5-15 ½	3 ½ years	1134±28 (TF) 1040±28 (PF) 1090±59 (NF)	451±14 (TF) 383±14 (PF) 427±30 (NF)	Total fastening (TF) 12,1% Partial fastening (PF) 43,3% No fastening (NF)	Cross-sectional study	Growth rate

association between dietary calcium intake and radial bone density among 1116 Caucasian girls (aged 11-15 years) at different levels of calcium intake in six European countries. There was no evidence of a relation between calcium intake and BMD at different levels of intake; although there was a positive association at calcium intake levels <600 mg/day (6.28, p=0.02). Nevertheless, the interaction was not significant and there was no consistent trend over intake categories. These results do not support the hypothesis that dietary calcium acts as a determinant of peak BMD in European young women, for a wide range of intake [15].

Kröger et al. studied the effect of puberty and genetic factors on the development of bone mass and density in a prospective study 65 children and adolescents (aged 7-20 years old). They didn't find any significant relationship between the increment rate of bone density and physical activity or calcium intake (<800 - >1200) [19].

Lloyd et al. studied in two longitudinal studies the relation of exercise and BMD on teenage. The first, one (year 2000) was conducted in 81 girls [20] Cumulative sports-exercise scores between ages 12 and 18 years were associated with hip BMD at age of 18 years (r = .42) but not related to the total body bone mineral gain. Time-averaged daily calcium intake ranged from 500 to 1500 mg/day in this cohort, was not associated with hip BMD at age 18 years or with total body bone mineral gain at age 12 through 18 years.

The second study (2002) included 75 healthy white female adolescents (aged 12-18 years) sports-exercise scores were correlated with BMD at the femoral neck and shaft. Average total daily calcium intake at age 12-20 years old ranged from 486 to 1958 mg/day and no associations ($p < 0.05$) were observed between bone measurements and calcium intake [22].

Kohlenberg-Mueller et al. in a cross-sectional study focused on the vegan diet in seven women and one man 19 to 24 years old indicating that neither calcium balance nor a single bone turnover biomarker has been significantly affected. Calcium intake levels (843±140 mg, p<0.01) were adequate despite the significant difference in calcium intake levels between plant foods and dairy [43].

Ho-Pham's et al. cross-sectional study compared bone health in 105 postmenopausal vegan Mahayana Buddhist nuns and 105 omnivorous postmenopausal women 50-85 years old. Nuns, which had been engaged the vegan diet at mean 33 years (range: 10-72 years), demonstrated on average very low (330 ± 205 vs. 682 ± 417 mg/day, p < 0.001mg) calcium daily intake. Lumbar Spine BMD was identical in both groups (0.74±0.14 vs. 0.77±0.14 g/cm^2; mean ± SD; p=0.18). Same results in Femoral Neck BMD (0.62±0.11 vs. 0.63±0.11 g/cm^2; p=0.35) as well as in Body BMD results were 0.88±0.11 vs. 0.90±0.12 g/cm^2; (p=0.31). Prevalence of osteoporosis (T scores ≤−2.5) at the femoral neck in vegans and omnivores was 17.1% and 14.3% (p=0.57), respectively. Therefore, veganism has no adverse effect on BMD [44].

In a randomized case-control study, milk intake of 1.5 L/day for 7 days was associated with higher calcium intake (p<0.0001) but decreased bone turnover in prepubertal 24 boys. Serum osteocalcin (s-OC) and C-terminal telopeptides of type I collagen (s-CTX) were significantly decreased in the milk group (-30.9%; -18.7%, respectively, p≤0.04) as well as bone-specific alkaline phosphatase (s-BAP) (p=0.06) [45].

Another randomized case-control prospective study examined the effects of increased milk consumption on bone (and body) composition in boys engaged with resistance training. Twenty-eight boys (13 to 17 years of age) were randomly assigned to consume, in addition to their habitual diet, 3 servings/day of 1% milk (n=14) or fruit juice not fortified with calcium (n=14) while followed a 12-week resistance training program. Only the "whole-body BMD" was been affected. The milk group showed two-fold greater increase than the juice group (0.028 vs 0.014 g/cm^2, respectively). Nevertheless, the juice group showed a greater increase at "whole-body BMC", after 12 weeks (2,667g vs 2,591g respectively, p<0.001). BMD and BMC were increased in both groups after six weeks, and there was a further increase at 12th week [46].

A cross-sectional study of 330 boys and girls aged 8 years no association was found between children's current calcium intake (1336 mg per day deriving from milk, fruit and vegetable consumption) and BMD. Only potassium and vegetable intakes, were negatively associated with total-body BMD (-0.14 and -0.15, respectively, p <0.05) [47].

A cross-sectional study of 176 Finnish girls aged 8-20 years-old had a seven-day Calcium intake Diary (CaD). Subjects were classified into three groups according to Tanner stage: prepubertal (Tanner 1; n=41), peripubertal (Tanner 2 and 3; n=54), and postpubertal (Tanner 4 and 5; n=81). No association was found between calcium intake and bone activity variables (BMD, BMC, and BMD femoral neck), but the high level of calcium intake in all age groups of the study T1+1018±361mg/d, T3 and T4=1059±460mg/d and T4 and T5=1231±565mg/d was likely to explain the lack of association. Namely, the fact that calcium intake that exceeds a certain level does not further contribute to bone mineralization [48].

A cross-sectional study in Central Japan's community (Mie) study the effects of dairy intake on bone health in a representative population sample of Japanese adult women (n = 1252, 19-80 years-old), high-school adolescent girls (n = 2651, 15-18 years-old) and boys (n = 2110, 15-18 years-old). These groups were examined according to the frequency of weekly milk consumption which was set at 4-5 times, 2-3 times and ≤ 1 time a week. Z-score dropped as the frequency of milk intake increased in all women. Specifically, it was 103.4% for once a week intake or less, 102.3% for 2 or 3 times a week, 102.8% for 4 to 5 times a week, and 101.2% for the daily intake. Bone density of the subjects was noticeably lower in the daily intake subgroup than that of the once a week or less, although not statistically significant ($p < 0.1$). This suggests that the bone density of adult women decreases as the frequency of milk intake is increasing. Also, the intake of milk did not affect the bone density of high school girls who avoided physical exercise (Z-score daily 95.3%, ≤1 time 95.2%, ($p < 0.1$)) [49].

The same study included 2110 Japanese boys, study the effect of milk intake on bone density on those abstained from physical activities in comparison to those systematically exercised was similar ($p < 0.1$). For no-exercise boys, Z-score showed a rise proportionally to the milk consumption, although the alteration was not significant (for daily milk intake was 95.9% and for ≤1 time was 93.3%, $p < 0.1$). In those Japanese boys cheese intake showed that Z-score was lowest in the daily intake group (systematic-exercise boys: daily 99.5% and ≤1 time 100.4%, no-exercise boys: daily 93.6% and ≤1 time 94.4%, ($p < 0.1$)). Same results for yoghurt in no-exercise high school girls (daily 93.8% and ≤1 time 95.1%, ($p < 0.1$)). Lastly, the cream seems to be beneficial for the bone health of girls without physical activity ($p = 0.05$) [49].

In a cross-sectional study in Netherlands examined the relation between physical activity, calcium intake from dairy products and BMC in 1359 children aged 7 to 11 years. No significant differences (in adjusted mean levels) in bone mineral content were found when "high" and "low" calcium intake groups were compared ("high" and "low" being defined by the upper and lowest decile of calcium intake). No evidence was found for any association between daily calcium intake and BMC in childhood, concluding that increased BMC was only detected in children with a high level of physical activity [50].

The study of 215 female twin pairs (122 monozygotic and 93 dizygotic) aged 10-26 years demonstrated the roles of constitutional and lifestyle factors on bone mass. 60% of total dietary calcium was derived from dairy products. In post-menarchial pairs, the detected difference in daily calcium intake level of intake (1041±674g/day) was associated with a univariate fashion with a relevant difference in total body BMC, although the robust estimation was considerably smaller. Across all pairs and after adjusting for menarchial status, height, lean mass, and fat mass, the coefficient for calcium intake became 0.04 -/+ 0.09%/100 mg. There were also found no associations within-pair relatively calcium intake and BMD at the lumbar or femoral sites [51].

In a 3-year prospective study of 179 Hong Kong boys and girls aged 12-13 years at baseline - reported that there was no influence of calcium intake (711.8 mg/day for boys and 580.8 mg/day for the girls) on BMD, BMC and bone mineral accretion. During the study period, mean BMC of the dominant forearm increased from 0.61 to 0.75 ($p < 0.001$) in the boys (25%) and from 0.64 to 0.73 ($p < 0.001$) in the girls (12.3%). Similarly, mean BMD of L2-L4 vertebrae was increased for both sexes from 0.63 to 0.77 ($p < 0.001$) in boys (24.2%) and from 0.75 to 0.87 ($p < 0.001$) in girls (14.5%). For girls, a higher age and an advanced pubertal stage at the beginning of the study resulted in a significantly ($p=0.03$) slower rate of increase in BMC of the forearm and BMD of the lumbar spine when compared with those girls with a less advanced pubertal stage at the beginning of the study. Comparing BMC of the forearm and BMD of the lumbar spine among boys and girls, rates of change in boys were significantly higher than those in girls ($p < 0.01$) [52].

In a study of 162 Icelandic Caucasian girls aged 13 and 15, reported a threshold effect intake on BMD of calcium intake 1293±452 mg/d in 13 year age group and 1082±382 mg/d calcium in 15 year age group (the intake of calcium from dairy products was 16.3% higher in younger girls as compared to older); above this, no further effect has been noticed. Univariate analysis showed no significant correlation between calcium intakes from milk and other dairy products and BMD or BMC in either age group [53].

A case-control study investigated for 2 years the relationship between dairy intake and the risk of fractures in 100 children (74 boys and 26 girls, aging 7-14 years old). They concluded that high consumption of cola sodas and noncarbonated beverages (including fruit juices) are positively and significantly associated with the probability of a fracture. On the other hand, consumption of either non-cola carbonated beverages or dairy products is unrelated. The latter is possibly due to misclassification of dairy intake or/and the fact that consumption of dairy products is generally high in adolescence and early adulthood [54].

A cohort of 581 pupils from Wales aged 7-9 investigated for the effect of milk supplement in childhood during growth on adult forearm Bone Mineral Content (BMC) and Bone Mineral Density (BMD) in a 14 years follow-up. They were randomized half of them to receive 190 ml of milk daily (or 228 mg of calcium) at their free school meal, regardless of that at home, on each school day for six terms in comparison to a control group given no milk. The total calcium intake in the two groups was for the Men: 1297±397, 1323±319 (Control) and for the Women: 933±278, 940±317 (Control). They found that BMD was positively associated with body weight ($p < 0.01$) current intakes of calcium (P less than 0.05), vitamin D (P less than 0.01) and sports activity during adolescence; inversely associated with alcohol consumption ($p < 0.05$). In multiple linear regression analysis, body weight and sports activity during adolescence were stronger determinants of female BMD than was diet [55].

In a 3-year prospective study 171 Finnish girls aged 9-15 years were investigated for calcium intake (1575±637 g/day) and BMD measured every 6 months for 3 years. There's no significant correlation between mean dietary calcium & vitamin D intake and BMD or BMAD at the lumbar spine or femoral neck regarding the overall study population. However, lumbar spine BMD was at 27% (0.030g/cm^2) greater in the highest than in the lowest vitamin D intake tertile in the girls with advanced sexual maturation (95% CI: 0.004, 0.056 g/cm^2 - p for trend for all tertiles = 0.016) [56].

Another 15-year longitudinal study (the "Amsterdam Growth and Health Study"), examined 84 males and 98 females (aged between 13 and 28). Multiple-regression analyses incorporating Calcium Intake By Body Height (CAIH), Weight-Bearing Activities (WBA), Body Weight (WT) and BMD indicated that calcium intake during adolescence and young adulthood was not a significant predictor of lumbar BMD at the age of 27 years for both sexes (males p<0.01, females p<0.001) [57].

The effects of calcium supplementation and dairy products consumption on bone and body composition were studied in 48 of pubertal girls from USA (aged 9 to 13). In this randomized intervention study, the control group consumed their usual diet (728±321 mg/day calcium) and the dairy group's diet was supplemented weekly with dairy products to at least 1200 mg calcium daily (1437±366 mg/day) over a period of 1 year. There were no differences between the two groups during the study as showed by bone mineral values (control group: 1508±167, dairy group: 1490±291gm) and lumbar spine bone density data (control: 0.665±0.077, dairy: 0.663±0.096 gm/cm^2). At the end of the year, either average lumbar spine bone density (control group: 0.748±0.084, dairy group: 0.772±0.086) or total body bone mineral content (control: 1617±152, dairy: 1695±317) did not differ significantly between groups [58].

Another randomized intervention study, 82 girls of United Kingdom 12 year old girls treated with daily consumption of 568 ml milk for 18 months follow-up. The control group received an average baseline of 150 ml of daily milk. Both groups demonstrated 9.6% increase for total body BMD of milk group (SD 1.9%; 95% confidence interval 9.0% to 10.2%) and control group 8.5% (2.7%; 7.6% to 9.4%); p=0.017) and total body BMC 27.0% (5.8%; 25.2% to 28.8%) v 24.1% (6.3%; 22.0% to 26.1%); p=0.009). Expressed in absolute terms, the respective increases were 0.090 (0.020; 0.084 to 0.096) v 0.081 (0.025; 0.072 to 0.089) g/cm^2 for total body BMD (p=0.021) and 428 (88; 398 to 452) v 391 (107; 358 to 430) g for total body BMC (p=0.035). Short term increases in calcium or dairy food intake in children or adolescents may not be sufficient to sustain an increase in bone mass over several decades [59].

At last, Chryssochoou et al. in a cross-sectional study investigated the relationships between Christian Orthodox Church's fasting and overall nutrition, growth and physical activity in children and adolescents from northern Greece. Among 609 children between 5 and 15.5 years old, 12.1% of them followed All Fasting periods (AF) without consuming dairy products (p=0.056) during the last 3.5 years and they didn't demonstrate any significant difference in their height (144.0±1.2 cm) in comparison to those that Partially Fasted (PF) (145.0±0.6 cm) or to the Never Fasted (NF) (145.1±0.6) (p=0.730). These children (NF) were found to have significantly higher calcium intake than full-fastened ones (1.151 versus 1.037 mg, respectively, p=0,017) [60].

Discussion

This study summarizes the published data on calcium balance regarding different types of dietary patterns. On the other hand, it presents the current knowledge on Orthodox Christian Church's diet as she combines features of other diets in proper timing as well as in a balanced combination of food ingredients.

Benefits from Calcium consumption are well-established regardless the source. Calcium intake - as the apparent rate of calcium absorption - doesn't significantly differ between vegan (26%) and lactovegetarian (24%) diet [43]. These rates are consistent with other studies on calcium balance that carried out with different kinds of non- vegetarian diet: 15% -36% [61], 27% [62], 16% -21% [63], 23%-31% [64] and 16% -24% [65].

The controversy about calcium's impact on musculoskeletal metabolism and more specifically on bone density shows significant interest. Studies on dairy-free vegetarian diets showed no (statistically significant) difference in BMD or in the degree of bone loss [66].

On the other side, the "Rotterdam study" points that fruit, vegetable, and dairy pattern with 135gr more milk as well as 29gr more yogurt, is responsible for high BMD, bending strength and more stable bones and hence for reduced osteoporotic fracture risk [HR (95% CI): 0.92 (0.89 - 0.96)] and hip fracture [HR (95% CI): 0.81 (0.70 - 0.93)] in women at the age of 55 [67].

Finally, others support that bone density and risk of bone fractures were found to be similar in omnivores and lacto-vegetarians [68,69].

The amount of calcium uptake is inversely related to the risk of fractures. Indeed sub-Saharan people with around 200 mg calcium daily consumption, they present lower bone fragility than those with double intake levels (i.e. Hong Kong, Singapore and Papua New Guinea) and even more than people who exceed 1000 mg (Norway, Sweden, Denmark, USA, UK, Ireland, New Zealand, Finland, etc.). Reasons for the wide geographic variation of fracture incidence still remain unknown [70] while causality can be found in high animal-derived protein intake, low vegetable and fruit intake, and low physical activity. The negative effect of menopause onto the musculoskeletal metabolism logically it should be accompanied by corresponding changes in bone density, though this has not yet been confirmed in populations where the daily calcium intake is significantly limited about 375 mg on average per day [44].

Even calcium and vitamin D3 daily intake for vegans is 55% and 63% respectively of those of omnivores; this doesn't adversely affect bone density or the frequency of fractures. In contrast, the higher intake of animal protein and lipids is associated with more bone loss than that of vegans [66].

On the daily milk consumption or calcium supplements during childhood and adolescence, some studies support that dietary or supplementary calcium does not affect spine & hip bone density [15,17-19,21,22,25,44,45,48,50,52,53,55,56,71-92] or bone turnover [45], even when the daily consumption of calcium exceeds 1000 mg [48,53,90].

Others studies advocate the opposite; total body BMC/BMD increase range from small [93] to significant [46,59,76,85,94-97], also they lack sufficient information about that beneficial effect.

Having on mind that the ideal vitamin D intake levels for the children and adolescents are 400-600 IU/day, alterations on its quantity as well as on that of other nutrients (such retinol) along with exercise habits could alter the effects of milk on BMD [18,87,89,98,99].

High intakes of calcium (>1400 mg/day) in women are associated with higher death rates from all causes and cardiovascular disease

[100]. Indeed, higher milk intake was associated with higher mortality as disclosed by cohort studies in both sexes with higher fracture incidence in women [101]. Moreover, there's a link between habitual consumption of milk & dairy products with increasing risk for prostate cancer [102-104].

Apart from all the above reported on calcium equilibrium and its interactions, non-animal sources of calcium such beverages from soy or rice, cereals, and fruit juices are of great biological and nutritional importance [105].

The content of oxalates or fibers (phytate/dietary) seems to be associated with low calcium intake [106-109]. Indeed, high oxalates in spinach and rhubarb show 5% [110] and 9% [111] absorption rates respectively, while other plant foods demonstrate higher: 41% for cabbage [112], 22% for beans [113] or 52% for Chinese cabbage and 48% for broccoli [114].

Regardless of vegan or lactovegetarian diet, calcium equilibrium isn't affected despite foods with high oxalates (spinach, beets), fiber-rich (whole grain bread, oats) or the calcium-rich mineral water [43]. Nevertheless, adequate calcium amounts in humans can be also achieved at intake levels far below the recommended [115-118].

Over and above, calcium bioavailability is of importance. In mineral water and milk is almost the same no matter the content [119-120] as a single dose of 172mg/l mineral water's calcium inhibits PTH secretion and bone resorption [121]. Higher bioavailability was observed in low-oxalate vegetables as broccoli, kale, cabbage and collards unlike in nuts, dried beans, and spinach [122,123].

Regular weight-bearing exercise and at least a normal age-related body weight in adolescence and young adulthood are of key importance in reaching the highest lumbar peak bone mass at the age of 27 years, regardless calcium intake [57], protein consumption or vitamin D levels [58].

Alternative nutritional lifestyles such as vegetarianism are popular the trend for any age group. An increasing rate of 2-5% of European adult population adopts a vegetarian eating pattern [124]. Concerns about global warming and sustainable food production as well as economic concerns are important incentives for someone to become vegetarian [105,125,126]. Most of them are young parents who introduce their children to vegan diet [105]. Moreover, vegan teenagers live in an otherwise omnivorous family [127]; published data (even limited) report no significant effect of vegan or vegetarian diet in growth during adolescence [128].

Among these various diets and under the general acceptance of the healthy eating habits that they introduce, Christian Orthodox Church (COC) diet introduces the "rationale of measure" instead of a complete abstinence from certain kind of foods. Fish is less frequently recommended while dairy and meat products are not allowed.

These fasting periods are well-defined and applied every year. Before long periods of fasting, such the 48-day long Lent, free consumption of all kind of food for three weeks is allowed. At the third week, only dairy products and fish (except meat) are allowed. During the week that follows the end of Lent (Easter Week) and the first 12 days after the Christmas fasting (The Twelve days) any kind of food is allowed to be consummated (Table 1) [28-30].

Papadaki et al. studied data on calcium intake alterations during one week of Lent. A double portion of all foods and drinks were chemically analyzed. Mean daily calcium intake was 533mg all from non-dairy products. Same chemical analysis for the non-fasting week after Easter reported 966mg/day [129].

A study conducted in children who fast, show a lower than the recommended daily intake of vitamin E and magnesium, but not for calcium and other minerals or trace elements which did not differ significantly between fasters (partially or completely) and non-fasters. These latter are ranked higher in daily energy intake, as they consume larger amounts of animal proteins and/or saturated fatty acids [6].

Conclusion

Overall balance and bioavailability of calcium are not significantly affected by the type of diet followed by children and adolescents. Christian Orthodox Church diet with its fasting may have a favorable effect on health as it provides adequate nutrition coverage as well as protection against numerous chronic diseases. In fact, periodic abstinence since childhood from dairy products meat and eggs tends to reduce the risk for future osteoporosis as it positively affects the bone mass density.

Far away from permanent food deprivations, which are not secondary to medical directives, one can argue that COC fasting is an integrated nutritional opportunity for a healthy lifestyle.

Acknowledgements

We thank Dr. Labadarios D., Prof. Emeritus of Stellenbosch University and Prof. Extraordinaire of University of Limpopo, South Africa, for his advice on the final writing of the Article. And Dr. Tornaritis E., Founder and Chairman of the Board of the Research and Education Institute of Child Health, Arh. Kiprianou 31, 2061, Strovolos, Cyprus, for the financial coverage of the publication of the Article.

References

1. Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride. Institute of Medicine. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Food and Nutrition Board. Washington, DC: National Academy Press. 1997.
2. Ilich JZ, Kerstetter JE. Nutrition in bone health revisited: a story beyond calcium. Journal of the American College of Nutrition. 2000; 19: 715-737.
3. Aoe S, Koyama T, Toba Y, Itabashi A, Takada Y. A controlled trial of the effect of milk basic protein (MBP) supplementation on bone metabolism in healthy menopausal women. Osteoporosis International. 2005; 16: 2123-2128.
4. Calcium requirements. Report of an FAO/WHO Expert Group. FAO/WHO Expert Group. Rome, Food and Agriculture Organization of the United Nations, 1962 (FAO Nutrition Meetings Report Series, No. 30.
5. National Institutes of Health. Consensus Development Panel on Optimal Calcium Intake. NIH Consensus Conference: optimal calcium intake. JAMA. 1994; 272: 1942-1948.
6. National Institutes of Health. Osteoporosis Prevention, Diagnosis, and Therapy. NIH Consensus Statement. 2000; 17: 1-45.
7. The Food Guide Pyramid. US Department of Agriculture. Human Nutrition Information Service. Hyattsville, MD. 1996.
8. US Department of Agriculture and US Department of Health and Human Services. Nutrition and Your Health: Dietary Guidelines for Americans. 5th ed. Washington, DC. US Department of Agriculture and US Department of

Health and Human Services. 2000 [Home and Garden Bull. No 232].

9. National Dairy Council. 2004.

10. Gerrior S. Nutrient Content of the US Food Supply. Washington, DC. US Department of Agriculture, Center of Nutrition Policy and Promotion. 2001 (Home Economics Research Report no.53).

11. Abelow BJ, Holford TR, Insogna KL. Cross-cultural association between dietary animal protein and hip fracture: a hypothesis. Calcif Tissue Int. 1992; 50: 14-18.

12. Report of a Joint Food and Agriculture Organization of the United Nations/ World Food Organization of the United Nations Expert Consultation. Human vitamin and mineral requirements. Bangkok, Thailand; September 1998.

13. Avenell A. Bone density and milk. Exercise and body size influence bone density. BMJ. 1994; 308: 1566.

14. Hegsted DM. Fractures, calcium, and the modern diet. American Journal of Clinical Nutrition. 2001; 74: 571-573.

15. Kardinaal AF, Ando S, Charles P, Charzewska J, Rotily M, Väänänen K, et al. Dietary calcium and bone density in adolescent girls and young women in Europe. Journal of Bone and Mineral Research. 1999; 14: 583-592.

16. Seppa K. Bone density and milk. Consider fat as well as calcium intake. BMJ. 1994; 308: 1566.

17. Weinsier RL, Krumdieck CL. Dairy foods and bone health: examination of the evidence. American Journal of Clinical Nutrition. 2000; 72: 681-689.

18. Feskanich D, Willett WC, Colditz GA. Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women. American Journal of Clinical Nutrition. 2003; 77: 504-511.

19. Kroger H, Kotaniemi A, Kröger L, Alhava E. Development of bone mass and bone density of the spine and femoral neck--a prospective study of 65 children and adolescents. Bone and Mineral. 1993; 23: 171-182.

20. Lloyd T, Chinchilli VM, Johnson-Rollings N, Kieselhorst K, Eggli DF, Marcus R, et al. Adult female hip bone density reflects teenage sports exercise patterns but not teenage calcium intake. Journal of Pediatrics. 2000; 106: 40-44.

21. Lloyd T, Taylor DS. Calcium intake and peak bone mass. Journal of the American Medical Women's Association. 2001; 56: 49-52.

22. Lloyd T, Beck TJ, Lin HM, Tulchinsky M, Eggli DF, Oreskovic TL, et al. Modifiable determinants of bone status in young women. Bone. 2002; 30: 416-421.

23. Lanou AJ, Berkow SE, Barnard ND. Calcium, dairy products, and bone health in children and young adults: a reevaluation of the evidence. Journal of Pediatrics. 2005; 115: 736-743.

24. Joint Food and Agriculture Organization of the United Nations/World Food Organization of the United Nations Expert Consultation on Diet, Nutrition, and the Prevention of Chronic Diseases. WHO Technical Report Series 916. Geneva, Switzerland: World Health Organization. 2003.

25. Whiting SJ, Healey A, Psiuk S, Mirwald R, Kowalski K, Bailey DA, et al. Relationship between carbonated and other low nutrient dense beverages and BMC of adolescents. Nutrition Research. 2001; 21: 1107-1115.

26. Institute of Medicine of the National Academy of Sciences. Dietary Reference Intakes for Calcium and Vitamin D. 2011.

27. Trepanowski JF and Bloomer RJ. The impact of religious fasting on human health. Nutrition Journal 2010; 9: 57.

28. Voloudakis EB. [Book in Greek]. Vypakis Publications, Edition B, Athens, 1999.

29. Kappa D. The Fasting. According to the teaching of our Church [Book in Greek]. Publishing Bookshops of the Holy Metropolis of Limassol, 1995. ISBN: 9607048091.

30. Koutsa Symeon, Archim. The Fasting of the Church. Why, when and how we are fast [Book in Greek]. Apostoliki Diakonia, Series Theory and Practice, Edition C, Athens, December 1991. ISBN: 9963794440.

31. Sarri KO, Tzanakis NE, Linardakis MK, Mamalakis GD, Kafatos AG. Effects of Greek Orthodox Christian Church fasting on serum lipids and obesity. BMC Public Health. 2003; 16: 3-16.

32. Sarri KO, Linardakis MK, Bervanaki FN, Tzanakis NE, Kafatos AG. Greek Orthodox fasting rituals: a hidden characteristic of the Mediterranean diet of Crete. British Journal of Nutrition. 2004; 92: 277-284.

33. Papadaki A, Vardavas C, Hatzis C, Kafatos A. Calcium, nutrient and food intake of Greek Orthodox Christian monks during a fasting and nonfasting week. Public Health Nutrition. 2008; 11: 1022-1029.

34. Sarri KO, Kafatos AG, Higgins S. Is religious fasting related to iron status in Greek Orthodox Christians? British Journal of Nutrition. 2005; 94: 198-203.

35. Sarri K, Bertsias G, Linardakis M, Tsibinos G, Tzanakis N, Kafatos A. The effect of periodic vegetarianism on serum retinol and alpha-tocopherol levels. International Journal for Vitamin and Nutrition Research. 2009; 79: 271-280.

36. Hakim I. Mediterranean diets and cancer prevention. Archives of Internal Medicine. 1998; 158: 1169-1170.

37. White R, Frank E. Health effects and prevalence of vegetarianism. The Western Journal of Medicine. 1994; 160: 465-471.

38. Whorton JC. Historical development of vegetarianism. American Journal of Clinical Nutrition. 1994; 59: 1103S-1109S.

39. Kafatos A, Kouroumalis I, Vlachonikolis I, Theodorou C, Labadarios D. Coronary-heart-disease risk-factor status of the Cretan urban population in the 1980s. American Journal of Clinical Nutrition. 1991; 54: 591-598.

40. Sabaté J. Religion, diet and research. British Journal of Nutrition. 2004; 92: 199-201.

41. Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, Gnardellis C, Lagiou P, Trichopoulos D, et al. Diet and overall survival in elderly people. BMJ. 1995; 311: 1457-1460.

42. Heany RP. The bone-remodeling transient: implications for the interpretation of clinical studies of bone mass change. Journal of Bone and Mineral Research. 1994; 9: 1515-1523.

43. Kohlenberg-Mueller K. and Raschka L. Calcium balance in young adults on a vegan and lactovegetarian diet. Journal of Bone and Mineral Metabolism. 2003; 21: 28-33.

44. Ho-Pham LT, Nguyen PL, Le TT, Doan TA, Tran NT, Le TA, et al. Veganism, bone mineral density, and body composition: a study in Buddhist nuns. Osteoporosis International. 2009; 20: 2087-2093.

45. Budek AZ, Hoppe C, Michaelsen KF, Molgaard C. High intake of milk, but not meat, decreases bone turnover in prepubertal boys after 7 days. European Journal of Clinical Nutrition. 2007; 61: 957-962.

46. Volek JS, Gomez AL, Scheett TP, Sharman MJ, French DN, Rubin MR, et al. Increasing fluid milk favorably affects BMD responses to resistance training in adolescent boys. Journal of the American Dietetic Association. 2003; 103: 1353-1356.

47. Jones G, Riley MD, Whiting SJ. Association between urinary potassium, urinary sodium, current diet, and bone density in prepubertal children. American Journal of Clinical Nutrition. 2001; 73: 839-844.

48. Uusi-Rasi K, Haapasalo H, Kannus P, Pasanen M, Sievänen H, Oja P, et al. Determinants of bone mineralization in 8 to 20 year old Finnish females. European Journal of Clinical Nutrition. 1997; 51: 54-59.

49. Yoshii S, Kamimotono Sh, Sawai Sh, Matsushita MB, Tanaka F, Rujkorakarn D, et al. Cross-sectional survey on the relationship between dairy product intake and bone density among adult women and high school students. Nutrition Research. 2007; 27: 618-624.

50. VandenBerg MF, DeMan SA, Witteman JC, Hofman A, Trouerbach WT, Grobbee DE. Physical activity, calcium intake, and BMC in children in the Netherlands. Journal of Epidemiology and Community Health. 1995; 49: 299-304.

51. Young D, Hopper JL, Nowson CA, Green RM, Sherwin AJ, Kaymakci B, et

al. Determinants of bone mass in 10 to 26-year-old females: a twin study. Journal of Bone and Mineral Research. 1995; 10: 558-567.

52. Cheng JC, Maffulli N, Leung SS, Lee WT, Lau JT, Chan KM. Axial and peripheral bone mineral acquisition: a 3-year longitudinal study in Chinese adolescents. Eur J Pediatr. 1999; 158: 506-512.

53. Kristinsson JO, Valdimarsson O, Steingrimsdottir L, Sigurdsson G. Relation between calcium intake, grip strength and BMD in the forearms of girls aged 13 and 15. J Intern Med. 1994; 236: 385-390.

54. Petridou E, Karpathios T, Dessypris N, Simou E, Trichopoulos D. The role of dairy products and non alcoholic beverages in bone fractures among school age children. Scand J Soc Med. 1997; 25: 119-125.

55. Fehily AM, Coles RJ, Evans WD, Elwood PC. Elwood PC. Factors affecting bone density in young adults. Am J Clin Nutr. 1992; 56: 579-586.

56. Lehtonen-Veromaa MK, Möttönen TT, Nuotio IO, Irjala KM, Leino AE, Viikari JS. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. Am J Clin Nutr. 2002; 76: 1446-1453.

57. Welten DC, Kemper HC, Post GB, Van Mechelen W, Twisk J, Lips P, et al. Weight-bearing activity during youth is a more important factor for peak bone mass than calcium intake. J Bone Miner Res. 1994; 9: 1089-1096.

58. Chan GM, Hoffman K, McMurry M. Effects of dairy products on bone and body composition in pubertal girls. J Pediatr. 1995; 126: 551-556.

59. Cadogan J, Eastell R, Jones N, Barker ME. Milk intake and bone mineral acquisition in adolescent girls: randomized, controlled intervention trial. BMJ. 1997; 315: 1255-1260.

60. Chryssochoou E, Linardakis M, Chatziagorou E, Tsanakas I, Kafatos AG. The effect of Christian Orthdox Church fasting on the health and growth of children and adolescents [Article in Greek]. Paediatriki. 2010; 73: 121-129.

61. McDonald JT, Margen S. Wine versus ethanol in human nutrition. III. Calcium, phosphorus, and magnesium balance. Am J Clin Nutr. 1979; 32: 823-833.

62. Gallagher JC, Riggs BL. Action of 1.25-dihydroxyvitamin D3 on calcium balance and bone turnover and its effect on vertebral fracture rate. Metabolism. 1990; 39: 30-34.

63. Spencer H, Norris C, Derler J, Osis D. Effect of oat bran muffins on calcium absorption and calcium, phosphorus, magnesium and zinc balance in men. J Nutr. 1991; 121: 1976-1983.

64. Hunt JR, Gallagher SK, Johnson LK, Lykken GI. High-versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. Am J Clin Nutr. 1995; 62: 621-632.

65. Haack VS, Chesters JG, Vollendorf NW, Story JA, Marlett JA. Increasing amounts of dietary fiber provided by foods normalizes physiologic response of the large bowel without altering calcium balance or fecal steroid excretion. Am J Clin Nutr. 1998; 68: 615-622.

66. Ho-Pham LT, Vu BQ, Lai TQ, Nguyen ND, Nguyen TV. Vegetarianism, bone loss, fracture and vitamin D: a longitudinal study in Asian vegans and non-vegans. Eur J Clin Nutr. 2012; 66: 75-82.

67. de Jonge EA, Kiefte-de Jong JC, Hofman A, Uitterlinden AG, Rivadeneira F, Franco OH, et al. Dietary patterns explaining differences in bone mineral density and hip structure in the elderly: the Rotterdam Study. Am J Clin Nutr. 2017; 105: 203-211.

68. Appleby P, Roddam A, Allen N, Key T. Comparative fracture risk in vegetarians and nonvegetarians in EPIC-Oxford. Eur J Clin Nutr. 2007; 61: 1400-1406.

69. New SA. Do vegetarians have a normal bone mass?. Osteoporos Int. 2004; 15: 679-688.

70. Prentice A. Diet, nutrition and the prevention of osteoporosis. Public Health Nutr. 2004; 7: 227-243.

71. Katzman DK, Bachrach LK, Carter DR, Marcus R. Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. J Clin Endocrinol Metab. 1991; 73: 1332-1339.

72. Lloyd T, Andon MB, Rollings N, Martel JK, Landis JR, Demers LM, et al. Calcium supplementation and BMD in adolescent girls. JAMA. 1993; 270: 841-844.

73. Lloyd T, Martel JK, Rollings N, Andon MB, Kulin H, Demers LM, et al. The effect of calcium supplementation and Tanner stage on bone density, content and area in teenage women. Osteoporosis International. 1996; 6: 276-283.

74. Winzenberg T, Shaw K, Fryer J, Jones G. Effects of calcium supplementation on bone density in healthy children: meta-analysis of randomized controlled trials. BMJ. 2006; 333: 775-780.

75. Taha W, Chin D, Silverberg AI, Lashiker L, Khateeb N, Anhalt H. Reduced spinal BMD in adolescents of an Ultra-Orthodox Jewish community in Brooklyn. Pediatrics. 2001; 107: 79.

76. Dibba B, Prentice A, Ceesay M, Stirling DM, Cole TJ, Poskitt EM. Effect of calcium supplementation on bone mineral accretion in Gambian children accustomed to a low-calcium diet. Am J Clin Nutr. 2000; 71: 544-549.

77. Nieves JW, Golden AL, Siris E, Kelsey JL, Lindsay R. Teenage and current calcium intake are related to bone mineral density of the hip and forearm in women aged 30–39 years. Am J Epidemiol. 1995; 141: 342-351.

78. Moro M, Van der Meulen MC, Kiratli BJ, Marcus R, Bachrach LK, Carter DR. Body mass is the primary determinant of midfemoral bone acquisition during adolescent growth. Bone. 1996; 19: 519-526.

79. Maggiolini M, Bonofiglio D, Giorno A, Catalano S, Marsico S, Aquila S, et al. The effect of dietary calcium intake on BMD in healthy adolescent girls and young women in southern Italy. Int J Epidemiol. 1999; 28: 479-484.

80. Bonofiglio D, Maggiolini M, Marsico S, Giorno A, Catalano S, Aquila S, et al. Critical years and stages of puberty for radial bone mass apposition during adolescence. Horm Metab Res. 1999; 31: 478-482.

81. Bonofiglio D, Maggiolini M, Catalano S, Marsico S, Aquila S, Giorno A, et al. Parathyroid hormone is elevated but bone markers and density are normal in young female subjects who consume inadequate dietary calcium. Br J Nutr. 2000; 84: 111-116.

82. Matkovic V, Fontana D, Tominac C, Goel P, Chesnut CH III. Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. Am J Clin Nutr. 1990; 52: 878-888.

83. Bonjour JP, Carrie AL, Ferrari S, Clavien H, Slosman D, Theintz G, et al. Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. J Clin Invest. 1997; 99: 1287-1294.

84. Johnston CC, Miller JZ, Slemenda CW, Teresa KR, Siu H, Joe C, et al. Calcium supplementation and increases in BMD in children. New England Journal of Medicine. 1992; 327: 82-87.

85. Lee WT, Leung SS, Wang SH, Xu YC, Zeng WP, Lau J, et al. Double-blind, controlled calcium supplementation and bone mineral accretion in children accustomed to a low-calcium diet. Am J Clin Nutr. 1994; 60: 744-750.

86. Lee WT, Leung SS, Leung DM, Tsang HS, Lau J, Cheng JC. A randomized double-blind controlled calcium supplementation trial, and bone and height acquisition in children. Br J Nutr. 1995; 74: 125-139.

87. Stear SJ, Prentice A, Jones SC, Cole TJ. Effect of a calcium and exercise intervention on the bone mineral status of 16–18-y-old adolescent girls. Am J Clin Nutr. 2003; 77: 985-992.

88. Yano K, Heilbrun LK, Wasnich RD, Hankin JH, Vogel JM. The relationship between diet and BMC of multiple skeletal sites in elderly Japanese-American men and women living in Hawaii. Am J Clin Nutr. 1985; 42: 877-888.

89. Fujiwara S, Kasagi F, Yamada M, Kodama K. Risk factors for hip fracture in a Japanese cohort. J Bone Miner Res. 1997; 12: 998-1004.

90. Nowson CA, Green RM, Hopper JL, Sherwin AJ, Young D, Kaymakci B, et al. A co-twin study of the effect of calcium supplementation on bone density during adolescence. Osteoporos Int. 1997; 7: 219-225.

91. Kalkwarf HJ, Khoury JC, Lanphear BP. Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. Am J Clin Nutr. 2003; 77: 257-265.

92. Whiting SJ, Lemke B. Excess retinal intake may explain the high incidence of osteoporosis in northern Europe. Nutr Rev. 1999; 57: 192-195.

93. De Fu Ma, Wei Zheng, Ming Ding, Yu Mei Zhang, Pei Yu Wang. Milk intake increases BMC through inhibiting bone resorption: Meta-analysis of randomized controlled trials. e-SPEN. 2013; 8: 1-7.

94. Wosje KS, Binkley TL, Fahrenwald NL, Specker BL. High bone mass in a female Hutterite population. J Bone Miner Res. 2000; 15: 1429-1436.

95. Teegarden D, Lyle RM, Proulx WR, Johnston CC, Weaver CM. Previous milk consumption is associated with greater bone density in young women. Am J Clin Nutr. 1999; 69: 1014-1017.

96. Sentipal JM, Wardlaw GM, Mahan J, Matkovic V. Influence of calcium intake and growth indexes on vertebral BMD in young females. Am J Clin Nutr. 1991; 54: 425-428.

97. Rubin K, Schirduan V, Gendreau P, Sarfarazi M, Mendola R, Dalsky G. Predictors of axial and peripheral BMD in healthy children and adolescents, with special attention to the role of puberty. J Pediatr. 1993; 123: 863-870.

98. Gennari C. Calcium and vitamin D nutrition and bone disease of the elderly. Public Health Nutr. 2001; 4: 547-559.

99. Nakamura K, Nashimoto M, Tsuchiya Y, Obata A, Miyanishi K, Yamamoto M. Vitamin D insufficiency in Japanese female college students: a preliminary report. Int J Vitam Nutr Res. 2001; 71: 302-305.

100. Michaëlsson K, Melhus H, Lemming EW, Wolk A, Byberg L. Long term calcium intake and rates of all cause and cardiovascular mortality: community based prospective longitudinal cohort study. BMJ. 2013; 346: 228.

101. Michaëlsson K, Wolk A, Langenskiold S, Basu S, Warensjo E, Melhus, et al. Milk intake and risk of mortality and fractures in women and men: cohort study. BMJ. 2014; 349: g6015.

102. Giovannucci E, Rimm EB, Wolk A, Ascherio A, Stampfer MJ, Colditz GA, et al. Calcium and fructose intake in relation to risk of prostate cancer. Cancer Res. 1998; 58: 442-447.

103. Giovannucci E, Liu Y, Stampfer MJ, Willet WC. A prospective study of calcium intake and incident and fatal prostate cancer. Cancer Epidemiol Biomarkers Prev. 2006; 15: 203-210.

104. Chan JM. Dairy products, calcium, and prostate cancer risk in the Physicians' Health Study. Am J Clin Nutr. 2001; 74: 549-554.

105. Van Winckel M, Vande Velde S, De Bruyne R, Van Biervliet S. Vegetarian infant and child nutrition, Clinical practice. Eur J Pediatr. 2011; 170: 1489-1494.

106. Levrat-Verny MA, Coudray C, Bellanger J, Lopez HB, Demigné C, Rayssiguier Y, et al. Whole wheat flour ensures higher mineral absorption and bioavailability than white wheat flour in rats. Br J Nutr. 1999; 82: 17-21.

107. Weaver CM, Proulx WR, Heaney R. Choices for achieving adequate dietary calcium with a vegetarian diet. Am J Clin Nutr. 1999; 70: 543S-548S.

108. Cheryan M. Phytic acid interactions in food systems. Crit Rev Food Sci Nutr. 1980; 13: 297-335.

109. Freeland-Graves J. Mineral adequacy of vegetarian diets. Am J Clin Nutr. 1988; 48: 859-862.

110. Heaney RP, Weaver CM, Recker RR. Calcium absorbability from spinach. Am J Clin Nutr. 1988; 47: 707-709.

111. Weaver CM, Heaney RP, Nickel KP, Packard PI. Calcium bioavailability from high oxalate vegetables: Chinese vegetables, sweet potatoes, and rhubarb. Journal of Food Science. 1997; 62: 524-525.

112. Heaney RP, Weaver CM. Calcium absorption from kale. Am J Clin Nutr. 1990; 51: 656-657.

113. Weaver CM, Heaney RP, Proulx WR, Hinders SM, Packard PT. Absorbability of calcium from common beans. Journal of Food Science. 1993; 58: 1401-1403.

114. Heaney RP, Weaver CM, Hinders SM, Martin B, Packard PT. Absorbability of calcium from brassica vegetables: broccoli, bok choy, and kale. Journal of Food Science. 1993; 58: 1378-1380.

115. Maierhofer WJ, Lemann J, Gray RW, Cheung HS. Dietary calcium and serum 1,25-(OH)2-vitamin D concentrations as determinants of calcium balance in healthy men. Kidney Int. 1984; 26: 752-759.

116. Greger JL, Baligar P, Abernathy RP, Bennett OA, Peterson T. Calcium, magnesium, phosphorus, copper, and manganese balance in adolescent females. Am J Clin Nutr. 1978; 31: 117-121.

117. Wisker E, Nagel R, Tanudjaja TK, Feldheim W. Calcium, magnesium, zinc, and iron balances in young women: effects of a low-phytate barley-fiber concentrate. Am J Clin Nutr. 1991; 54: 553-559.

118. Begum A, Pereira SM. Calcium balance studies on children accustomed to low calcium intakes. Br J Nutr. 1969; 23: 905-911.

119. Wynckel A, Hanrotel C, Wuillai A, Chanard J. Intestinal calcium absorption from mineral water. Miner Electrolyte Metab. 1997; 23: 88-92.

120. Heaney RP. Absorbability and utility of calcium in mineral waters. Am J Clin Nutr. 2006; 84: 371-374.

121. Guillemant J, Le HT, Accarie C, Du Montcel ST, Delabroise AM, Arnaud MJ, et al. Mineral water as a source of dietary calcium: acute effects on parathyroid function and bone resorption in young men. Am J Clin Nutr. 2000; 71: 999-1002.

122. Weaver CM. Calcium bioavailability and its relation to osteoporosis. Proc Soc Exp Biol Med. 1992; 200: 157-160.

123. Craig WJ, Mangels AR. Position of the American Dietetic Association: vegetarian diets. J Am Diet Assoc. 2009; 109: 1266-1282.

124. http://en.wikipedia.org/wiki/Vegetarianism_by_country # Ευρώπης.

125. White RF, Seymour J, Frank E. Vegetarianism among US women physicians. Journal of the American Dietetic Association. 1999; 99: 595-598.

126. Lea E, Worsley A. The cognitive contexts of beliefs about the healthiness of meat. Public Health Nutrition. 2002; 5: 37-45.

127. American Dietetic Association, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada. Vegetarian diets. Canadian Journal of Dietetic Practice and Research. 2003; 103: 748-765.

128. Sabaté J, Lindsted K, Harris RD, Johnston PK. Anthropometric parameters of schoolchildren with different life-styles. American Journal of Diseases of Children. 1990; 144: 1159-1163.

129. Papadaki A, Valsta LM, Lampi AM, Peñalvo J, Adlercreutz H, Vardavas C, et al. Differences in nutrient intake during a Greek Orthodox Christian fasting and non-fasting week, as assessed by a food composition database and chemical analyses of 7-day weighed food samples. Journal of Food Composition and Analysis. 2011; 24: 22-28.

Austin J Nutri Food Sci - Volume 5 Issue 3 - 2017
ISSN : 2381-8980 | www.austinpublishinggroup.com

Citation: Rodopaios NE, Manolarakis GE, Peppas C and Kafatos A. Is Periodic Abstinence from Dairy Products for Half a Year, Detrimental to Bone Health of Children and Adolescents?. Austin J Nutri Food Sci. 2017; 5(3): 1094.

Archives of Osteoporosis (2019) 14: 68
https://doi.org/10.1007/s11657-019-0625-y

ORIGINAL PAPER

Effect of periodic abstinence from dairy products for approximately half of the year on bone health in adults following the Christian Orthodox Church fasting rules for decades

Nikolaos E. Rodopaios[1] · Vassilis Mougios[2] · Anna Konstantinidou[3] · Stelios Iosifidis[4] · Alexandra-Aikaterini Koulouri[1] · Eleni Vasara[5] · Sousana K. Papadopoulou[3] · Petros Skepastianos[6] · Emmanouil Dermitzakis[7] · Maria Hassapidou[3] · Anthony G. Kafatos[1]

Received: 15 March 2019 / Accepted: 10 June 2019 / Published online: 27 June 2019

Abstract

Summary Christian Orthodox Church (COC) fasting is characterized by periodic abstinence from animal foods (including dairy products). We found that, despite this, older individuals adhering to COC fasting for decades did not differ in bone mineral density, bone mineral content, or prevalence of osteoporosis at five sites from non-fasting controls.

Purpose The present observational study investigated whether adherence to COC fasting, characterized by periodic abstinence from animal foods (including dairy products), affects bone health and the prevalence of osteoporosis in older individuals.

Methods Participants were 200 men and women, of whom 100 had been following the fasting rules of the COC for a median of 31 years and 100 were non-fasters, all aged 50 to 78 years. Participants underwent measurements of bone mineral density (BMD) and bone mineral content (BMC) at the lumbar spine, right hip, left hip, right femoral neck, and left femoral neck; completed a 3-day food intake record and food frequency questionnaire; and provided blood samples for biochemical measurements.

Results Fasters did not differ from non-fasters in demographic characteristics, anthropometric measures, BMD, BMC, or prevalence of osteopenia or osteoporosis at any of the five sites measured ($P > 0.05$). Fasters had lower daily calcium intake than non-fasters (median 532 vs 659 mg, $P = 0.010$), daily protein intake (0.67 vs 0.71 g/kg, $P = 0.028$), and consumption of dairy and soy products (10.3 vs 15.3 servings per week, $P < 0.001$). Groups did not differ in serum calcium, vitamin D, or urea concentrations.

Conclusions Despite lower calcium intake and lower consumption of dairy and soy products, older individuals adhering to COC fasting did not differ in BMD, BMC, or prevalence of osteoporosis from controls. Thus, periodic abstinence from dairy and, generally, animal products does not seem to compromise bone health in older individuals.

Keywords Bone mineral density · Calcium · Christian Orthodox Church fasting · Dairy · Osteoporosis · Vitamin D

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11657-019-0625-y) contains supplementary material, which is available to authorized users.

✉ Nikolaos E. Rodopaios
nikow1966@yahoo.gr

[1] Department of Social Medicine, Preventive Medicine and Nutrition, Medical School, University of Crete, Heraklion, Greece

[2] Laboratory of Evaluation of Human Biological Performance, School of Physical Education and Sport Science at Thessaloniki, Aristotle University of Thessaloniki, Thessaloniki, Greece

[3] Department of Nutrition and Dietetics, Alexander Technological and Educational Institute of Thessaloniki, Sindos, Greece

[4] Department of Nutrition Science and Dietetics, Harokopio University, Athens, Greece

[5] Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece

[6] Department of Medical Laboratory Studies, Alexander Technological and Educational Institute of Thessaloniki, Sindos, Greece

[7] Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland

Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass and deterioration of bone microarchitecture, with consequent increased bone fragility and susceptibility to fracture [1]. Osteoporosis is a major public health concern, affecting 200 million worldwide [2] and causing 9 million fractures per year, which are accompanied by substantial pain and suffering, impaired quality of life, disability, and even death [3]. Since it is a disease associated primarily with old age, its prevalence is expected to rise with improvements in life expectancy.

Although genetic and hormonal factors influence skeletal health, proper nutrition is considered essential for the prevention of osteoporosis [4]. In this context, dietary lifestyles that are biased toward specific food sources and/or exclude other food sources offer the opportunity to examine the dependence of bone health on nutrition. One such case is the lifelong periodic fasting implemented by the Christian Orthodox Church (COC).

In COC lifestyle, faith is expressed, among other things, through abstinence from certain foods—predominantly of animal origin except for seafood and snails—for certain periods and days during the year. The total days of COC fasting range from 159 to 197 (average, 178) and include five main periods, three important religious days, Wednesdays, and Fridays, as detailed in Supplemental Table 1. However, to facilitate replenishment of nutrients in the body after prolonged fasting, the COC has set two periods, comprising a total of 47 days, of no food restriction (not even on Wednesdays or Fridays).

A fundamental characteristic of COC fasting rules is the avoidance of meat, poultry, eggs, and dairy products. Consumption of olive oil, wine, and fish is generally permitted during fasting, except on Wednesdays and Fridays, unless these fall on the 47 days during which there is no food restriction. Seafood, such as oysters, mussels, shrimp, cuttlefish, octopus, squid, crab, and lobster, as well as snails, are permitted during all fasting days on which oil consumption is allowed. These characteristics of COC fasting are reminiscent of the Mediterranean diet [5].

At the same time, the characteristics of COC fasting raise concern about possible consequences on bone health. In particular, the exclusion of dairy products (the best source of calcium) and eggs (a good source of cholecalciferol) from the diet during approximately half of the year, as well as the abstention from fish (another good source of cholecalciferol) during approximately 5 months, may compromise calcium status in the body [6]. Therefore, the present study sought to investigate the role of COC fasting on bone mass maintenance and prevalence of osteoporosis among men and women above 50 years of age. To the best of our knowledge, there are no other studies assessing the potential impact of so long periodic abstinence from dairy products on bone health or osteoporosis.

Methods

Participants and study period

This study is part of a larger cross-sectional study aimed at investigating the effects of COC fasting on health (including bone health) by comparing fasting to non-fasting individuals (hereafter referred to as fasters and non-fasters). Because, as mentioned, we are unaware of any similar study, we could not perform a power analysis to determine the necessary sample size. Thus, we intuitively chose to recruit at least 400 adults, equally divided between fasters and non-fasters. This number is much higher than the sample sizes of 120 and 59 in two previous studies that examined the association of COC fasting with serum lipids and obesity [7] and with iron status [8], respectively.

We further decided to divide our sample equally between the ages of < 50 and ≥ 50, with at least 100 fasters and 100 non-fasters in each age group. The present study focuses on the older group; the sample sizes comply with the estimate of Ho-Pham and coworkers [9] that a sample size of 91 vegetarians and 91 omnivores was required to have a power of 0.80 in detecting a difference of 0.05 g/cm^2 in bone mineral density (BMD) at the confidence interval of 95%. Although COC fasters are not vegetarians, the aforementioned study was as close to our study as we could possibly find.

Participants were recruited on a voluntary basis in the province of Thessaloniki, Greece, between September 2013 and October 2015 through churches, a monastery, and public centers for the elderly. Of the 218 individuals who consented to participate, 15 were excluded because they did not meet the inclusion criteria, which were the absence of chronic disease, absence of morbid obesity (BMI $\geq$ 40 kg/m^2), no dietary supplementation, no medication, and (in the case of the fasting group) adherence to COC fasting for at least 10 years. Three more individuals were excluded because they did not complete all measurements. The remaining 200 participants were 100 fasters and 100 non-fasters. The fasters included 68 women and 32 men; the non-fasters included 66 women and 34 men.

To ensure that the data regarding the fasters were as representative of the entire year as possible, we did not perform measurements during periods of fasting or periods of no food restriction. Rather, all measurements and blood sampling were performed on Saturdays during the periods characterized by fasting on Wednesdays and Fridays only.

Anthropometric and cardiac parameters

Body weight was measured on a digital scale to the nearest 0.1 kg with the participant wearing light clothing and no shoes. Standing height was measured with a portable stadiometer to the nearest centimeter. Resting heart rate and blood pressure were measured with an electronic blood pressure monitor (Omron, Hoffman Estates, IL) in sitting position after 10 min of rest.

Bone health parameters

BMD and bone mineral content (BMC) were evaluated using dual-energy X-ray absorptiometry (Lunar DPX Bravo, GE Healthcare, equipped with the GE Lunar enCORE software, v. 13.5) at the lumbar spine (L2–L4), right hip, left hip, right femoral neck, and left femoral neck. Osteoporosis was diagnosed as BMD of 2.5 standard deviations (*T* score) or more below the young adult reference mean, whereas osteopenia was defined as a *T* score between − 1.0 and − 2.5 [10]. Participants were also asked about any history of bone fracture.

Biochemical parameters

Six milliliters of blood was drawn from a forearm vein in sitting position between 8 and 10 a.m., after 12 h of fast. Blood samples were placed in test tubes without anticoagulant, left to clot at room temperature, and centrifuged at 1500*g* for 10 min. Serum was separated from other blood components, divided into Eppendorf tubes, and stored at − 80 °C until analyzed for calcium, vitamin D (specifically, 25-hydroxyvitamin D), and urea (as an index of protein intake). Calcium was measured through the arsenazo III method in a Mindray BS-300 Chemistry Analyzer. Vitamin D was measured through chemiluminescent microparticle immunoassay in an Abbott Architect i2000SR analyzer. Urea was measured through the urease–glutamate dehydrogenase method in a Mindray BS-300 Chemistry Analyzer. The coefficients of variation for the three analytes were 5%, 7%, and 3%, respectively, and the laboratory carrying out these measures was involved in a nationwide external quality control program.

Dietary assessment

Dietary calcium, vitamin D, protein, and alcohol intakes were assessed through interviewer-based recalls of food intake over 3 days, which included a Wednesday or Friday (during which the fasters obeyed fasting), another weekday, and a weekend day. Participants were interviewed about all foods and liquids consumed during those days. Food intake records were analyzed using the Food Processor Nutrition Analysis software (ESHA, Salem, OR).

In addition to the food intake records, a semi-quantitative food frequency questionnaire with 140 questions was used to assess the participants' dietary habits during a period of 1 month. Frequency of consumption was based on a typical portion of each food and beverage. This questionnaire was then used to assess the consumption of dairy products (milk, yogurt, cheese, butter, and dairy cream) and soy products (soy milk and soy cheese) as good sources of calcium. Soy products were included to take into account 20 participants, 17 fasters and 3 non-fasters, who obtained part of their calcium intake from them. The questionnaire was also used to evaluate adherence to the Mediterranean diet through the MedDietScore [11], an 11-item index that produces a score ranging from 0 to 55 (0 to 5 for each item); a higher score suggests a greater adherence to the traditional Mediterranean diet.

Physical activity and smoking habits

Participants were asked to rank their habitual physical activity as very low, low, moderate, high, or very high on a 5-point scale. Additionally, they were asked what type of physical activity they practiced for distinction of those who practiced high-intensity/impact weight-bearing exercise from those who did not. Finally, they were asked about their smoking habits. Those who smoked one or more cigarettes per day were classified as smokers.

Ethics

The study protocol was approved by the Bioethics Committee of the Alexander Technological Educational Institute of Thessaloniki, and the study was conducted according to the Declaration of the World Medical Association of Helsinki (1989). Each participant was informed about the aims, benefits, and potential risks of the study and signed a consent form before data collection and blood sampling.

Statistical analysis

The Kolmogorov–Smirnov test and histogram charts were used to assess the normality of distribution of continuous variables. The distribution of most variables differed significantly from normal in at least one of the two groups (fasters or non-fasters). Thus, for the sake of uniformity, we report all continuous variables as median (interquartile range) and compared groups by using the non-parametric Mann–Whitney *U* test throughout. The few variables that warranted a *t* test did not produce a different outcome from the Mann–Whitney *U* test. Nominal variables are reported as percentages and were tested through the χ^2 test.

Statistical analysis was performed using the SPSS, version 25 (SPSS, Chicago, IL). All statistical tests and corresponding

P values were two-sided, and the level of statistical significance was set at 0.05.

Results

Demographic characteristics

Fasters were 58.2 (54.0–62.3) years old (maximum 77.6) and had been observing COC fasting for 31 (10–63) years, starting at the age of 25 (20–46). Fourteen had started fasting before adulthood (between the ages of 10 and 15). Non-fasters were 56.4 (53.2–61.8) years old (maximum 77.3). All women were postmenopausal, with menopause having occurred at the age of 50 (45–52) in fasters and 49 (47–50) in non-fasters. The two groups did not differ in any of the aforementioned parameters ($P > 0.05$).

Anthropometric and cardiac parameters

Body weight, height, BMI, resting heart rate, systolic blood pressure, and diastolic blood pressure of the participants are shown in Table 1. The two groups did not differ in any anthropometric or cardiac parameter ($P > 0.05$) except for diastolic blood pressure ($P = 0.007$), which was thus more favorable in fasters.

Bone health parameters

The two groups did not differ significantly in BMD (Fig. 1) or BMC (Fig. 2) at the lumbar spine, right hip, left hip, right femoral neck, or left femoral neck. Likewise, the 14 fasters who started fasting before adulthood did not differ significantly from the 86 who started as adults in any of these parameters. The frequency of osteoporosis and osteopenia at these sites (Table 2) was independent of group ($P > 0.05$), as shown by χ^2 tests where all expected frequencies were at least 5% to ensure validity. Likewise, the frequency of osteoporosis and osteopenia was independent of sex. The average prevalence of osteoporosis was 5% (ranging from 0 to 16% across sites, groups, and sexes), while the average prevalence of osteopenia was 35% (ranging from 22 to 50%). Eighty-eight fasters and 82 non-fasters were free of osteoporosis at all five sites, although this difference was not statistically significant. Twenty participants from each group, all women, had had bone fractures.

Biochemical and dietary parameters

The serum calcium, 25-hydroxyvitamin D, and urea concentrations are shown in Table 3. The two groups did not differ significantly in calcium or vitamin D, whereas the difference in urea was marginally significant ($P = 0.095$). Daily dietary calcium, vitamin D, and protein intakes, based on the 3-day recalls, are also shown in Table 3. The differences in calcium and protein intake were significant ($P = 0.010$ and 0.028, respectively).

The lower calcium intake of fasters compared to non-fasters was corroborated by the analysis of the food frequency questionnaire: fasters consumed 10.3 (7.6–16.3) servings of dairy and soy products per week, whereas non-fasters consumed 15.3 (10.6–21.0) servings ($P < 0.001$). Finally, the corresponding MedDietScore was the same for the two groups: 30 (27–34) and 30 (27–33), respectively. Daily alcohol consumption was 0 (0–0) g in both groups, since most participants (89 fasters and 76 non-fasters) reported none.

Physical activity and smoking habits

Fasters did not differ from non-fasters in habitual physical activity (Fig. 3). Only seven fasters and eight non-fasters practiced high-intensity/impact weight-bearing exercises. Fasters smoked considerably less than non-fasters, since only three fasters were smokers, as compared to 36 non-fasters ($P < 0.001$). Fasters smoked 0 (0–0) cigarettes daily, whereas non-fasters smoked 0 (0–5) cigarettes daily.

Table 1 Demographic, anthropometric, and cardiac parameters of the participants (median and interquartile range)

Parameter	Fasters ($n = 100$)	Non-fasters ($n = 100$)
Gender (female/male)	68/32	66/34
Age (years)	58.2 (54.0–62.3)	56.4 (53.2–61.8)
Body weight (kg)	75.5 (68.8–84.0)	76.7 (66.5–84.3)
Height (m)	1.63 (1.58–1.69)	1.63 (1.59–1.71)
BMI (kg/m^2)	28.0 (25.8–31.4)	28.1 (25.0–30.5)
Resting heart rate (bpm)	69 (62–76)	70 (64–76)
Systolic blood pressure (mmHg)	130 (123–133)	129 (126–133)
Diastolic blood pressure (mmHg)	80 (74–84)	82 (78–85)*

*$P = 0.007$, significantly different from fasters according to Mann–Whitney *U* test

Discussion

In the present study, we have examined differences in a number of parameters related to bone health (specifically, BMD, BMC, prevalence of osteopenia and osteoporosis, and history of bone fracture) between individuals of both sexes, aged 50 to 78 years, who had been adhering to periodic fasting according to the rules of the COC for at least 10 years and controls who did not restrict their diet. Our main finding is that bone health did not differ between groups even though fasters had lower consumption of dairy and soy products, as well as lower calcium and protein intakes (but not lower food consumption overall, as evidenced by the absence of difference in BMI). Although previous studies [7, 8] have addressed the impact of COC fasting on health indices (specifically, the lipidemic profile, BMI, and iron status), this is the first study, to the best of our knowledge, relating COC fasting to the prevalence of chronic diseases such as osteoporosis.

Sufficient consumption of dairy products is generally recommended due their high contents of high-quality protein, minerals, and vitamins, with most countries suggesting 2 to 3 servings per day for the adult population [12]. In our study, consumption of dairy and soy products was below these recommendations for most fasters: division of the weekly intakes by 7 yielded 1.5 (1.1–2.3) servings per day for fasters and 2.2 (1.5–3.0) servings per day for non-fasters. Yet, fasters did not differ from non-fasters in any of the bone health parameters assessed. These findings challenge the view that dairy consumption is beneficial for bone health and constitute a useful addition to recent controversial findings showing an association of higher milk and total dairy consumption with a lower risk of hip fracture in older adults [13], no clear association of milk or total dairy consumption with the risk of hip fracture [14], and an attenuation of age-related cortical bone loss in consumers of fermented dairy products (such as yogurt) but not in consumers of milk or ripened cheese [15]. It is thus possible that bone health depends on other factors than the intermittent intake of specific foods.

A recent meta-analysis has concluded that, compared with omnivores, vegetarians and vegans have lower BMD at the femoral neck and lumbar spine, while vegans have higher fracture rates [16]. These conclusions, combined with the findings of the present study, suggest that, although abolition of many or all foods of animal origin for life (vegetarianism and veganism, respectively) is detrimental to bone health, periodic abstinence from these foods for about half of the year (COC fasting) does not.

In accordance with the lower consumption of dairy and soy products, calcium intake by fasters was lower than that by non-fasters. Nevertheless, most fasters obtained more calcium (median 532 mg/day) than the recommended minimum of 400–500 mg/day for the prevention of osteoporosis [17]. This may explain the fact that fasters did not differ from non-fasters in BMD, BMC, or fracture

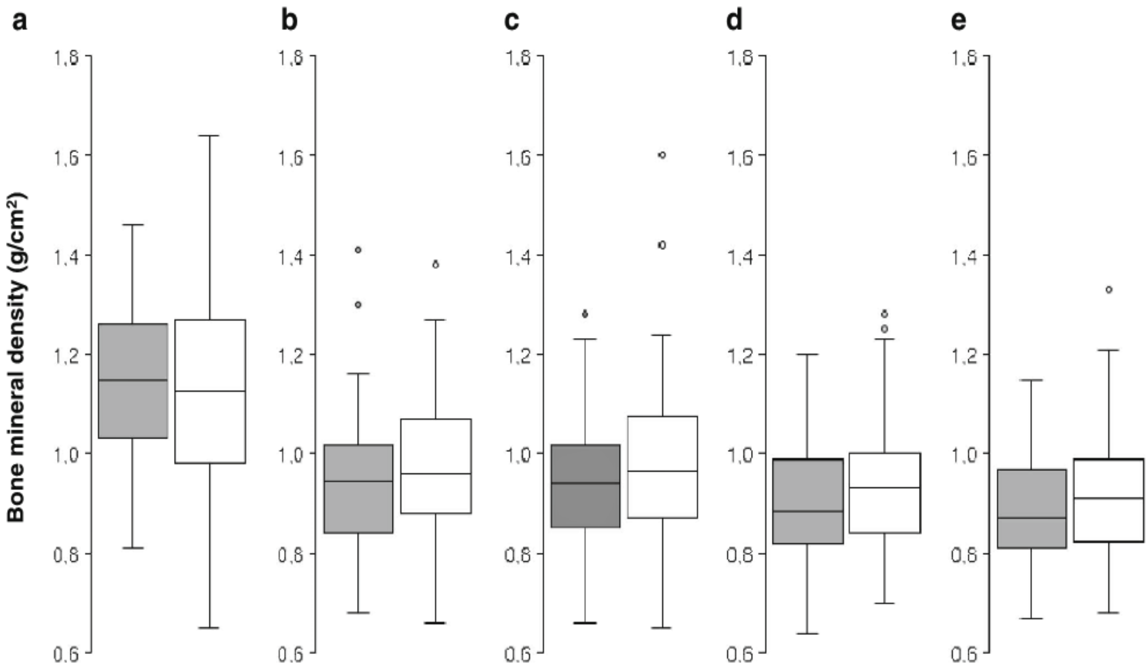


Fig. 1 Box plots of bone mineral density at the lumbar spine (**a**), right hip (**b**), left hip (**c**), right femoral neck (**d**), and left femoral neck (**e**) of fasters (gray boxes) and non-fasters. Each box represents the interquartile range, and the center line represents the median. Whiskers are extended to the most extreme data point that is no more than 1.5 times the interquartile range from the edge of the box (Tukey style). Dots represent outliers. No significant difference between groups was found (Mann–Whitney *U* test, $P > 0.05$).

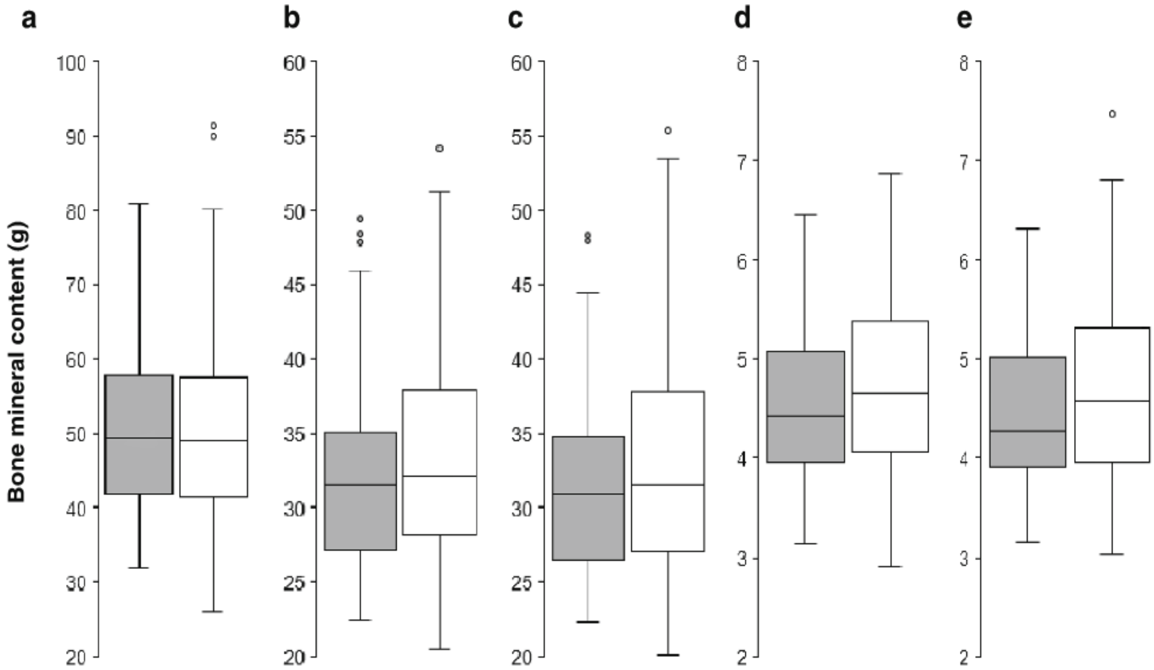


Fig. 2 Box plots of bone mineral content at the lumbar spine (**a**), right hip (**b**), left hip (**c**), right femoral neck (**d**), and left femoral neck (**e**) of fasters (gray boxes) and non-fasters. See Fig. 1 for description of each plot's

rate. Considering the fact that the DRI for calcium for older adults is 1000–1200 mg [18], our findings suggest that not as much calcium is needed to optimize BMD and BMC and prevent osteoporosis.

In addition to calcium, fasters had lower intakes of two more nutrients related to bone health, that is, vitamin D and protein [19–21], although the difference in vitamin D did not reach statistical significance, despite being numerically large (due to the wide dispersion of the individual values), and although sunshine exposure is usually the most important determinant of circulating 25-hydroxyvitamin D levels. These findings can also be explained by the lower consumption of dairy products and, generally, foods of animal origin by fasters. As with calcium, however, these lower intakes were not reflected in did not prove enough to cause a difference between fasters and non-fasters in the bone health parameters assessed in this study.

Despite a significant difference in dietary calcium intake, the serum calcium concentration did not differ between fasters and non-fasters. This may be explained by the presence of a very efficient calcium homeostatic system in the body [22]. Likewise, despite a significant difference in dietary protein intake, the serum urea concentration did not differ between fasters and non-fasters. However, differences in both parameters were numerically small (6 to 10%) and in the same direction (that is, both protein intake and serum urea concentration were higher in the non-fasters). Finally, the serum vitamin D concentration and vitamin D intake did not differ significantly between groups and were numerically higher in non-fasters. In all, the biochemical data seem to be in accordance with the dietary ones.

An interesting finding of our study was the lower diastolic blood pressure of fasters compared to non-fasters. This suggests that some aspect of their lifestyle is more favorable. At

Table 2 Prevalence of osteoporosis and osteopenia at five sites

Site	Osteoporosis (%)		Osteopenia (%)		Normal (%)	
	Fasters	Non-fasters	Fasters	Non-fasters	Fasters	Non-fasters
Lumbar spine	6	15	28	31	66	54
Right hip	3	3	38	28	59	69
Left hip	3	2	35	30	62	68
Right femoral neck	7	1	39	33	54	66
Left femoral neck	5	1	47	43	48	56

Table 3 Biochemical and dietary parameters of the participants (median and interquartile range)

Parameter	Fasters ($n = 100$)	Non-fasters ($n = 100$)
Serum calcium (mg/dL)	9.7 (9.4–10.1)	9.7 (9.4–9.9)
Serum 25-hydroxyvitamin D (ng/mL)	16.5 (12.7–20.8)	18.2 (12.7–23.2)
Serum urea (mg/dL)	29 (24–36)	32 (25–40)
Daily dietary calcium intake (mg)	532 (347–752)	659 (474–857)*
Daily dietary vitamin D intake (μg)	1.20 (0.41–2.79)	1.69 (0.65–3.30)
Daily dietary protein intake (g)	0.67 (0.46–0.84)	0.71 (0.58–0.93)*

*$P < 0.05$, significantly different from fasters according to Mann–Whitney U test

present, it is not clear what that aspect might be. Further analyses are under way, which might shed light on this difference.

A limitation of the present study is the one always associated with studies involving self-reporting of lifestyle parameters. Therefore, the possibility exists of misreporting (either under- or over-reporting) food intakes, physical activity, and smoking habits. However, there is no reason to suspect that fasters misreported these parameters in a way different from non-fasters. Additionally, because of the variation in dietary habits of fasters over the year, the possibility exists that some of the measured endpoints may fluctuate depending on when they are measured (although it is difficult to think that the primary endpoints, such as BMD, BMC, and prevalence of osteopenia and osteoporosis, can fluctuate during a year). Nevertheless, it would be desirable to perform the same measurements in future studies during periods of fasting and periods of no food restriction in addition to the "intermediate" period assessed in the present study.

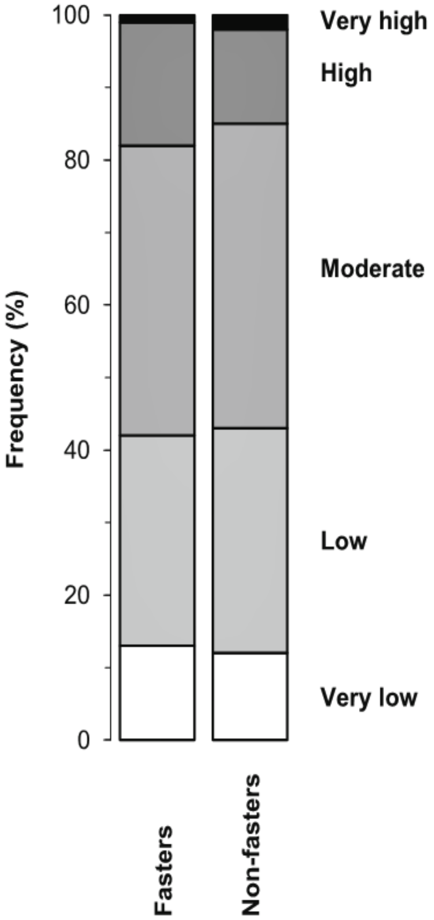


Fig. 3 Distribution of physical activity levels in fasters and non-fasters. Groups did not differ significantly (χ^2 test, $P > 0.05$).

In conclusion, despite lower calcium intake and lower consumption of dairy and soy products, older men and women adhering to COC fasting did not differ from peers who did not fast in any of the indices of bone health examined. Thus, periodic abstinence from dairy and, in general, animal products does not seem to harm bone health in older individuals. This finding may prove useful in designing healthy diets for people who wish, or need, to have limited intake of such foods.

Acknowledgments We are grateful to Metropolitan Varnavas of Neapolis and Stavroupolis, Thessaloniki; Metropolitan Georgios of Kitros, Katerini, and Platamon; Hieromonk Father Luke Kipouros of Holy Trinity Monastery, Panorama, Thessaloniki; Professor Emeritus of the School of Theology, Aristotle University of Thessaloniki, Dimitrios Tselegidis; Archimandrite Nikodemos Skrettas-Plexidas; and Archpriest and Professor of the School of Pastoral and Social Theology, Aristotle University of Thessaloniki, Athanasios Gikas for their help in collecting the study sample. We also thank Hieromonk Archimandrite Eulogios Tsalapatanis for information about COC fasting.

Compliance with ethical standards

Ethics The study protocol was approved by the Bioethics Committee of the Alexander Technological Educational Institute of Thessaloniki, and the study was conducted according to the Declaration of the World Medical Association of Helsinki (1989). Each participant was informed about the aims, benefits, and potential risks of the study and signed a consent form before data collection and blood sampling.

Conflicts of interest None.

References

1. Pisani P, Renna MD, Conversano F, Casciaro E, Di Paola M, Quarta E, Muratore M, Casciaro S (2016) Major osteoporotic fragility

fractures: risk factor updates and societal impact. World J Orthop 7: 171–181
2. Lin JT, Lane JM (2004) Osteoporosis—a review. Clin Orthop Relat Res 425:126–134
3. Johnell O, Kanis JA (2006) An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int 17:1726–1733
4. Kruger MC, Wolber FM (2016) Osteoporosis: modern paradigms for last century's bones. Nutrients. 8:376
5. Sarri KO, Linardakis MK, Bervanaki FN, Tzanakis NE, Kafatos AG (2004) Greek Orthodox fasting rituals: a hidden characteristic of the Mediterranean diet of Crete. Br J Nutr Aristotle University of Thessaloniki 92:277–284
6. Papadaki A, Vardavas C, Hatzis C, Kafatos A (2008) Calcium, nutrient and food intake of Greek Orthodox Christian monks during a fasting and non-fasting week. Public Health Nutr 11:1022–1029
7. Sarri KO, Tzanakis NE, Linardakis MK, Mamalakis GD, Kafatos AG (2003) Effects of Greek orthodox Christian church fasting on serum lipids and obesity. BMC Public Health 3:16
8. Sarri KO, Kafatos AG, Higgins S (2005) Is religious fasting related to iron status in Greek Orthodox Christians? Br J Nutr 94:198–203
9. Ho-Pham L, Nguyen P, Le T, Doan T, Tran N, Le T, Nguyen T (2009) Veganism, bone mineral density, and body composition: a study in Buddhist nuns. Osteoporos Int 20:2087–2093
10. WHO (2004) WHO scientific group on the assessment of osteoporosis at primary health care level. WHO, Geneva
11. Panagiotakos DB, Pitsavos C, Stefanidis C (2006) Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis 16:559–568
12. Weaver CM (2014) How sound is the science behind the dietary recommendations for dairy? Am J Clin Nutr 99:1217S–1222S
13. Feskanich D, Meyer HE, Fung TT, Bischoff-Ferrari HA, Willett W (2018) Milk and other dairy foods and risk of hip fracture in men and women. Osteoporos Int 29:385–396
14. Bian S, Hu J, Zhang K, Wang Y, Yu M, Ma J (2018) Dairy product consumption and risk of hip fracture: a systematic review and meta-analysis. BMC Public Health 18:165
15. Biver E, Durosier-Izart C, Merminod F, Chevalley T, van Rietbergen B, Ferrari SL, Rizzoli R (2018) Fermented dairy products consumption is associated with attenuated cortical bone loss independently of total calcium, protein, and energy intakes in healthy postmenopausal women. Osteoporos Int 29:1771–1782
16. Iguacel I, Miguel-Berges L, Gómez-Bruton A, Moreno LA, Julián C (2018) Veganism, vegetarianism, bone mineral density, and fracture risk: a systematic review and meta-analysis. Nutr Rev 77:1–18
17. WHO (2003) Diet, nutrition and the prevention of chronic diseases. WHO Technical Report Series No. 916. WHO, Geneva
18. Ross AC, Taylor CL, Yaktine AL, Del Valle HB (2011) The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine. 634 p
19. Dolan E, Sale C (2019) Protein and bone health across the lifespan. Proc Nutr Soc 78:45–55
20. Rizzoli R, Biver E, Bonjour J-P, Coxam V, Goltzman D, Kanis JA, Lappe J, Rejnmark L, Sahni S, Weaver C et al (2018) Benefits and safety of dietary protein for bone health. Osteoporos Int:1–16
21. Durosier-Izart C, Biver E, Merminod F, van Rietbergen B, Chevalley T, Herrmann FR, Ferrari SL, Rizzoli R (2017) Peripheral skeleton bone strength is positively correlated with total and dairy protein intakes in healthy postmenopausal women. Am J Clin Nutr 105:513–525
22. Houillier P, Froissart M, Maruani G, Blanchard A (2006) What serum calcium can tell us and what it can't. Nephrol Dial Transplant 21:29–32

European Journal of Pediatrics (2020) 179:645–651
https://doi.org/10.1007/s00431-019-03542-1

ORIGINAL ARTICLE

Bone status of young adults with periodic avoidance of dairy products since childhood

Nikolaos E. Rodopaios[1] · Vassilis Mougios[2] · Alexandra-Aikaterini Koulouri[1] · Eleni Vasara[3] · Sousana K. Papadopoulou[4] · Petros Skepastianos[5] · Emmanouil Dermitzakis[6] · Maria Hassapidou[4] · Anthony G. Kafatos[1]

Received: 15 August 2019 / Revised: 28 November 2019 / Accepted: 3 December 2019 / Published online: 23 December 2019

Abstract

Proper nutrition throughout childhood and adolescence is crucial for normal bone development. We investigated whether adherence to Christian Orthodox Church fasting is characterized by periodic avoidance of animal foods (including dairy products), since childhood affects stature or bone health in young adults. This cross-sectional study included 200 healthy men and women, aged 18–35, of whom 100 had been following religious fasting for a median of 14 years, starting at the age of 10, and 100 were non-fasters. Measurements included body height; bone mineral density and bone mineral content at the lumbar spine, right hip, left hip, right femoral neck, and left femoral neck; prevalence of bone fracture; serum biochemical parameters; food and nutrient intake; and physical activity and smoking habits. Fasters did not differ from non-fasters in anthropometric measures (including height), bone mineral density and content, or prevalence of low bone mineral density at any of the five sites measured; number of bone fractures; or serum calcium or 25-hydroxyvitamin D concentrations ($P > 0.05$). Fasters had lower daily calcium and protein intakes, as well as lower dairy consumption than non-fasters. Groups did not differ in physical activity, and fasters smoked less than non-fasters.

Conclusion: Despite lower calcium intake and lower dairy product consumption, individuals adhering to religious fasting since childhood did not differ in height, bone mineral density and content, or prevalence of fractures from controls. Therefore, periodic abstention from dairy and, generally, animal products since childhood does not seem to compromise bone health in young adults.

Communicated by Mario Bianchetti

✉ Nikolaos E. Rodopaios
nikow1966@yahoo.gr

Vassilis Mougios
mougios@auth.gr

Alexandra-Aikaterini Koulouri
alexkoulou@yahoo.com

Eleni Vasara
evasara@bio.auth.gr

Sousana K. Papadopoulou
souzpapa@gmail.com

Petros Skepastianos
pskep@otenet.gr

Emmanouil Dermitzakis
emmanouil.dermitzakis@unige.ch

Maria Hassapidou
mnhas@nutr.teithe.gr

Anthony G. Kafatos
kafatos@med.uoc.gr

1 Department of Social Medicine, Preventive Medicine and Nutrition, Medical School, University of Crete, Heraklion, Greece

2 Laboratory of Evaluation of Human Biological Performance, School of Physical Education and Sport Science at Thessaloniki, Aristotle University of Thessaloniki, Thessaloniki, Greece

3 Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece

4 Department of Nutritional Sciences and Dietetics, International Hellenic University, Thermi, Greece

5 Department of Medical Laboratory Studies, International Hellenic University, Thermi, Greece

6 Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland

What is Known:

- *Bone health is an important determinant of overall health and longevity.*
- *Proper nutrition throughout childhood and adolescence is crucial for normal bone development.*
- *Adequate intake of dairy products is considered important due to their high calcium content.*

What is New:

- *Young adults with limited calcium intake and dairy product consumption, due to adherence to the fasting rules of the Christian Orthodox Church since childhood, do not differ in height or indices of bone health from non-fasting controls.*

Keywords Adulthood · Bone mineral density · Calcium · Childhood · Christian Orthodox Church fasting · Dairy

Abbreviations:

BMC Bone mineral content
BMD Bone mineral density
COC Christian Orthodox Church

Introduction

Bone health is an important determinant of overall health and longevity. Maximizing peak bone mass by young adulthood is considered crucial for reducing the risk of bone fracture and osteoporosis in later life [1]. In fact, longitudinal studies show that measures of bone health, such as bone mineral density (BMD) and bone mineral content (BMC), track strongly from childhood to adolescence and young adulthood [2–4]. This stresses the importance of ensuring optimal bone development during childhood and adolescence.

Among the many factors determining bone mass and strength development in youth, nutrition (in the sense of both individual nutrient intake and food patterns) plays a pivotal role [5]. This is why unusual food patterns, emphasizing specific foods and/or excluding others, during childhood and adolescence can provide valuable insight into the dependence of bone development on nutrition. A case in point is the periodic fasting dictated by the Christian Orthodox Church (COC), which consists in abstention from certain foods—mostly of animal origin notwithstanding seafood and snails—for specific periods and days during the year [6] and has many characteristics of the Mediterranean diet [7].

Because COC bars dairy products (the best dietary source of calcium) and eggs (a good source of cholecalciferol) for approximately half of the year, as well as fish (another good source of cholecalciferol) for approximately 5 months, it may lower calcium status in the body, with consequences on bone health, especially during development. We recently showed that older adults who had been fasting for decades did not differ in BMD, BMC, or prevalence of osteoporosis from non-fasting peers despite lower calcium intake [6]. However, most (86%) had started fasting as adults. To address the question whether fasting in childhood and adolescence affects bone mass in adulthood, we investigated the effect of COC fasting since childhood on stature and bone health of young men and women.

Material and methods

Participants and study design

This is part of a larger cross-sectional study, conducted in the province of Thessaloniki, Greece, between September 2013 and October 2015 and aimed at examining the effects of COC fasting on health (including bone health) by comparing fasting to non-fasting individuals (hereafter referred to as fasters and non-fasters) of both sexes. We chose to include at least 400 participants, equally divided between fasters and non-fasters, as well as between the ages of 18–35 and ≥ 50, with at least 100 subjects in each subgroup. The present study refers to the younger group, while findings on the older group have been published [6]. The study protocol was approved by the Bioethics Committee of the Alexander Technological Educational Institute of Thessaloniki.

Participants were recruited on a voluntary basis from the Aristotle University of Thessaloniki, the (then) Alexander Technological Educational Institution of Thessaloniki (presently International Hellenic University), churches, and two monasteries. Of the 236 persons who agreed to participate, 25 were excluded because, at their first visit, they were found not to meet all the inclusion criteria, which were the absence of chronic disease, absence of morbid obesity (BMI ≥ 40 kg/m^2), no dietary supplementation, no medication, and (in the case of the fasters) observance of COC fasting for at least 10 years or from the age of 10. Eleven more individuals were excluded because they did not complete all measurements. That left 200 subjects (100 fasters and 100 non-fasters).

To make certain that data regarding fasters were as representative of the whole year as possible, no measurements were done during periods of fasting or no food restriction. Instead, measurements and blood sampling were performed on Saturdays during periods characterized by fasting on Wednesdays and Fridays only.

Anthropometric and cardiac parameters

Body weight was measured on a digital scale, and height was measured to the nearest centimeter with a stadiometer. Resting heart rate and blood pressure were measured with an electronic blood pressure monitor (Omron, Hoffman Estates, IL).

Bone status parameters

BMD and BMC were measured through dual-energy X-ray absorptiometry at five sites, as described [6]. BMD was considered low if it was 2.0 standard deviations (Z-score) or more below the mean of a healthy population of the same age and sex; BMD was considered normal if it was above that limit [8]. Subjects were asked whether they had had any bone fracture.

Biochemical parameters

Participants provided 6 mL of venous blood after 12 h of fast while seated. Serum, produced after clotting, was analyzed for calcium, vitamin D (specifically, 25-hydroxyvitamin D), and urea (as an index of protein intake), as described [6].

Dietary assessment

Dietary calcium, vitamin D, protein, and alcohol intakes were assessed through interviewer-based recalls of food intake, as described [6]. Moreover, the subjects' dietary habits during a period of one month were assessed through a food frequency questionnaire to estimate the consumption of dairy and soy products as good sources of calcium, as well as adherence to the Mediterranean diet through the MedDietScore [9].

Physical activity and smoking habits

Subjects were asked about the amount and type of physical activity to help us pinpoint high-impact weight-bearing exercises that better promote BMD [10]. Finally, they were interviewed about their smoking habits, and those smoking at least one cigarette daily were considered smokers.

Statistical analysis

Continuous variables were examined for normality of distribution with the Kolmogorov-Smirnov test and histogram charts. Because the distribution differed significantly from normal in most cases and to facilitate comparisons across parameters, we report all continuous variables as median (interquartile range) and compared groups by the non-parametric Mann-Whitney U test. (None of the few variables that warranted a t test produced a different outcome.) Nominal variables are reported as percentages and were tested through the χ^2 test.

The SPSS, version 25 (SPSS, Chicago, IL), was used in all statistical analyses. All statistical tests and corresponding P values were two-sided, and statistical significance was declared at $P < 0.05$.

Results

Demographic characteristics

Fasters were 26.7 (22.2–33.4) years old and had been observing COC fasting for 14 (10–21) years, starting at the age of 10 (10–12). Non-fasters were 24.0 (21.0–28.9) years old. Although significant ($P = 0.004$), this difference of 2.7 years between groups is not expected to have any bearing on the results.

Anthropometric and cardiac parameters

Distribution of sexes, body weight, height, BMI, resting heart rate, systolic blood pressure, and diastolic blood pressure did not differ between groups ($P > 0.05$, Table 1).

Bone status parameters

No significant difference between groups was found in BMD (Fig. 1) or BMC (Fig. 2) at the lumbar spine, right hip, left hip, right femoral neck, or left femoral neck. The frequency of low and normal BMD at these sites (Table 2) was independent of group ($P > 0.05$), as shown by the χ^2 test. The average prevalence of low BMD was 4% (ranging from 2 to 9% across sites and groups). Eighty-nine fasters and 88 non-fasters had normal BMD at all 5 sites (non-significant difference). Fifteen fasters and 13 non-fasters, all women, reported that they had had bone fractures (non-significant difference). These had occurred in the upper limbs (8 and 6, respectively), lower limbs (6 in each group), and shoulder (one in each group).

Biochemical and dietary parameters

Table 3 presents the serum calcium, 25-hydroxyvitamin D, and urea concentrations of fasters and non-fasters. The differences in calcium or 25-hydroxyvitamin D were not significant, whereas the difference in urea was ($P = 0.004$). Table 3 also presents the daily calcium, vitamin D, protein, and alcohol intakes, based on the 3-day recalls. The differences in calcium, protein, and alcohol intakes were significant (P = 0.010, 0.022, and 0.005, respectively), whereas the difference in vitamin D intake was marginally significant ($P = 0.095$). In all cases, values were higher in non-fasters.

Analysis of the food frequency questionnaires showed that fasters consumed 11.5 (7.5–17.0) servings of dairy products per week, whereas non-fasters consumed 14.0 (9.0–22.4) servings ($P = 0.039$). When the consumption of soy products by 33 participants (32 fasters and one non-faster) was added, the difference between groups was dampened: fasters consumed 12.0 (7.5–18.5) servings of dairy and soy products per week, whereas non-fasters still consumed 14.0 (9.0–

Table 1 Demographic, anthropometric, and cardiac parameters of the participants (median and interquartile range)

Parameter	Fasters (n = 100)	Non-fasters (n = 100)
Sex (female/male)	63/37	60/40
Body weight (kg)	67.5 (57.2–80.1)	66.9 (58.0–81.7)
Height (m)	1.68 (1.62–1.75)	1.69 (1.63–1.78)
BMI (kg/m^2)	23.9 (21.3–27.5)	23.5 (20.8–26.2)
Resting heart rate (bpm)	69 (63–77)	72 (65–82)
Systolic blood pressure (mmHg)	120 (113–130)	123 (114–130)
Diastolic blood pressure (mmHg)	76 (68–81)	76 (71–87)

22.4) servings (P = 0.103). Weekly alcohol consumption was 2 (0–4) drinks in fasters and 4 (2–6) in non-fasters (P < 0.001). Finally, the MedDietScore was 30 (26–33) and 29 (26–32), respectively (P = 0.643). These values indicate moderate adherence to the traditional Mediterranean diet, since the MedDietScore ranges from 0 (no adherence) to 55 (full adherence) [9].

Physical activity and smoking habits

Habitual physical activity did not differ between fasters and non-fasters (Fig. 3). Twenty-nine fasters and 32 non-fasters practiced high-impact weight-bearing exercises (non-significant difference). Only seven fasters were smokers, compared with 22 non-fasters (P = 0.003). Smokers did not differ significantly from non-smokers in any bone status parameter.

Discussion

We have investigated differences in indices of bone status (specifically, stature, BMD, BMC, prevalence of low BMD, and history of bone fracture) between young adults of both sexes, who had been practicing periodic religious fasting for at least 10 years or from the age of 10, and non-fasting controls. We found that none of these indices differed between groups despite fasters having lower dairy consumption and calcium intake. We are unaware of other research on the potential impact of prolonged periodic abstinence from dairy products since childhood on bone status. Longitudinal studies for up to 17 years have found that bone mass tracks strongly from childhood all the way to young adulthood, with tracking correlation coefficients ranging from 0.6 to 0.9 [2–4]. These findings indicate that early levels of bone mass are important for later bone health and reinforce the relevance of the present study.

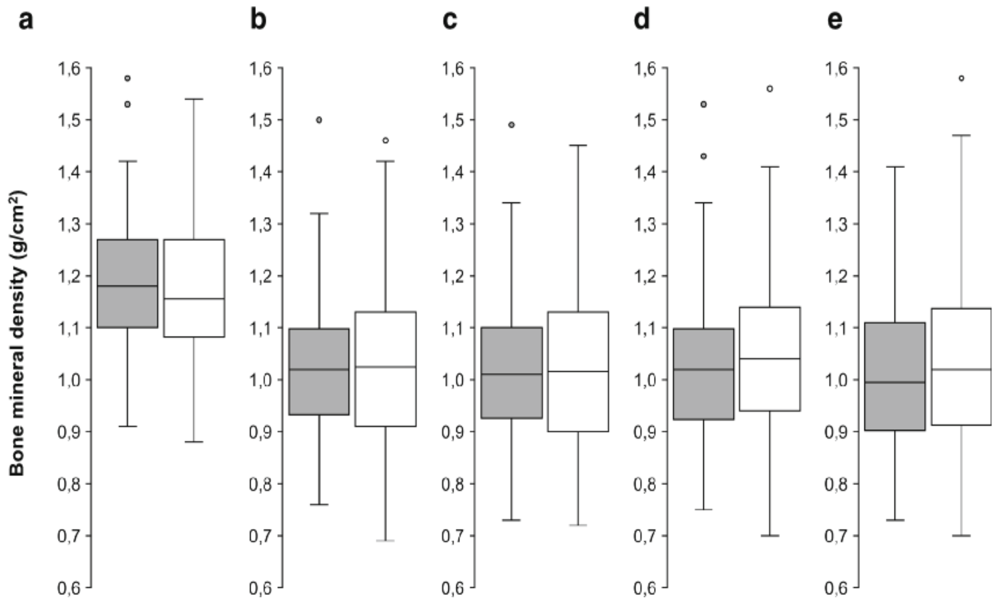


Fig. 1 Box plots of bone mineral density at the lumbar spine (**a**), right hip (**b**), left hip (**c**), right femoral neck (**d**), and left femoral neck (**e**) of fasters (gray boxes) and non-fasters. Each box represents the interquartile range, and the center line represents the median. Whiskers are extended to the most extreme data point that is no more than 1.5 times the interquartile range from the edge of the box (Tukey style). Dots represent outliers. No significant difference between groups was found (Mann-Whitney U test, P > 0.05)

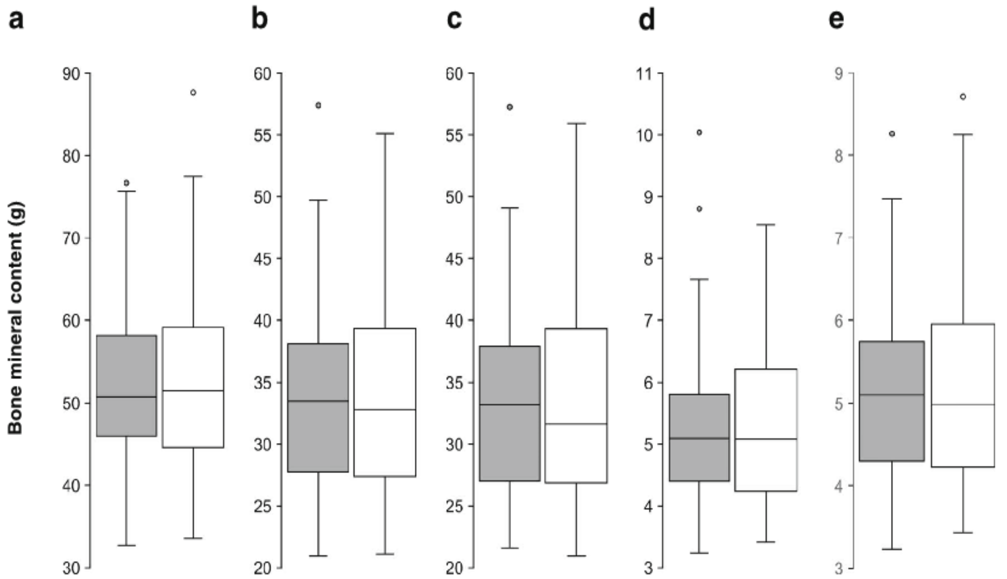


Fig. 2 Box plots of bone mineral content at the lumbar spine (**a**), right hip (**b**), left hip (**c**), right femoral neck (**d**), and left femoral neck (**e**) of fasters (gray boxes) and non-fasters. See Fig. 1 for description of each plot's elements. No significant difference between groups was found (Mann-Whitney *U* test, $P > 0.05$)

A recent position statement and systematic review on peak bone mass development and lifestyle factors have concluded that the best evidence (grade A) is available for positive effects of calcium intake and physical activity [5]. In our study, the latter did not differ between groups, whereas the former was lower in fasters. Nevertheless, this did not seem to affect bone development or, in general, bone health. It appears that this lower calcium intake was still sufficient for good bone status.

In accordance with the lower calcium intake, consumption of dairy products was lower by fasters compared with non-fasters. Evidence for a positive effect of dairy consumption on bone mass is considered good [5], and most countries suggest 2–3 servings per day [11]. In the present study, dairy consumption was below these recommendations for most fasters, as can be seen when one divides the weekly intakes reported under the "Results" section by 7, which yields 1.6 (1.1–2.4) servings per day. By comparison, non-fasters consumed 2.0 (1.3–3.2) servings per day; thus, half of them met the minimum recommendation. The lack of differences between groups in the bone status parameters assessed questions the importance of dairy products for bone health. Thus, our findings add to recent controversial findings ranging from a positive to no association of dairy consumption with indices of bone health [12–14].

Table 2 Prevalence of low and normal bone mineral density values at five sites

Site	Low (%)		Normal (%)	
	Fasters	Non-fasters	Fasters	Non-fasters
Lumbar spine	7	9	93	91
Right hip	2	4	98	96
Left hip	4	3	96	97
Right femoral neck	3	3	97	97
Left femoral neck	2	3	98	97

Fasters had lower intakes of two more nutrients that have been linked to bone status, namely, vitamin D and protein [5, 15]. These differences are apparently due to the lower consumption of dairy products and, in general, foods of animal origin by fasters. As with calcium, however, these differences were not reflected in a difference between groups regarding bone status. Of the biochemical parameters measured, the serum calcium concentration did not differ between fasters and non-fasters (despite the significant difference in calcium intake), indicative of the strong calcium homeostasis in the body [16]. On the other hand, the serum urea concentration was lower in fasters, in accordance with their lower protein intake.

Among the lifestyle factors differing between fasters and non-fasters were alcohol consumption and smoking. Despite drinking more alcohol, non-fasters, as well as fasters, had all light to moderate consumption, not exceeding two drinks per day. Because such consumption is generally reported to be beneficial for bone health, unlike heavy consumption [17], one cannot argue that the higher alcohol consumption by non-fasters had a detrimental effect on bone status, one that could have counterbalanced a detrimental effect of lower calcium and dairy consumption in fasters, resulting in no difference between groups. Likewise, it seems unlikely that the higher prevalence of smoking among non-fasters could have counterbalanced the lower calcium and dairy consumption by

Table 3 Biochemical and dietary parameters of the participants (median and interquartile range)

Parameter	Fasters (n = 100)	Non-fasters (n = 100)
Serum calcium (mg/dL)	9.7 (9.4–10.1)	9.8 (9.5–10.0)
Serum 25-hydroxyvitamin D (ng/mL)	17.6 (12.8–24.0)	19.2 (15.8–23.8)
Serum urea (mg/dL)	25 (19–30)	28 (23–35)*
Daily calcium intake (mg)	532 (350–799)	663 (444–920)*
Daily vitamin D intake (μg)	1.25 (0.50–2.22)	1.82 (0.56–2.97)
Daily protein intake (g/kg)	0.73 (0.59–0.96)	0.83 (0.67–1.05)*
Daily alcohol intake (g)	0.0 (0.0–0.0)	0.0 (0.0–7.2)*

* $P < 0.05$, significantly different from fasters according to Mann-Whitney U test

fasters: first, because the evidence for a detriment of smoking on bone health is limited and inconsistent [5, 18]; and second, because smokers did not differ from non-smokers in any of the bone status parameters measured.

The findings of the present study are in accordance with those of our previous study [6], which showed that older men and women (50 years and over) observing religious fasting for a median of 31 years did not differ in BMD, BMC, prevalence of osteoporosis, or history of bone fracture from non-fasting controls despite having lower dairy consumption and calcium intake. At the same time, it has the same limitations, that is, (1) the possibility of participants misreporting (either under- or over-reporting) food intakes, physical activity, and smoking habits (although it seems unlikely that one group misreported differently from the other) and (2) the possible fluctuation of some parameters during periods of fasting and periods of no food restriction in fasters, which could be remedied by performing multiple measurements over the year in future studies.

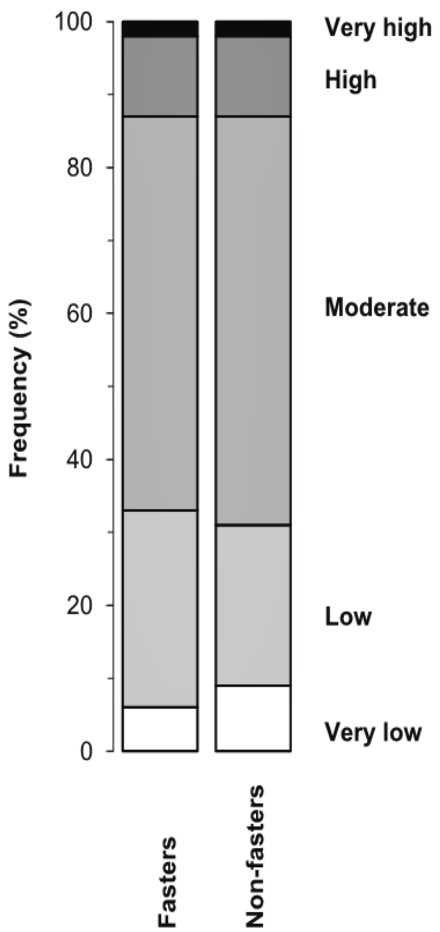


Fig. 3 Distribution of physical activity levels in fasters and non-fasters. Groups did not differ significantly (χ^2 test, $P > 0.05$)

In conclusion, young men and women adhering to COC fasting since childhood did not differ from peers who did not fast in stature or any of the indices of bone health examined, despite lower calcium intake and lower consumption of dairy products. Taken together with those of our recent study in older individuals, our findings show that periodic abstinence from dairy and, in general, animal products over decades, even starting in childhood, does not compromise bone health. This conclusion may be exploited for the formulation of healthy dietary plans for persons who desire, or need, to have low intakes of such foods.

Acknowledgments We are grateful to Metropolitan Varnavas of Neapolis and Stavroupolis, Thessaloniki; Metropolitan Georgios of Kitros, Katerini, and Platamon; Hieromonk Father Luke Kipouros of Holy Trinity Monastery, Panorama, Thessaloniki; Nun, Melani Chatzipatera, Mother Superior of Assumption of Virgin Monastery, Panorama, Thessaloniki; Professor Emeritus of the School of Theology, Aristotle University of Thessaloniki, Dimitrios Tselegidis; Archimandrite Nikodemos Skrettas-Plexidas; and Archpriest Athanasios Gikas; Professors of the School of Pastoral and Social Theology, Aristotle University of Thessaloniki, for their help in collecting the study sample.

Authors' contributions N. Rodopaios conceptualized and designed the study, collected data, analyzed data, drafted the initial manuscript, and critically reviewed and revised the manuscript.

V. Mougios analyzed data, drafted the initial manuscript, and critically reviewed and revised the manuscript.

A-A Koulouri designed the study and collected data, and critically revised the manuscript.

E. Vasara collected and analyzed data, and critically revised the manuscript.

S. Papadopoulou and Dr Skepastianos collected data, and critically revised the manuscript.

E. Dermitzakis and Hassapidou provided essential materials data, and critically revised the manuscript.

A Kafatos conceptualized and designed the study, coordinated and supervised data collection, reviewed and critically revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

References

1. Golden NH, Abrams SA, Committee on Nutrition (2014) Optimizing bone health in children and adolescents. Pediatrics 134(4):e1229–e1243. https://doi.org/10.1542/peds.2014-2173
2. Rønne M, Heidemann M, Schou A, Laursen JO, Bojesen AB, Lylloff L, Husby S, Wedderkopp N, Mølgaard C (2018) Tracking of bone mass from childhood to puberty: a 7-year follow-up. The CHAMPS study DK. Osteoporos Int 29(8):1843–1852. https://doi.org/10.1007/s00198-018-4556-z
3. Wren TAL, Kalkwarf HJ, Zemel BS et al (2014) Longitudinal tracking of dual-energy X-ray absorptiometry bone measures over 6 years in children and adolescents: persistence of low bone mass to maturity. J Pediatr 164(6):1280–1285.e2. https://doi.org/10.1016/j.jpeds.2013.12.040
4. Yang Y, Wu F, Winzenberg T, Jones G (2018) Tracking of areal bone mineral density from age eight to young adulthood and factors associated with deviation from tracking: a 17-year prospective cohort study. J Bone Miner Res 33(5):832–839. https://doi.org/10.1002/jbmr.3361
5. 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(4):1281–1386. https://doi.org/10.1007/s00198-015-3440-3
6. Rodopaios NE, Mougios V, Konstantinidou A, Iosifidis S, Koulouri AA, Vasara E, Papadopoulou SK, Skepastianos P, Dermitzakis E, Hassapidou M, Kafatos AG (2019) Effect of periodic abstinence from dairy products for approximately half of the year on bone health in adults following the Christian Orthodox Church fasting rules for decades. Arch Osteoporos 14(1):68–68. https://doi.org/10.1007/s11657-019-0625-y
7. Sarri KO, Linardakis MK, Bervanaki FN, Tzanakis NE, Kafatos AG (2004) Greek Orthodox fasting rituals: a hidden characteristic of the Mediterranean diet of Crete. Br J Nutr 92:277–284. https://doi.org/10.1079/BJN20041197
8. Lewiecki EM, Gordon CM, Baim S, Leonard MB, Bishop NJ, Bianchi ML, Kalkwarf HJ, Langman CB, Plotkin H, Rauch F, Zemel BS, Binkley N, Bilezikian JP, Kendler DL, Hans DB, Silverman S (2008) International Society for Clinical Densitometry 2007 adult and pediatric official positions. Bone 43(6):1115–1121. https://doi.org/10.1016/j.bone.2008.08.106
9. Panagiotakos DB, Pitsavos C, Stefanidis C (2006) Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis 16:559–568. https://doi.org/10.1016/j.numecd.2005.08.006
10. Xu J, Lombardi G, Jiao W, Banfi G (2016) Effects of exercise on bone status in female subjects, from young girls to postmenopausal women: an overview of systematic reviews and meta-analyses. Sports Med 46(8):1165–1182. https://doi.org/10.1007/s40279-016-0494-0
11. Weaver CM (2014) How sound is the science behind the dietary recommendations for dairy? Am J Clin Nutr 99(5):1217S–1222S. https://doi.org/10.3945/ajcn.113.073007
12. Bian S, Hu J, Zhang K, Wang Y, Yu M, Ma J (2018) Dairy product consumption and risk of hip fracture: a systematic review and meta-analysis. BMC Public Health 18:165. https://doi.org/10.1186/s12889-018-5041-5
13. Biver E, Durosier-Izart C, Merminod F, Chevalley T, van Rietbergen B, Ferrari SL, Rizzoli R (2018) Fermented dairy products consumption is associated with attenuated cortical bone loss independently of total calcium, protein, and energy intakes in healthy postmenopausal women. Osteoporos Int 29:1771–1782
14. Feskanich D, Meyer HE, Fung TT, A B-FH, C WW (2018) Milk and other dairy foods and risk of hip fracture in men and women. Osteoporos Int 29:385-396.
15. Dolan E, Sale C (2019) Protein and bone health across the lifespan. Proc Nutr Soc 78:45–55. https://doi.org/10.1017/S0029665118001180
16. Houillier P, Froissart M, Maruani G, Blanchard A (2006) What serum calcium can tell us and what it can't. Nephrol Dial Transplant 21(1):29–32. https://doi.org/10.1093/ndt/gfi268
17. Gaddini GW, Turner RT, Grant KA, Iwaniec UT (2016) Alcohol: a simple nutrient with complex actions on bone in the adult skeleton. Alcohol Clin Exp Res 40(4):657–671. https://doi.org/10.1111/acer.13000
18. Strozyk D, Gress TM, Breitling LP (2018) Smoking and bone mineral density: comprehensive analyses of the third National Health and Nutrition Examination Survey (NHANES III). Arch Osteoporos 13(1):16. https://doi.org/10.1007/s11657-018-0426-8

European Journal of Clinical Nutrition (2020) 74:1736–1742
https://doi.org/10.1038/s41430-020-0667-7

ARTICLE

Food and health

The significant effect on musculoskeletal metabolism and bone density of the Eastern Mediterranean Christian Orthodox Church fasting

Nikolaos E. Rodopaios [1] · George E. Manolarakis [1] · Alexandra-Aikaterini Koulouri [1] · Eleni Vasara [2] · Sousana K. Papadopoulou [3] · Petros Skepastianos [4] · Emmanouil Dermitzakis [5] · Maria Hassapidou [3] · Manolis K. Linardakis [1] · Anthony G. Kafatos [1]

Received: 9 January 2020 / Revised: 19 May 2020 / Accepted: 20 May 2020 / Published online: 1 June 2020

Abstract

Objective Nutritional disorders cause secondary osteoporosis as well as musculoskeletal metabolism dysfunction. The Christian Orthodox Church's fasting in Mediterranean countries such Greece and Cyprus, or M.C.O.C. diet consists of self-restraint from food and/or food categories for 180 ± 19 days of total, especially of animal protein and dairy products. This case-control study attempts to investigate the effect of this fasting pattern on musculoskeletal metabolism and bone density.
Design One hundred fasters (or M.C.O.C. diet followers; 68 women and 32 men, mean 59 ± 6.5) with 32 years average fasting time and 100 non-fasters (66 females and 34 males, mean 58.1 ± 6.8; the control group of Mediterranean diet followers) over the age of 50, including menopausal women, were interviewed, as well as physically and laboratory examined along with DEXA measurements of the L2-4 vertebrae and hips. Nutrition data gathered through a 3-day food record during a non-fasting period, while energy intakes calculated on a daily food consumption basis.
Results Given the overall low incidence of osteoporosis in the Mediterranean diet, it appears to be more than three times higher than that of M.C.O.C. diet despite the periodic restriction of food intake of animal origin into a slightly hypothermic pattern, which in turn is characterized by increased consumption of multicolored vegetable foods.
Conclusions Abstinence from dairy products and meat does not adversely affect musculoskeletal metabolism or bone density. M.C.O.C. diet seems to be "healthy" eating habit for the musculoskeletal system, as future studies expected to confirm.

Introduction

World's population is steadily aging. By 2050, people over the age of 60 probably will double, reaching 22%, while the elderly will be tripled [1].

After the age of 50, almost one in two females and one in five men will suffer fragility fracture [2]. In USA, over 2 million osteoporotic fractures per year cost up to $ 17 billion, predicting an increase of over 87% in the 65–74 age group, which is expected to reach 50% of prevalence by 2025 [3], as the age-adjusted incidence of fractures increases exponentially during the seventh decade of life [4].

Aging is accompanied by changes in the dietary status and metabolism of the musculoskeletal system and is clinically manifested either as a reduction in bone density, i.e., osteoporosis, or in muscle mass, i.e., sarcopenia, and/or as muscle-strength impairment, i.e., dynapenia [5]. The key point is the inability to maintain the energy balance

✉ Nikolaos E. Rodopaios
nikow1966@yahoo.gr

1 Department of Social Medicine, Preventive Medicine and Nutrition, Medical School, University of Crete, Heraklion, Greece

2 Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece

3 Department of Nutritional Sciences and Dietetics, International Hellenic University, Thermi, Greece

4 Department of Medical Laboratory Studies, International Hellenic University, Thermi, Greece

5 Department of Genetic Medicine and Development, Faculty of Medicine, University of Geneva, Geneva, Switzerland

resulting in frailty [6]. In fact, osteoporosis is observed in 20% of malnourished (undernourished) elders [7], while 30% of the frail elder women have severe osteoporosis and sarcopenia [8].

For this reason, a balanced diet with the essential for bone health foods contributes to the prevention of osteoporosis [9]. Such a diet is the "Mediterranean" that appears to have beneficial effects on bone health [10]. A special variation of this is the diet of the Greek Orthodox Christian Church (M.C.O.C. diet) as it is considered a healthy eating habit [11], where abstinence from dairy and animal proteins lasts for 180 ± 19 days a year.

The purpose of this case-control study is to investigate the effects of the Hellenic (Greek) or Mediterranean C.O.C. fasting on bone density and musculoskeletal metabolism in men and women in menopause, over 50 years of age.

Methods

The permission of the Ethics Committee of the Alexandrian Technological Educational Institute as well the written consent given by the participants made the study feasible.

The study group (M.C.O.C. diet followers) consisted of 100 fasters (68 women and 32 men, mean 59 ± 6.5), with 32 years average fasting time and the control group (typical Mediterranean diet followers) of 100 non-fasters (66 females and 34 males, mean 58.1 ± 6.8). Interviews gathered personal information along with medical and nutritional history.

In addition to the food intake records, a semiquantitative food frequency questionnaire with 140 questions was used to assess the participants' dietary habits during a period of one month. Frequency of consumption was based on a typical portion of each food and beverage. This questionnaire was also used to evaluate adherence to the Mediterranean diet through the MedDietScore [12], an 11-item index that produces a score ranging from 0 to 55 (0–5 for each item); a higher score suggests a greater adherence to the traditional Mediterranean diet.

The post-hoc analysis of the data obtained showed strength of 88.6%. Therefore, the total number of 200 individuals (100 in each group) is considered to meet the requirements for proper statistical processing and reliable results.

Of the 218 people who agreed to participate, 15 were excluded because they did not meet the integration criteria, which were the absence of chronic disease, the absence of morbid obesity (BMI ≥ 40 kg/m^2), no dietary supplement, no medication and adherence to COC fasting for at least 10 years. Three more people were excluded due to the lack of complete data.

The participants were recruited on a voluntary basis and came from the province of Thessaloniki, in particular through parishes, two monasteries and public centers for the care of the elders.

The fasting group was formed by people dedicated to fasting for more than 10 years. Also, no intervention was performed and the statistical processing was based exclusively on measurement data.

Everyone underwent basic physical assessment, height, body weight, and BMI measurements. Biochemical blood tests calculated key-metabolic markers and DEXA tests analyzed body composition (fat and lean mass) as well as BMD, BMC, *T*-score, and *Z*-score of L2-4 vertebrae and hips.

Nutrition history through a 3-day food record during a non-fasting period provided data of macro-, micronutrient, and calorie intakes processed by Food Processor Nutrition Analysis software. Finally, energy intakes calculated on a daily food consumption mode.

To ensure that the data regarding the fasters were as representative of the entire year as possible, we did not perform measurements during periods of fasting or periods of no food restriction. Rather, all measurements and blood sampling were performed on Saturdays during the periods characterized by fasting on Wednesdays and Fridays only.

Including a statement confirming that informed consent was obtained from all subjects.

Results

The IBM SPSS 25.0 software performed statistical process. A comparative analysis conducted between fasters and non-fasters via Chi-square and Student *T* test, having previously set an acceptable level of significance of 5%.

In short, fasters showed better *T*-scores for the lumbar spine, as opposed to the right femoral neck, not only for normal bone density levels, but also for osteoporosis. In terms of statistical significance:

1. Overall lumbar spine osteoporosis incidence was as low as 9% (18 individuals out of 200), namely 4% of fasters and the—more than three times higher—14% of non-fasters.
2. The exactly opposite was observed for the right hips as 5% of fasters demonstrated femoral neck osteoporosis versus null of the non-fasters. (Figs. 1 and 2).
3. Among non-fasters, 35% were smokers instead of 4% of fasters.
4. Non-fasters exceeded in the following intakes: macronutrients (protein, total, monounsaturated and polyunsaturated fat and dietary fibers), vitamins (B3, B12, D3, E, and folic acid), minerals and trace minerals (calcium, ferrum, magnesium, manganese, phosphorus, and selenium).

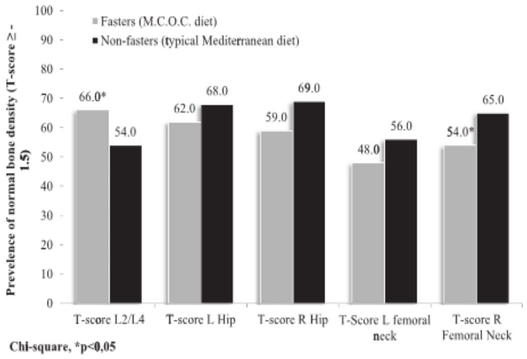


Fig. 1 Columns of prevalence of normal bone density (*T*-score ≥ 1.5) at the lumbar spine (L2/L4), left hip (L Hip), right hip (R Hip), left femoral neck (L femoral neck), and right femoral neck (R Femoral Neck) of fasters (gray boxes) and non-fasters (black boxes). A comparative analysis conducted between fasters and non-fasters via Chi-square and Student *T* test, having previously set an acceptable level of significance of 5%. Fasters showed better *T*-scores for the lumbar spine (Chi-square, $p < 0.05$), as opposed to the right femoral neck, not only for normal bone density levels.

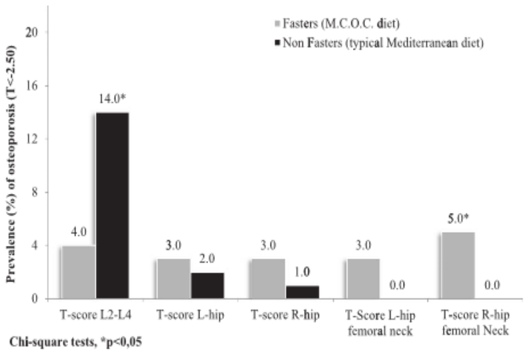


Fig. 2 Columns of prevalence (%) of osteoporosis at the lumbar spine (L2/L4), left hip (L Hip), right hip (R Hip), left femoral neck (L femoral neck), and right femoral neck (R Femoral Neck) of fasters (gray columns) and non-fasters (black columns). A comparative analysis conducted between fasters and non-fasters via Chi-square and Student *T* test, having previously set an acceptable level of significance of 5%. Overall lumbar spine osteoporosis incidence was as low as 9%, namely 4% of fasters and the—more than three times higher—14% of non-fasters (Chi-square, $p < 0.05$). The exactly opposite was observed for the right hips as 5% of fasters demonstrated femoral neck osteoporosis versus null of the non-fasters.

5. As for potassium, an inverse correlation detected for those within the range of sufficiency, i.e., 1600–2000 mg (32.7% of fasters vs 13.2% of non-fasters), despite the fact that daily intake over 2000 mg recorded the 50.9% of non-fasters and the 41.82% of fasters (X^2 and Mann–Whitney). (Table 1).
6. The corresponding MedDietScore was the same for the two groups: 30 (27–34) and 30 (27–33), respectively. The weight in the two groups did not differ, for faster was 75.5 kg (68.8–84.0) and for the non-fasters was 76.7 kg (66.5–84.3).

Table 1 Nutritional intakes.

Macro- and micronutrient intakes	Fasters (C.O.C. diet) ($n = 53$)		Non-fasters (Mediterranean diet) ($n = 55$)		
	Mean	Median	Mean	Median	*p* value
Proteins (g)	51.0	49.0	59.5	57.1	0.040
Total fat (g)	79.5	73.5	90.9	89.5	0.005
Polysaturated fat (g)	8.9	8.5	10.8	9.7	0.033
Dietary fibers (g)	18.0	17.0	25.0	22.0	0.005
B3 vitamin (% RDA)	56.5	42.0	74.8	64.0	0.004
B12 vitamin (mcg)	2.7	1.9	5.3	2.5	0.007
B12 vitamin (% RDA)	114.7	79.5	219.7	105.0	0.010
D3 vitamin (mcg)	1.9	0.9	5.3	2.3	0.001
D3 vitamin (% RDA)	20.1	9.5	36.8	21.0	0.002
E vitamin (% RDA)	46.0	40.5	65.3	48.5	0.029
Folic acid (%RDA)	44.5	40.0	57.5	48.0	0.001
Calcium (%RDA)	565	511	763	686	0.014
Calcium (mg)	48.2	42.5	64.4	57.0	0.016
Ferrum (mg)	8.5	7.9	11.0	9.4	0.021
Magnesium (mg)	163.3	159.0	216.2	186.7	0.024
Manganese (%RDA)	53.5	42.0	71.3	59.0	0.013
Manganese (mg)	1.1	0.9	1.5	1.2	0.024
Phosphorus (%RDA)	110.6	105.0	138.3	126.0	0.027
Phosphorus (mg)	785.0	735.0	971.0	880.0	0.026
Potassium (1.6–2.0 g)	32.7%		13.2%		0.016
Selenium (%RDA)	72.2	65.0	99.5	78.0	0.035
Selenium (mg)	40.6	34.4	55.9	43.0	0.027

In contrast to the above, the blood levels of all nutrients did not show statistically significant differences between the two study groups.

Discussion

The present study investigated the possible effects of the M.C.O.C. diet on musculoskeletal metabolism, particularly on bone density in individuals over 50 years and in post-menopausal women. Fasting, for Orthodox Christianity, is the consumption of "dry food" with no meat, fish, eggs, dairy, oil, or wine, once a day, and even at 9 o'clock according to the ecclesiastical timetable of the Holy Sacraments (around 3:00 p.m.). In fact, this nutritional habit is a variation of the typical Mediterranean diet with the approved beneficial effects on the human musculoskeletal system [10]. It consists of bread, fruit, legumes, nuts, seafood, snails, and vegetables during the fasting periods [13], resembling to a vegetarianism variant or Dietary Restriction type.

The existence of cyclic alternations of fasting periods with those of restriction-free on food consumption is the main difference from the classic Mediterranean pattern. In addition, the same restrictions do not apply to all fasts of the year. For example, during Christmas fasting, fish can be consumed all days except Wednesdays, Fridays, and the final week until Christmas Eve.

From a spiritual point of view, fasting in combination with self-restraint (i.e., limitation of quantities consumed) helps believers to appease their passions, strengthen their morale and cleanse their bodies from the toxins, to wit, this behavior is not a mere abstinence from certain types of food, but also a "physical exercise" with mental benefits [14].

The majority of participants were overweight and obese, as the proportion of normal BMI subjects was 16% and 25% for fasters and non-fasters respectively, with more than half being above the upper-normal waist circumference levels (58.2% and 55%). These are in line with the observations of Sarri et al. where no BMI reduction observed in the largest part of the sample [15].

Everyone engaged a sub-caloric diet, as the minimum daily requirement of 1600 Kcal (IOM, 2005) reached by the 67.56% of fasters and the 37.14% of non-fasters. Regarding men, 83.3% and 88.8% respectively, reached the threshold of 2000 Kcal, all consistent with relevant published data [16].

There was evidence of a low-protein intake in about 60% of individuals; 65.4% of the fasters and 58.5% of the non-fasters reported an average daily intake below 0.8 g/kg BW as 0.8–1.5 g/kg BW is the range of sufficiency for adequate BMD [17]. These findings confirm previously published data [18]. However, calculations in mass units, daily protein intake provided evidence of sufficiency, as over 25 g/24 h recorded the 94.5% and 98.1% respectively.

It has been reported that high protein intake is a risk factor for osteoporosis as the increase of the acidic "load" promotes catabolism of calcium phosphate crystals (including those of hydroxyapatite), resulting to increased blood calcium and hypercalciuria [19]. On the other hand, reduction of dietary protein can cause decreased calcium absorption, leading to secondary hyperparathyroidism [20]. In our study, both blood calcium levels as the relevant biochemical markers were within the normal range.

The Institute of Medicine recommends 800 mg/day calcium as estimated average requirement for men of 51 through 71 years old and 1000 mg/day for women over 51 and men aged 71 and older, while RDAs are 1000 and 1200 mg/day, respectively [21]. Our study showed that calcium ≤800 mg/24 h measured in 81.8% of fasters and in 62.26% of non-fasters. Calcium above 1000 mg/24 h recorded by 5.45% and 13.2%, respectively. Similarly, 1.85 and 11.3% noted daily calcium intake above 100% RDA, compatible with those already published [14, 22].

RDA percentage for phosphorus ranged to satisfactory levels, as the 100% achieved the >63% of fasters and the 68% of non-fasters. Indeed, unaltered levels of phosphorus intake have also reported in M.C.O.C. fasters [23].

Despite the differences in macro- and micronutrient intakes and the almost steady small amounts consumed by fasters, the blood tests did not show statistically significant fluctuations. Noteworthy, serum 25 (OH) D3 ranged at low levels as a daily intake of less than 100% RDA noted in 95% of fasters. Indeed, >20 ng/ml found in 29.51% of fasters and in 41% of non-fasters. Concerning B12, low intake levels were found in 40.7% and 54.7%, respectively, as for vitamin C in 64.8 and 56.6%, all the above similar to the already known data [15].

Alkaline phosphatase found to be within the normal range in 77.42% of fasters and among non-fasters, 80.64% in males and 18.3% in females. Menopause could explain the difference between women and men. Nevertheless, there was no laboratory evidence of bone catabolic activity.

Besides the M.C.O.C. fasts, relevant published data regarding other religious fastings are in line with our findings. In a comparative study between Catholic nuns and Buddhists following a hybrid vegan/lactovegetarian diet, no differences found between daily intakes and biochemical markers or even somatometric changes. The same work points to the "agreement" between other studies conducted on vegetarians of other "neighboring" doctrines such as Chinese and Korean Taoists and/or Protestants, such as Seventh Day Adventists [24]. Table 2 provides comparative data regarding fasting features among various doctrines.

DEXA measurements showed a statistically significant difference in favor of fasters; where the incidence of osteoporosis was 4% versus 14% of the non-fasters, although the overall 9% confirm the already known beneficial effect of the Mediterranean diet on bone metabolism. The statistically significant difference in bone density of the right hips versus the left ones attributed to right-handed participants and, therefore, not taken into account.

As mentioned, few studies have so far focused on the relationship between "religion-related" eating habits and bone density disorders. In one of those conducted in elder vegetarians, vegans, lactovegetarians, and omnivores, lower BMD found in femoral neck and Ward's triangle of vegetarians, as compared with the latter, but not in the spine [25]. These are consistent with our findings as well with antecedent studies as Lau at al. have reported. There was also agreement in correlations between daily intakes and somatometric data.

Several studies assess possible BMD changes through the prism of classical dietary patterns. As stated, though there is no statistically significant correlation between the Mediterranean diet and the bone metabolism's biochemical markers, it appears

Table 2 Concise features of the fasts of various religious doctrines.

	Eastern Christian Orthodoxy (C.O.C. fasting) [11–17, 23]	Christian Catholicism [33]	Seventh day adventism (protestantism)	Islam [34]	Buddhism/Taoism
Fasting type	Periodic fasting (abstinence of certain foods) on Mediterranean pattern, slightly CR	Hybrid (calorie restriction/ vegetarianism), not necessarily CR	Biblical diet and various types of veganism/vegetarianism/semi-vegetarianism (pesco-)/CR [24, 36]	Intermittent fasting (time-restricted feeding)	Various types Buddhists' Temple food: vegetarianism/semi-vegetarianism (lacto-/ ovo-), not necessarily CR [24] Taoists' bigu diet: IF-to-CR [37]
Annual duration	180 ± 19 days	40 days	Throughout the year [36]	One month	Throughout the year [24]
Abstinence of	Meat, fish, eggs, dairies, oil, wine	Meat	Pigs, shellfish, vultures/meat [38]	Food and water	Meat (Taoists and Buddhists), five pungent vegetables, alcohol, high amounts of processed food (Buddhists), [24] grain (Taoists) [37]
Physical activity	Regular (no restriction)	Regular (no restriction)	Sabbatarianism (24 h drawback) [38, 39]	Regular (no restriction)	Reduced (prayer)-to-regular
BMI	Increased	Normal-to-high	Low-to-normal [36, 38]	Normal [34]- to-high [35]	Normal [37]-to-high, higher than omnivores (i.e., Koreans) [24]
Bone Health	Positive impact	No negative impact	Positive impact [38, 40]	Controversial (beneficial on PTH and Calcium [34] /early osteoporosis [41])	Controversial (no negative impact/ osteoporosis) [25, 42]

that adoption of these dietary habits ensures optimum bone density with minimization of the fracture risk [10].

Other studies examine the effect of dietary interventions for specific purposes, e.g., weight loss in obesity. A recent study examining the effect of Calorie Restriction versus Intermittent Fasting and vegetarian/vegan patterns has concluded that BMD—but not bone quality—is negatively affected mainly in vegans since they do not exercise systematically, as compared with omnivores [26]. There is no data on the level of physical activity in this document, and this is a limitation.

A similar meta-analysis indicates that in relation to meat-eaters, vegetarians and vegans as well demonstrate a correspondingly lower BMD as well as a fracture risk of 32% [27]. Again, the lack of FRAX data did not allow conclusions to be drawn, and this is another one limitation.

On ending up the attempts for comparative evaluation of our study's findings, a mention should be made to the valid CALERIE study. Despite the methodological differences (i.e., study design, inclusion/exclusion criteria) that exceeded the obvious similarities such as bone health/metabolism [28], this comparison enhanced the importance of our conclusions.

Indeed, while BMD results from CALERIE ranged from non-statistically significant increases in obese young adults [29] to almost unchanged in nonobese young adults and obese elders [30], our study showed a statistically significant increase in BMD in overweight and obese men and postmenopausal women aged 50+ years without the use of dietary supplements and despite low levels of vitamin D3.

According to the anthropology of nutrition, geographic peculiarities determine the availability of raw materials, forming the basic dietary pattern [31]. So far, C.O.C. diet in Mediterranean countries seems to be unique among other C.O.C. nations around the world with regard to antioxidant load and detoxification potential of the body especially during the non-fasting periods as there are time-intervals where the antioxidant consumption overrides the low consumption of animal fats. For example, the "north-European" Russians who consume more animal fat than the "southern" Greeks do. However, there is flexibility in choosing through a "wide" group of foods, e.g., of vegetable/animal origin with some exceptions (fish, oil, wine). The latter becomes important, as these foods are beneficial for the prevention or treatment of osteoporosis, as modern therapies with fewer drugs propose [32]. Incidentally, the lack of data on oxidative stress is another limitation, as low-protein intake does not exclusively represent the total oxidative potential.

The strength of this study is that it contemplates a less-studied eating habit in the light of the musculoskeletal metabolism and osteoporosis in a crucial age of over 50, and especially in menopausal women. This is, as it seems, a novelty in the field of Nutrition and Orthopedics.

Conclusion

The M.C.O.C. diet based on the Mediterranean pattern has a favorable effect bone metabolism and bone density. The - overall low - prevalence of osteoporosis in the Mediterranean diet was over the threefold that of the M.C.O.C. diet. It turns out that abstinence from dairy products and meat does not affect bone density or iron levels and generally does not adversely affect musculoskeletal metabolism.

There were also significant differences in daily consumption—excluding potassium—of proteins, fats (including polyunsaturated), dietary fibers, vitamins (B3, B12, folate, D3, E) and minerals (calcium, manganese selenium, iron, phosphorus) in favor of traditional Mediterranean diet. However, the "periodic" M.C.O.C. diet, which helps in cell detoxification and—potentially—in "hormonal" balance, seems to be a healthy lifestyle habit, along with the low incidence of smoking in fasters.

In conclusion, the periodic restriction of dietary intake of animal origin into a slightly hypocaloric diet, combined with increased consumption of plant foods of all "colors", seems to affect beneficially the musculoskeletal metabolism, perhaps better than the exclusive consumption of dietary supplements. Future research expected to enrich our knowledge in this unique nutritional model.

Acknowledgements We are grateful to Metropolitan Varnavas of Neapolis and Stavroupolis, Thessaloniki; Metropolitan Georgios of Kitros, Katerini, and Platamon; Hieromonk Father Luke Kipouros of Holy Trinity Monastery, Panorama, Thessaloniki; Nun, Melani Chatzipatera, Mother Superior of Assumption of Virgin Monastery, Panorama, Thessaloniki; Professor Emeritus of the School of Theology, Aristotle University of Thessaloniki, Dimitrios Tselegidis; Archimandrite Nikodemos Skrettas-Plexidas; and Archpriest Athanasios Gikas; Professors of the School of Pastoral and Social Theology, Aristotle University of Thessaloniki, for their help in collecting the study sample.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. WHO (2014) Interesting facts about ageing. https://www.who.int/ageing/about/facts/en/
2. Sambrook P, Cooper C. Osteoporosis. Lancet. 2006;367:2010–8. https://doi.org/10.1016/S0140-6736(06)68891-0
3. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. J Bone Min Res. 2007;22:465–75. https://doi.org/10.1359/jbmr.061113
4. Kanis JA, McCloskey EV, Johansson H, Cooper C, Rizzoli R, Reginster JY. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Inter. 2013;24:23–57. https://doi.org/10.1007/s00198-012-2074-y
5. Lang PO, Michel JP, Zekry D. Frailty syndrome: a transitional state in a dynamic process. Gerontology. 2009;55:539–49. https://doi.org/10.1159/000211949
6. Rockwood K, Mitnitski A. Frailty in relation to the accumulation of deficits. J Gerontol. 2007;62A:722–7. https://doi.org/10.1093/gerona/62.7.722
7. Van Bokhorst-de van der Schueren MAE, Lonterman-Monasch S, de Vries OJ, Danner SA, Kramer MHH, et al. Prevalence and determinants for malnutrition in geriatric outpatients. Clin Nutr. 2013;32:1007–11. https://doi.org/10.1016/j.clnu.2013.05.007
8. Frisoli A, Chaves PH, Ingham SJM, Fried LP. Severe osteopenia and osteoporosis, sarcopenia, and frailty status in community-dwelling older women: results from the Women's Health and Aging Study (WHAS) II. Bone. 2011;48:952–7. https://doi.org/10.1016/j.bone.2010.12.025
9. Higgs J, Derbyshire E, Styles K. Nutrition and osteoporosis prevention for the orthopaedic surgeon: A wholefoods approach. EFORT Open Rev. 2017;2:300–8. https://doi.org/10.1302/2058-5241.2.160079
10. Romero Pérez A, Rivas Velasco A. Adherence to Mediterranean diet and bone health. Nutr Hosp. 2014;29:989–96. https://doi.org/10.3305/nh.2014.29.5.7332
11. Chliaoutakis JE, Drakou I, Gnardellis C, Galariotou S, Carra H, Chliaoutaki M. Greek Christian orthodox ecclesiastical lifestyle: could it become a pattern of health-related behavior. Preventive Med. 2002;34:428–35. https://doi.org/10.1006/pmed.2001.1001
12. Panagiotakos DB, Pitsavos C, Stefanidis C. Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis. 2006;16:559–68.
13. Sarri KO, Tzanakis NE, Linardakis MK, Mamalakis GD, Kafatos AG. Effects of Greek orthodox christian church fasting on serum lipids and obesity. BMC Public Health. 2003;3:1–8. https://doi.org/10.1186/1471-2458-3-16
14. Papadaki A, Vardavas C, Hatzis C, Kafatos A. Calcium, nutrient and food intake of Greek Orthodox Christian monks during a fasting and non-fasting week. Public Health Nutr. 2008;11:1022–9. https://doi.org/10.1017/S1368980007001498
15. Sarri KO, Linardakis MK, Bervanaki FN, Tzanakis NE, Kafatos AG. Greek Orthodox fasting rituals: a hidden characteristic of the Mediterranean diet of Crete. BJN. 2004;92:277–84. https://doi.org/10.1079/bjn20041197
16. Sarri KO, Kafatos AG, Higgins S. Is religious fasting related to iron status in Greek Orthodox Christians? BJN. 2005;9:198–203. https://doi.org/10.1079/bjn20051472
17. Sarri K, Bertsias G, Linardakis M, Tsibinos G, Tzanakis N, Kafatos A. The effect of periodic vegetarianism on serum retinol and??-tocopherol levels. Int J Vitam Nutr Res. 2009;79:271–80. https://doi.org/10.1024/0300-9831.79.5.271
18. Darling AL, Millward DJ, Torgerson DJ, Hewitt CE, Lanham-New SA. Dietary protein and bone health: a systematic review and meta-analysis. Am J Clin Nutr. 2009;90:1674–92. https://doi.org/10.3945/ajcn.2009.27799
19. Kerstetter JE, O'Brien KO, Insogna KL. Dietary protein, calcium metabolism, and skeletal homeostasis revisited. Am J Clin Nutr. 2003;78:584S–592S. https://doi.org/10.1093/ajcn/78.3.584S
20. Kerstetter JE, O'Brien KO, Caseria DM, Wall DE, Insogna KL. The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. J Clin Endocrinol Metab. 2005;90:26–31. https://doi.org/10.1210/jc.2004-0179
21. Ross AC, Manson JAE, Abrams SA, Aloia JF, Brannon RM, Clinton SK, et al. The 2011 report on dietary reference intakes for

calcium and vitamin D from the Institute of Medicine: What clinicians need to know. J Clin Endocrinol Metab. 2011;96:53–58. https://doi.org/10.1210/jc.2010-2704
22. Rizzoli R, Abraham C, Brandi M-L. Nutrition and bone health: turning knowledge and beliefs into healthy behaviour. Curr Med Res Opin. 2014;30:131–41. https://doi.org/10.1185/03007995.2013.847410
23. Sarri K, Linardakis M, Codrington C, Kafatos A. Does the periodic vegetarianism of Greek Orthodox Christians benefit blood pressure? Preventive Med. 2007;44:341–8. https://doi.org/10.1016/j.ypmed.2006.11.009
24. Lee Y, Krawinkel M. Body composition and nutrient intake of Buddhist vegetarians. Asia Pac J Clin Nutr. 2009;18:265–71.
25. Lau EMC, Kwok T, Woo J, Ho SC. Bone mineral density in Chinese elderly female vegetarians, vegans, lacto-vegetarians and omnivores. Eur J Clin Nutr. 1998;52:60–64. https://doi.org/10.1038/sj.ejcn.1600516
26. Veronese N, Reginster J-Y. The effects of calorie restriction, intermittent fasting and vegetarian diets on bone health. Aging Clin Exp Res. 2019;31:1–6. https://doi.org/10.1007/s40520-019-01174-x
27. Iguacel I, Miguel-Berges ML, Gómez-Bruton A, Moreno LA, Julián C. Veganism, vegetarianism, bone mineral density, and fracture risk: A systematic review and meta-analysis. Nutr Rev. 2019;77:1–18. https://doi.org/10.1093/nutrit/nuy045
28. Rickman AD, Williamson DA, Martin CK, Gilhooly CH, Stein RI, Bales CW, et al. The CALERIE study: design and methods of an innovative 25% caloric restriction intervention. Contemp Clin Trials. 2011;32:874–81. https://doi.org/10.1016/j.cct.2011.07.002
29. Redman LM, Rood J, Anton SD, Champagne C, Smith SR, Ravussin E. Pennington Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) Research Team. Calorie restriction and bone health in young, overweight individuals. Arch Intern Med. 2008;168:1859–66. https://doi.org/10.1001/archinte.168.17.1859
30. Villareal DT, Fontana L, Das SK, Redman L, Smith SR, Saltzman E, et al. Effect of two-year caloric restriction on bone metabolism and bone mineral density in non-obese younger adults: a randomized clinical trial. J Bone Min Res. 2016;31:40–51. https://doi.org/10.1002/jbmr.2701
31. Mintz SW, Du Bois CM. The anthropology of food and eating. Ann Rev Anthropol. 2002;31:99–119. https://doi.org/10.1146/annurev.anthro.32.032702.131011
32. Liu H, Bian W, Liu S, Huang K. Selenium protects bone marrow stromal cells against hydrogen peroxide-induced inhibition of osteoblastic differentiation by suppressing oxidative stress and ERK signaling pathway. Biol Trace Elem Res. 2012;150:441–50. https://doi.org/10.1007/s12011-012-9488-4
33. Sarkic N, López JH, López-costas O, Anglade AG. Eating in silence: isotopic approaches to nuns' diet at the convent of Santa Catalina de Siena (Belmonte, Spain) from the sixteenth to the twentieth century. Archaeological Anthropological Sci. 2019;11:3895–911. https://doi.org/10.1007/s12520-018-0734-3
34. Bahijri SM, Ajabnoor GM, Borai A, Al-aama JY, Chrousos GP. Effect of ramadan fasting in Saudi Arabia on serum bone profile and immunoglobulins. Ther Adv Endocrinol Metab. 2015;6:223–32. https://doi.org/10.1177/2042018815594527
35. El-Desouki MI, R.A. Sulimani RA. High prevalence of osteoporosis in Saudi men. Saudi Med J. 2007;28:774–7. https://www.researchgate.net/profile/Riad_Sulimani/publication/6372636_High_prevalence_of_Osteoporosis_in_Saudi_men/links/00b49529ad7fb9b766000000.pdf
36. Le LT, Sabaté J. Beyond meatless, the health effects of vegan diets: findings from the adventist cohorts. Nutrients. 2014;6:2131–47. https://doi.org/10.3390/nu6062131
37. Zhonghua XUE. Explore the role and function of bigu on taoism health preservation. 2015;11:90–93. https://doi.org/10.3968/7639
38. Banta JE, Lee JW, Hodgkin G, Yi Z, Fanica A, Sabate J. The Global Influence of the Seventh-Day Adventist Church on Diet. Religions. 2018;9: 251. https://doi.org/10.3390/rel9090251.
39. Lousuebsakul-Matthews V, Thorpe D, Knutsen R, Beeson WL, Fraser GE, Knutsen SF. Impact of walking and running on the heel bone: the adventist health study-2. J Sci Res Rep. 2015;7:165–177. https://doi.org/10.9734/JSRR/2015/17962
40. Thorpe DL, Knutsen SF, Beeson WL, Rajaram S, Fraser GE. Effects of meat consumption and vegetarian diet on risk of wrist fracture over 25 years in a cohort of peri- and postmenopausal women. Public Health Nutr. 2008;11:564–72. https://doi.org/10.1017/S1368980007000808
41. Sadat-Ali M, Al-Habdan IM, Al-Turki HA, Azam MQ. An epidemiological analysis of the incidence of osteoporosis and osteoporosis-related fractures among the Saudi Arabian population. Ann Saudi Med. 2012;32:637–41. https://doi.org/10.5144/0256-4947.2012.637
42. Chiu J, Lan S, Yang C, Wang P, Yao W, Su I, et al. Long-term vegetarian diet and bone mineral density in postmenopausal Taiwanese women. Calcif Tissue Int. 1997;60:245–9. https://doi.org/10.1007/PL00005812

INTERNATIONAL JOURNAL OF FOOD SCIENCES AND NUTRITION
https://doi.org/10.1080/09637486.2020.1856795

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Check for updates

Dietary protein intake from different animal and plant sources plays a minor role in the bone health of adults with or without intermittent fasting for decades

Nikolaos E. Rodopaios[a], Vassilis Mougios[b], Alexandra-Aikaterini Koulouri[a], Eleni Vasara[c], Sousana K. Papadopoulou[d], Petros Skepastianos[e], Maria Hassapidou[d] and Anthony G. Kafatos[a]

[a]Department of Social Medicine, Preventive Medicine and Nutrition, Medical School, University of Crete, Heraklion, Greece; [b]Laboratory of Evaluation of Human Biological Performance, School of Physical Education and Sport Science at Thessaloniki, Aristotle University of Thessaloniki, Thessaloniki, Greece; [c]Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece; [d]Department of Nutritional Sciences and Dietetics, International Hellenic University, Thermi, Greece; [e]Department of Medical Laboratory Studies, International Hellenic University, Thermi, Greece

ABSTRACT

We examined whether bone health is related to protein intake from different sources by utilising a distinct, rare dietary pattern: avoidance of animal foods for approximately half of the year according to Christian Orthodox Church fasting. Four-hundred adults, of whom 200 had been following religious fasting for a median of 15 years and 200 were non-fasters, underwent anthropometry, measurements of bone mineral density (BMD) and bone mineral content (BMC), and completed a food frequency questionnaire. Groups did not differ significantly in anthropometric measures, BMD, or BMC. Fasters had higher consumption of seafood and lower consumption of red meat, poultry-eggs, dairy products, and grains-cereals than non-fasters. Protein intake from these food groups exhibited similar differences; overall, fasters had lower protein intake than non-fasters. BMD and BMC were positively, though weakly, correlated with red meat and poultry-egg consumption. Thus, protein intake seems to play a minor (if any) role in bone health.

ARTICLE HISTORY
Received 18 September 2020
Revised 21 November 2020
Accepted 22 November 2020

KEYWORDS
Bone mineral content; bone mineral density; dairy; grains; meat; periodic fasting

Introduction

The integrity and health of osseous tissue are important for the overall health, well-being, quality of life, and longevity of an individual. Osteoporosis, on the other hand, by augmenting bone fragility and susceptibility to fracture, is associated with considerable pain, impaired quality of life, disability, and even death (Pisani et al. 2016; Tarrant and Balogh 2020). Justifiably then, numerous studies have sought to elucidate how bone status can be improved.

Among the factors that may have an impact on skeletal health and integrity, dietary protein intake remains one of the most controversial. This is partly due to the presence of several conflicting factors that, at least in theory, may lead to negative or positive effects of dietary protein on bone status (Dolan and Sale 2019). Dietary protein has been shown to be a powerful regulator of calcium metabolism (Kerstetter et al. 1997). On one hand, a high protein intake may increase the renal acid load, which induces calcium loss from bone to neutralise pH and promotes bone demineralisation (Carnauba et al. 2017). On the other hand, dietary protein is needed for bone formation, since protein constitutes approximately one quarter of human bone mass (Boskey 2013), upregulates anabolic hormones and growth factors with osteogenic action (Bonjour 2016), and promotes muscle growth (Witard et al. 2016), thus indirectly benefiting bone by placing increased mechanical load on it. Moreover, increased dietary protein increases intestinal calcium absorption (Kerstetter et al. 2003; Kerstetter et al. 2005; Calvez et al. 2012), thus counteracting the potentially negative effect of acidification.

The aforementioned conflicting factors may explain the variable findings of studies that have examined the association of protein intake with bone health and integrity. As an example, a recent meta-analysis has found a small positive effect of high protein intake on bone mineral density (BMD) at one skeletal site but not at other sites (Shams-White et al. 2017). Analysis of the association between dietary patterns

CONTACT Nikolaos E. Rodopaios nikow1966@yahoo.gr Protomagias 25, Evosmos, Thessaloniki, 56224, Greece
Supplemental data for this article can be accessed at https://doi.org/10.1080/09637486.2020.1856795.

characterised by consumption of different protein sources (such as meat vs fish) has also produced variable findings, as detailed in Discussion section (Perna et al. 2017).

One such dietary pattern is the periodic fasting advocated by the Christian Orthodox Church (COC), which consists in avoidance of certain foods (mainly of animal origin except seafood and snails) for certain periods and days (totalling approximately half of the year) and shares many characteristics with the Mediterranean diet (Rodopaios et al. 2019). As such, COC fasting is low in red meat, poultry, eggs, and dairy products, while being high in seafood. Foods predominating in the fasters' diet during fasting days include cereals, pulses, vegetables, and fruits. Foods predominating in the fasters' diet during non-fasting days and in the non-fasters' diet at all times include milk, cheese, and dishes of all sorts based on red meat, whereas consumption of vegetables and fruits is lower.

Because of its particularly demanding character, full adherence to COC fasting rules is nowadays rare. We have recently shown that men and women fully adhering to COC fasting for decades, whether being older (Rodopaios et al. 2019) or younger (Rodopaios et al. 2020), did not differ in indices of bone integrity, such as BMD, bone mineral content (BMC), and prevalence of osteopenia, osteoporosis, and bone fracture, from non-fasting counterparts.

The aim of the present work was to contribute to the clarification of the role of dietary protein in bone integrity and health. To this end, we took advantage of the aforementioned lack of differences in bone status between fasters and non-fasters to investigate whether protein intake or source differed or not between groups in the total sample of our two previous studies (Rodopaios et al. 2019, 2020). The former possibility (differences in protein intake or source despite no differences in bone status) would not favour a role of protein in bone integrity or health, whereas the latter possibility (no differences in protein intake or source, paralleled by no differences in bone status) would leave such a role open.

Materials and methods

Participants and study period

This cross-sectional study included 400 adults, equally divided between fasters and non-fasters. The study was conducted between September 2013 and October 2015, with each participant being assessed once. Participants were recruited on a voluntary basis from the Aristotle University of Thessaloniki, the (then) Alexander Technological Educational Institution of Thessaloniki (presently International Hellenic University), churches, monasteries, and public centres for the elderly, all located in the province of Thessaloniki, Greece. Of the 454 individuals who consented to participate, 40 were excluded because, at their first visit, they were found not to meet the inclusion criteria, which were the absence of chronic disease, absence of morbid obesity (BMI $\geq 40\,kg/m^2$), no dietary supplementation, and no medication. Additionally, to qualify as fasters, they were needed to declare full adherence to COC fasting without interruption for at least the past 10 years or (for those who were 18 or 19 years old) from the age of 10; to qualify as non-fasters, they were needed to declare no avoidance of any food.

Fourteen more individuals were excluded because they did not complete all the measurements described below, thus leaving 400 participants (200 fasters and 200 non-fasters). The fasters included 131 women and 69 men; the non-fasters included 126 women and 74 men. The participant flow diagram is shown in Figure 1.

Full adherence to COC fasting requires avoidance of foods of animal origin (except seafood and snails) during five periods of the year (totalling 101–131 days, depending on when Easter falls) and allows unrestricted eating during two other periods (totaling 47 days). The remainder of the year consists in "moderate" fasting, that is, avoidance of animal source foods, oil, and wine on Wednesdays and Fridays only. The total days of fasting range from 159 to 197 (average, 178). To ensure that the data regarding the fasters were as representative of the entire year as possible,

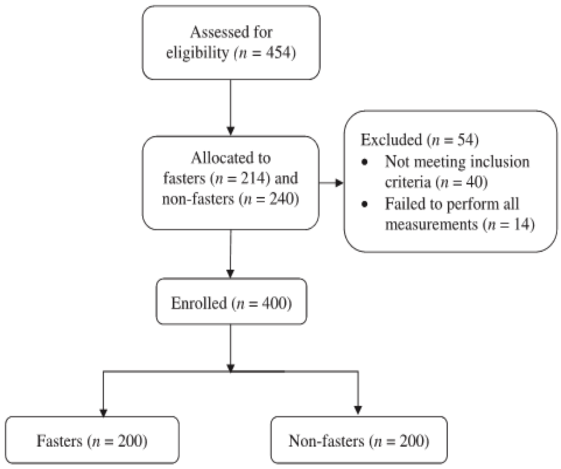


Figure 1. Participant flow diagram of the study.

no measurements were performed during the five periods of fasting or the two periods of no food restriction. Rather, all measurements, interviews, and blood sampling were performed during periods of moderate fasting, as presented in detail in Supplemental Table 1.

Anthropometric parameters

Body weight was measured on a digital scale to the nearest 0.1 kilogram with the participant wearing light clothing and no shoes. Standing height was measured with a portable stadiometer to the nearest centimetre.

Menstrual history and contraceptive use

The female participants were interviewed about whether they had regular (21–35 days) or irregular menstrual cycle, whether they had entered menopause and, if so, at what age. Additionally, they were asked whether they were using oral contraceptives.

Bone status parameters

BMD and BMC were evaluated using dual-energy X-ray absorptiometry (Lunar DPX Bravo, GE Healthcare, equipped with the GE Lunar enCORE software v. 13.5) at the lumbar spine (L2-L4), right hip, left hip, right femoral neck, and left femoral neck. Subjects were also asked about any history of bone fracture.

Dietary assessment

The participants' dietary habits were assessed through a validated semi-quantitative food frequency questionnaire (Papadopoulou et al. 2008) comprising 14 food groups (red meat; poultry and eggs; seafood; dairy products; pulses; grains and cereals; nuts and seeds; vegetables; fruits; oils and fats; sweets; beverages; alcoholic drinks; and composite dishes), which was administered by a certified dietitian. Participants were asked to report the frequency of consumption of foods in portions per week on average over the past month. Questionnaire data were then used to calculate the consumption of the following protein sources: red meat; poultry and eggs; seafood; dairy products; pulses; and grains and cereals. Portion sizes were set according to the National Nutrition Guide for Greek Adults (Institute of Preventive Medicine Environmental and Occupational Health 2014) and are presented in Table 1. Protein intake was assessed on the basis of the protein content of these foods (Food Standards Agency 2002; Trichopoulou 2004). It should be noted that grains and cereals were included in the list despite their rather low protein contents because they made a considerable quantitative contribution to the total protein intake of the participants, as will be shown under Results section.

The food frequency questionnaire was also used to assess alcohol intake in drinks per week.

Biochemical parameters

Blood samples were drawn into test tubes without anticoagulant from a forearm vein in sitting position between 8 and 10 a.m., after 12 hours of fast. Samples were left to clot at room temperature and centrifuged at 1,500 *g* for 10 minutes. Serum was removed and analysed for urea, creatinine, and uric acid through the urease – glutamate dehydrogenase, sarcosine oxidase, and uricase-peroxidase methods, respectively, in a Mindray BS-300 Chemistry Analyser. The coefficients of variation for the three analytes were 3%, 3%, and 1%, respectively, and the laboratory carrying out the measures participated in a nationwide external quality control program.

Table 1. Portion sizes and protein content of the foods whose intake was assessed in the study.

Food group	Foods	Portion size (g)	Protein content (g/portion)
Red meat	Beef, pork, lamb, goat, rabbit, game (cooked)	140	35.0
	Cold cuts	30	5.2
Poultry and eggs	Chicken, turkey (cooked)	140	36.0
	Eggs	60	7.4
Seafood	Fish, molluscs, shellfish (cooked)	140	21.0
Dairy	Milk, yogurt	240	11.3
	cheese	30	5.2
	dairy cream	120	3.8
Pulses	Beans, lentils, soybeans (cooked)	180	24.5
Grains and cereals	Bread	40	3.7
	Rusks	60	6.1
	Bagels, breadsticks, pita bread	30	2.7
	Pasta, rice, frumenty (cooked)	220	7.3
	Sweet corn (cooked)	50	1.4
	Breakfast cereals	35	3.2

Physical activity and smoking habits

Subjects were asked to rank their habitual physical activity as very low, low, moderate, high, or very high on a 5-point scale. Additionally, they were asked what type of physical activity they practiced to let us identify high-intensity/impact weight-bearing exercises that better promote BMD (Xu et al. 2016). Finally, they were interviewed about their smoking habits; those smoking one or more cigarettes daily were considered smokers.

Ethics

The study protocol was approved by the Bioethics Committee of the Alexander Technological Educational Institute of Thessaloniki, and the study was conducted according to the Declaration of the World Medical Association of Helsinki (1989). Each participant was informed about the aims, benefits, and potential risks of the study and signed a consent form before data collection and blood sampling.

Statistical analysis

The Kolmogorov–Smirnov test and histogram charts were used to assess the normality of distribution of continuous variables. The distribution of most variables differed significantly from normal in at least one of the two groups (fasters or non-fasters). Thus, for the sake of uniformity, we report all continuous variables as median (interquartile range) and compared groups by using the non-parametric Mann–Whitney *U* test throughout. The few variables that warranted a *t* test did not produce a different outcome from the Mann–Whitney *U* test. Nominal variables were tested through the χ^2 test. Correlation analysis was performed by determining Spearman's ρ correlation coefficient.

Statistical analysis was performed using the SPSS version 25 (SPSS, Chicago, IL). All statistical tests and corresponding *p* values were two-sided, and statistical significance was declared at $p < 0.05$.

Results

Demographic characteristics

Fasters were 43.0 (26.7–58.2) years old (ranging from 18.2 to 77.6). They had been observing COC fasting for 15 (10–32) years, starting at the age of 15 (10–26). Non-fasters were 43.0 (24.0–56.5) years old (ranging from 18.0 to 77.3).

Menstrual history and contraceptive use

In the fasting group, 53 women had regular menstrual cycle, 10 had irregular menstrual cycle, and 68 were postmenopausal (totaling 131). The corresponding values in the non-fasting group were 42, 18, and 66 (totaling 126). Menopause had occurred at the ages of 50 (45–52) and 49 (47–50), respectively. The two groups did not differ in any of the aforementioned parameters ($p > 0.05$). Only two fasters (and none of the non-fasters) were taking oral contraceptives.

Anthropometric parameters

Body weight of fasters and non-fasters was 72.9 (62.9–81.4) kg and 71.2 (60.5–84.0) kg, respectively. The corresponding values were 1.66 (1.60–1.73) m and 1.66 (1.61–1.74) m for height; and 26.6 (23.0–29.5) kg/m^2 and 25.6 (22.7–29.1) kg/m^2 for BMI. The two groups did not differ in any anthropometric parameter ($p > 0.05$).

Bone health parameters

The two groups did not differ significantly in BMD (Figure 2) or BMC (Figure 3) at the lumbar spine, right hip, left hip, right femoral neck, or left femoral neck. Thirty-five fasters (17.5%) and 33 non-fasters (16.5%), all women, reported that they had had bone fractures (non-significant difference). These had occurred in the upper limbs (17 in each group), lower limbs (17 and 13, respectively), and shoulder (one and three, respectively).

Dietary parameters

Table 2 presents the weekly frequency of consumption of protein sources by fasters and non-fasters. The latter had significantly higher consumption of red meat, poultry-eggs, dairy products, and grains-cereals, whereas the former had higher consumption of seafood. The same differences between groups were found in daily protein intake (Table 3). In addition, the sum of protein intake from these sources was higher in non-fasters. In both groups, dairy and grains-cereals were the major protein sources, followed, in sequence, by red meat, poultry-eggs, seafood, and pulses.

Weekly alcohol consumption was 0 (0–2) drinks in fasters and 2 (0–6) drinks in non-fasters ($p < 0.001$).

Biochemical parameters

In agreement with the higher meat and protein intakes by non-fasters, their serum urea, creatinine,

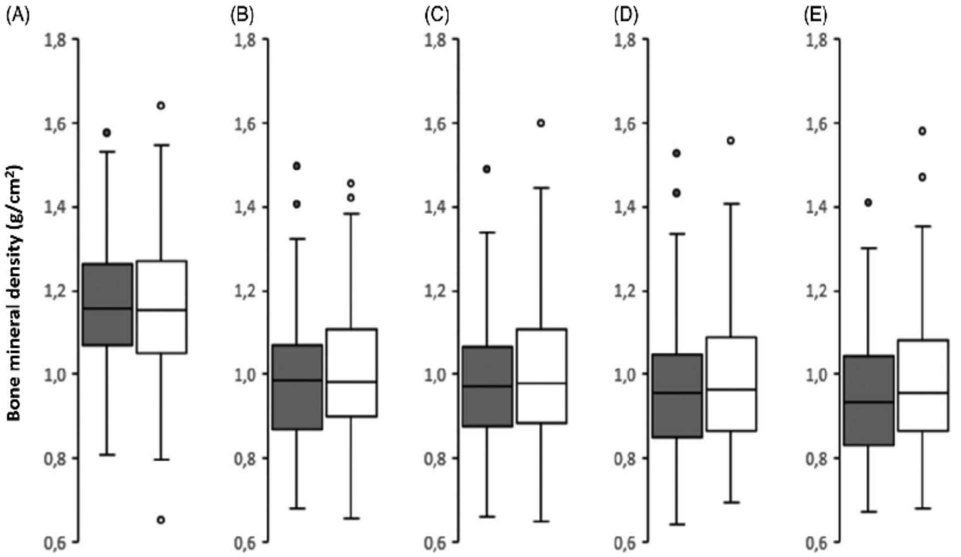


Figure 2. Box plots of bone mineral density at the lumbar spine (A), right hip (B), left hip (C), right femoral neck (D), and left femoral neck (E) of fasters (gray boxes) and non-fasters. See Figure 1 for description of the graph elements. No significant difference between groups was found (Mann–Whitney U test, $p > 0.05$).

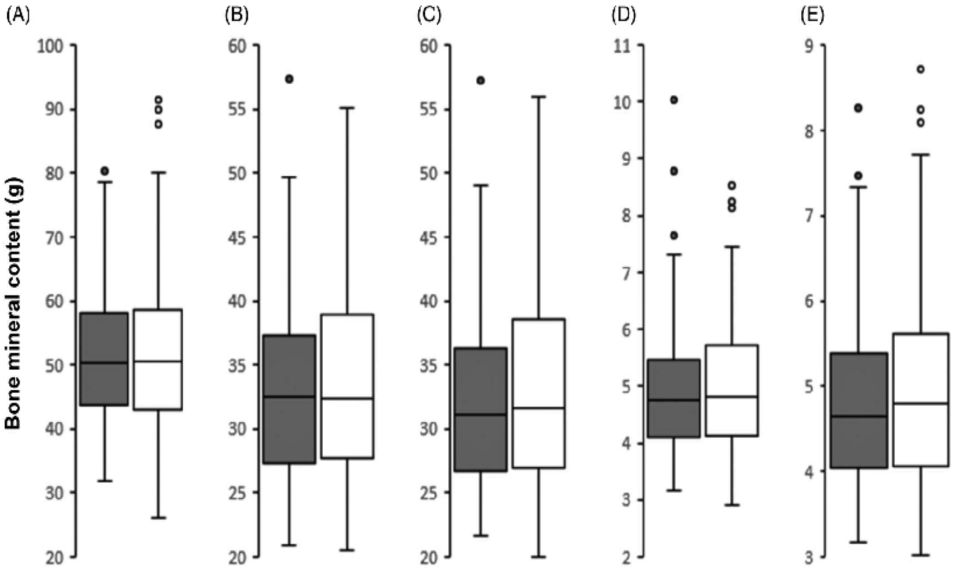


Figure 3. Box plots of bone mineral content at the lumbar spine (A), right hip (B), left hip (C), right femoral neck (D), and left femoral neck (E) of fasters (gray boxes) and non-fasters. See Figure 1 for description of the graph elements. No significant difference between groups was found (Mann–Whitney U test, $p > 0.05$).

Table 2. Frequency of consumption of food groups that are good protein sources by the participants of the study (portions/week).

Food group	Fasters ($n = 200$)	Non-fasters ($n = 200$)	p Value
Red meat	2.0 (1.0–3.5)	3.0 (1.5–4.5)	<0.001
Poultry and eggs	3.5 (2.5–5.5)	4.5 (3.0–7.0)	0.001
Seafood	2.0 (1.0–2.5)	1.5 (1.0–2.0)	0.010
Dairy	11.5 (7.5–17.9)	15.0 (11.5–21.5)	<0.001
Pulses	1.5 (1.5–1.5)	1.5 (1.5–1.5)	0.050
Grains and cereals	19.0 (15.0–27.0)	21.8 (15.5–30.4)	0.017

and uric acid concentrations were numerically higher compared to fasters (Table 4), although only the difference in urea reached statistical significance.

Physical activity and smoking habits

Fasters did not differ from non-fasters in habitual physical activity level or percentage of subjects practicing high-intensity/impact weight-bearing exercises ($p > 0.05$). Fasters smoked considerably less than non-fasters, since only 10 fasters were smokers, as compared to 58 non-fasters ($p < 0.001$). Fasters did not differ from non-fasters in any bone status parameter.

Correlation between bone status and protein source

We examined whether BMD or BMC correlated with protein source independent of group (to increase the power of the analysis). Results are shown in Tables 5 and 6. Both BMD and BMC were positively correlated with red meat and poultry-egg consumption. Seafood consumption exhibited either negative or no significant correlation with the two bone parameters. Dairy consumption displayed only two positive correlations, whereas pulse and grain-cereal consumption was not correlated with any bone parameter. All significant correlations were weak, explaining only 1.0%–2.8% of the variation in bone parameters. Because of this and because of the lack of normal distribution in most of the bone and dietary parameters, we did not proceed to multiple regression analysis.

Table 3. Daily protein intake from food groups that are good protein sources by the participants of the study (g/kg body weight).

Food group	Fasters ($n = 200$)	Non-fasters ($n = 200$)	p Value
Red meat	0.12 (0.07–0.20)	0.16 (0.10–0.23)	<0.001
Poultry and eggs	0.11 (0.06–0.13)	0.13 (0.10–0.16)	<0.001
Seafood	0.09 (0.05–0.12)	0.08 (0.04–0.12)	0.031
Dairy	0.17 (0.10–0.27)	0.22 (0.14–0.32)	<0.001
Pulses	0.07 (0.06–0.10)	0.07 (0.06–0.09)	0.121
Grains and cereals	0.18 (0.12–0.26)	0.20 (0.14–0.29)	0.049
Sum	0.78 (0.63–1.02)	0.91 (0.72–1.18)	<0.001

Table 4. Serum concentrations of metabolites related to protein and meat intake in the participants of the study.

Metabolite	Fasters ($n = 200$)	Non-fasters ($n = 200$)	p Value
Urea (mg/dL)	27.0 (21.0–33.0)	30.0 (24.0–36.8)	0.001
Creatinine (mg/dL)	0.90 (0.83–1.03)	0.94 (0.83–1.06)	0.102
Uric acid (mg/dL)	4.3 (3.5–5.3)	4.5 (3.7–5.5)	0.343

Discussion

The present study examined the relationship of dietary protein intake and source with a number of parameters related to bone integrity and health (namely, BMD, BMC, and prevalence of bone fracture) of 400 men and women by taking advantage of a rare, a priori defined, dietary pattern (full adherence to religious fasting), characterised by periodic avoidance of foods from animal sources. Movassagh and Vatanparast (2017), in their scoping review stress that dietary-pattern approaches in the study of bone health take into account contributions from various aspects of the diet and can thus complement single-nutrient and single-food studies.

Fasters did not differ from non-fasters in demographic characteristics or anthropometric measures, thus providing a sound basis for comparing the main study outcomes between groups. The dietary analysis performed showed distinct differences between fasters and non-fasters in the consumption of the major protein sources, i.e. dairy products, grains-cereals, red meat, and poultry-eggs, all in favour of non-fasters. Conversely, fasters had higher consumption of seafood, a minor protein source in both groups. As a result of these differences, daily protein intake was

Table 5. Correlation between frequency of consumption of food groups that are good protein sources and bone mineral density at five sites in the participants of the study ($n = 400$).

	Lumbar	Right hip	Left hip	Right femoral neck	Left femoral neck
Red meat	ns	0.149 (0.003)	0.150 (0.003)	0.136 (0.006)	0.168 (0.001)
Poultry and eggs	ns	0.142 (0.004)	0.131 (0.009)	0.161 (0.001)	0.139 (0.005)
Seafood	ns	−0.106 (0.034)	−0.122 (0.015)	−0.132 (0.008)	−0.160 (0.001)
Dairy	ns	ns	ns	ns	ns
Pulses	ns	ns	ns	ns	ns
Grains and cereals	ns	ns	ns	Ns	ns

Values are Spearman's ρ, followed by p value in parentheses. ns: not significant ($p > 0.05$).

Table 6. Correlation between frequency of consumption of food groups that are good protein sources and bone mineral content at five sites in the participants of the study ($n = 400$).

	Lumbar	Right hip	Left hip	Right femoral neck	Left femoral neck
Red meat	0.136 (0.006)	0.156 (0.002)	0.150 (0.003)	0.159 (0.001)	0.152 (0.002)
Poultry and eggs	0.109 (0.029)	0.110 (0.028)	0.100 (0.046)	0.162 (0.001)	0.143 (0.004)
Seafood	ns	ns	ns	ns	ns
Dairy	ns	ns	ns	0.115 (0.021)	0.101 (0.044)
Pulses	ns	ns	ns	ns	ns
Grains and cereals	ns	ns	Ns	ns	ns

Values are Spearman's ρ, followed by p value in parentheses. ns: not significant ($p > 0.05$).

higher in non-fasters. Nevertheless, groups did not differ in BMD, BMC, or incidence of bone fracture.

The connection of dietary protein intake with bone integrity and bone health is not clear. Dolan and Sale (2019), summarising systematic reviews and meta-analyses by Darling et al. (2009) and Shams-White et al. (2017), conclude that higher protein intakes have a small positive impact on BMD. In particular, Darling et al. (2009) found a weak positive relationship of dietary protein intake with BMD and BMC, only explaining 1–2% of the variation in BMD. Likewise, the meta-analysis of randomised controlled trials and prospective cohort studies by Shams-White et al. (2017) reported a small positive effect (0.52%) of higher, compared to lower, protein intakes (defined differently in each analysed study) on BMD at the lumbar spine but no effect at other sites (that is, total hip, femoral neck or total body). Similarly, Rizzoli et al. (2018), summarising systematic reviews and meta-analyses on the benefits and risks of dietary protein intakes for bone health in adults, concluded that high protein intakes may be beneficial in reducing bone loss and hip fracture risk, provided calcium intakes are adequate. Variation in protein intakes within the "normal" range accounted for 2–4% of BMD variance. Such small differences can also be seen in most of the panels of Figures 2 and 3 in the present paper, where the median BMD and BMC of non-fasters was 1%–3% higher than that of fasters. Although these differences did not reach statistical significance in the present single study, our data can make a useful contribution to future meta-analyses examining the connection between dietary protein intake and bone health.

To further examine the possible connection of protein source with bone status, correlation analysis was performed, which produced weak (explaining just 1%–3% of the variation in BMD or BMC) positive results for red meat, poultry-eggs, and (partly) dairy products, weak negative results for seafood, as well as no significant correlation for pulses and grains-cereals. Again, there are controversies in the literature regarding the connection of specific protein sources with bone status. In agreement with the present findings, Durosier-Izart et al. (2017) reported that bone strength was positively associated with total, animal, and dairy protein intakes but not with vegetable protein intake in healthy postmenopausal women. Nevertheless, Wallace and Frankenfeld (2017), in their systematic review and meta-analysis, conclude that, when it comes to the beneficial effect of high protein intake on BMD, there are no differences between animal and plant proteins, although data in this area are scarce.

Regarding seafood consumption, the present findings differ from those of Li et al. (2017) who reported that Chinese men with high fish consumption (without consideration for portion size) had a lower prevalence of osteoporosis. Of interest is the study of Choi and Park (2016) who found a weak positive association (with correlation coefficients ranging from 0.047 to 0.181) with BMD in Koreans (who consumed seafood 23 times per month on average) but not in Americans (who consumed seafood only 5 times per month on average). The authors suggest that a minimum seafood intake might be necessary for a substantial effect on bone status. Although comparison of their findings with ours is hampered by the fact that they used times of consumption, not portions, a median of 1.5 portions per week (for the entire sample in the present study) places the Greek participants closer to Americans than to Koreans.

Also of interest is the systematic review by Perna et al. (2017) on the association between dietary patterns of meat (including processed meat and poultry) and fish consumption with BMD or fracture risk. The review in question included 37 studies with a total of 432,924 subjects. The meat patterns were positively associated with bone health in the minority of studies (encompassing 4% of subjects), whereas the remainder of the studies showed either no association (48% of subjects) or negative association (48% of subjects). The fish patterns were positively associated with bone health in the minority of studies again (encompassing 13% of subjects), whereas the remaining studies showed either no association (84% of subjects) or negative association (3% of subjects). The authors note, however, that negative effects of meat diets on bone were seen in the setting of a Western diet, not in Mediterranean or Asian diets. These conclusions highlight how complex the interrelationship of the different food components is in determining bone health.

The biochemical measures employed in the present study corroborate the dietary data. Specifically, serum urea, an accepted index of dietary protein intake (Wu 2006) was significantly higher in non-fasters than fasters, as was protein intake. Serum creatinine and uric acid, known indices of meat consumption (Choi et al. 2005; Wu 2006) were also higher, although not significantly, in non-fasters. Biochemical data do not usually accompany dietary data in similar studies; thus, we consider them an important part of the present study.

Alcohol consumption and smoking were two lifestyle factors differing between fasters and non-fasters, both being higher in the latter. Despite drinking more alcohol, non-fasters (as well as fasters) had all light to moderate consumption, that is, less than two drinks per day. Because such consumption is thought to be beneficial for bone health (as opposed to heavy consumption) (Gaddini et al. 2016), it seems unlikely that the higher alcohol consumption by non-fasters had a detrimental effect on bone status, such that could counterbalance a detrimental effect of lower protein intake by fasters, resulting in no differences between groups. Likewise, it seems unlikely that the higher prevalence of smoking among non-fasters could have counterbalanced the lower protein intake by fasters. This is because the evidence for a detriment of smoking on bone health is limited and inconsistent (Weaver et al. 2016; Strozyk et al. 2018) and because smokers did not differ from non-smokers in any of the bone status parameters measured.

The main strength of the present study is the examination of a relatively large group of individuals who followed a distinct, rare dietary pattern (COC fasting). Their marked differences in food intake from non-fasting controls enabled a robust testing of the relation between bone health and protein intake from different animal and plant sources. On the other hand, as with all studies based on self-reported lifestyle parameters, the present study is limited by the likelihood of misreporting food intakes. However, given the similarity of the two groups in demographic and anthropometric characteristics, it seems improbable that fasters misreported differently from non-fasters. Moreover, whilst the participants have declared full adherence to COC fasting in previous years, there is no confirmation of this, understandably, so there was a reliance on self-report, which is not ideal but was the only feasible option in this circumstance. In addition, the variation in the fasters' diet over the year may lead to fluctuations in some endpoints, making their values dependent on when they are measured (although it is difficult to think that BMD and BMC can fluctuate during a year). An additional limitation is the lack of snapshots of physical activity, nutrition, and other contributing lifestyle factors over the years. Finally, a limitation of the study is that the participants lost during the study (14 of the 414 eligible ones) might have substantially different profiles from those of the 400 who completed the study. However, the proportion of the former to the latter (3.5%) is rather small; therefore, the chance of their having a significant effect on the outcomes of the study should also be considered small.

Conclusion

Despite having lower protein intakes, men and women who had been periodically avoiding animal source foods for a median of 15 years did not differ in BMD, BMC, or prevalence of bone fracture from controls with no food restrictions. BMD and BMC exhibited only weak (if any) correlations with consumption of protein-rich foods. Thus, protein intake seems to play a minor (if any) role in bone health. These findings may aid in delineating the role of dietary protein in bone health and producing practical recommendations on the matter. Future studies could employ assessment of fasters during periods of fasting and periods of no food restriction (in addition to the moderate-fasting period chosen in the present study) for a more accurate characterisation of the relation between nutrient intake and bone health.

Acknowledgements

The authors are grateful to Metropolitan Varnavas of Neapolis and Stavroupolis, Thessaloniki; Metropolitan Georgios of Kitros, Katerini, and Platamon; Hieromonk Father Luke Kipouros of Holy Trinity Monastery, Panorama, Thessaloniki; Nun, Melani Chatzipatera, Mother Superior of Assumption of Virgin Monastery, Panorama, Thessaloniki; Professor Emeritus of the School of Theology, Aristotle University of Thessaloniki, Dimitrios Tselegidis; Archimandrite Nikodemos Skrettas-Plexidas; and Archpriest Athanasios Gikas; Professors of the School of Pastoral and Social Theology, Aristotle University of Thessaloniki, for their help in collecting the study sample. The authors have received permission from those named in the acknowledgement.

Disclosure statement

The authors have no conflicts of interest to report.

Funding

The study was funded by funds of the authors' institutions.

ORCID

Nikolaos E. Rodopaios http://orcid.org/0000-0001-7710-6231
Vassilis Mougios http://orcid.org/0000-0002-8430-7231

References

Bonjour JP. 2016. The dietary protein, IGF-I, skeletal health axis. Horm Mol Biol Clin Investig. 28:39–53.
Boskey AL. 2013. Bone composition: relationship to bone fragility and antiosteoporotic drug effects. Bonekey Rep. 2:447.

Calvez J, Poupin N, Chesneau C, Lassale C, Tomé D. 2012. Protein intake, calcium balance and health consequences. Eur J Clin Nutr. 66:281–295.

Carnauba RA, Baptistella AB, Paschoal V, Hübscher GH. 2017. Diet-induced low-grade metabolic acidosis and clinical outcomes: a review. Nutrients. 9:e538.

Choi E, Park Y. 2016. The association between the consumption of fish/shellfish and the risk of osteoporosis in men and postmenopausal women aged 50 years or older. Nutrients. 8:113.

Choi HK, Liu S, Curhan G. 2005. Intake of purine-rich foods, protein, and dairy products and relationship to serum levels of uric acid: The third National Health and Nutrition Examination Survey. Arthritis Rheum. 52: 283–289.

Darling AL, Millward DJ, Torgerson DJ, Hewitt CE, Lanham-New SA. 2009. Dietary protein and bone health: a systematic review and meta-analysis. Am J Clin Nutr. 90:1674–1692.

Dolan E, Sale C. 2019. Protein and bone health across the lifespan. Proc Nutr Soc. 78:45–55.

Durosier-Izart C, Biver E, Merminod F, van Rietbergen B, Chevalley T, Herrmann FR, Ferrari SL, Rizzoli R. 2017. Peripheral skeleton bone strength is positively correlated with total and dairy protein intakes in healthy postmenopausal women. Am J Clin Nutr. 105:513–525.

Food Standards Agency. 2002. McCance & Widdowson's the composition of foods. Cambridge (UK): Royal Society of Chemistry.

Gaddini GW, Turner RT, Grant KA, Iwaniec UT. 2016. Alcohol: a simple nutrient with complex actions on bone in the adult skeleton. Alcohol Clin Exp Res. 40:657–671.

Institute of Preventive Medicine Environmental and Occupational Health. 2014. Εθνικός διατροφικός οδηγό ς για ενή λικες, Greek (National Nutrition Guide for Greek Adults). Athens (Greece): Labrakis Press Organization.

Kerstetter JE, Caseria DM, Mitnick ME, Ellison AF, Gay LF, Liskov TAP, Carpenter TO, Insogna KL. 1997. Increased circulating concentrations of parathyroid hormone in healthy, young women consuming a protein-restricted diet. Am J Clin Nutr. 66:1188–1196.

Kerstetter JE, O'Brien KO, Caseria DM, Wall DE, Insogna KL. 2005. The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. J Clin Endocrinol Metab. 90:26–31.

Kerstetter JE, O'Brien KO, Insogna KL. 2003. Dietary protein, calcium metabolism, and skeletal homeostasis revisited. Am J Clin Nutr. 78:584S–592S.

Li X, Lei T, Tang Z, Dong J. 2017. Analyzing the association between fish consumption and osteoporosis in a sample of Chinese men. J Health Popul Nutr. 36:13.

Movassagh EZ, Vatanparast H. 2017. Current evidence on the association of dietary patterns and bone health: a scoping review. Adv Nutr. 8:1–16.

Papadopoulou SK, Barboukis V, Dalkiranis A, Hassapidou M, Petridou A, Mougios V. 2008. Validation of a questionnaire assessing food frequency and nutritional intake in Greek adolescents. Int J Food Sci Nutr. 59:148–154.

Perna S, Avanzato I, Nichetti M, D'Antona G, Negro M, Rondanelli M. 2017. Association between dietary patterns of meat and fish consumption with bone mineral density or fracture risk: a systematic literature. Nutrients. 9:1029.

Pisani P, Renna MD, Conversano F, Casciaro E, Di Paola M, Quarta E, Muratore M, Casciaro S. 2016. Major osteoporotic fragility fractures: risk factor updates and societal impact. World J Orthop. 7:171–181.

Rizzoli R, Biver E, Bonjour J-P, Coxam V, Goltzman D, Kanis JA, Lappe J, Rejnmark L, Sahni S, Weaver C, et al. 2018. Benefits and safety of dietary protein for bone health-an expert consensus paper endorsed by the European Society for Clinical and Economical Aspects of Osteopororosis, Osteoarthritis, and Musculoskeletal Diseases and by the International Osteoporosis Foundation. Osteoporos Int. 29:1933–1948.

Rodopaios NE, Mougios V, Konstantinidou A, Iosifidis S, Koulouri AA, Vasara E, Papadopoulou SK, Skepastianos P, Dermitzakis E, Hassapidou M, Kafatos AG. 2019. Effect of periodic abstinence from dairy products for approximately half of the year on bone health in adults following the Christian Orthodox Church fasting rules for decades. Arch Osteoporos. 14:68.

Rodopaios NE, Mougios V, Koulouri AA, Vasara E, Papadopoulou SK, Skepastianos P, Dermitzakis E, Hassapidou M, Kafatos AG. 2020. Bone status of young adults with periodic avoidance of dairy products since childhood. Eur J Pediatr. 179:645–651.

Shams-White MM, Chung M, Du M, Fu Z, Insogna KL, Karlsen MC, LeBoff MS, Shapses SA, Sackey J, Wallace TC, Weaver CM. 2017. Dietary protein and bone health: a systematic review and meta-analysis from the National Osteoporosis Foundation. Am J Clin Nutr. 105: 1528–1543.

Strozyk D, Gress TM, Breitling LP. 2018. Smoking and bone mineral density: comprehensive analyses of the third National Health and Nutrition Examination Survey (NHANES III). Arch Osteoporos. 13:16.

Tarrant SM, Balogh ZJ. 2020. The global burden of surgical management of osteoporotic fractures. World J Surg. 44: 1009–1019.

Trichopoulou A. 2004. Πίνακες σύνθεσης τροφίμων και ελληνικών φαγητών, Greek (Composition tables of foods and Greek dishes). Athens (Greece): Parisianou.

Wallace TC, Frankenfeld CL. 2017. Dietary protein intake above the current RDA and bone health : a systematic review and meta-analysis. J Am Coll Nutr. 36:481–496.

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.

Witard OC, Wardle SL, Macnaughton LS, Hodgson AB, Tipton KD. 2016. Protein considerations for optimising skeletal muscle mass in healthy young and older adults. Nutrients. 8:181.

Wu A. 2006. Tietz clinical guide to laboratory tests. Philadelphia (PA): Saunders.

Xu J, Lombardi G, Jiao W, Banfi G. 2016. Effects of exercise on bone status in female subjects, from young girls to postmenopausal women: an overview of systematic reviews and meta-analyses. Sports Med. 46:1165–1182.