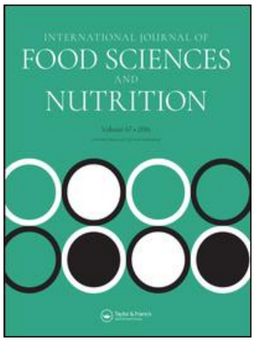

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RESEARCH ARTICLE

Calcium intake from different food sources in Italian women without and with non-previously diagnosed osteoporosis

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ABSTRACT
An adequate calcium and vitamin D intake may play a role in preventing osteoporosis, but the contribution of the different food sources of calcium with regards to the risk of osteoporosis been barely explored. This observational study evaluated the calcium intake through a food frequency questionnaire in 126 adult women with not previously diagnosed osteoporosis undergoing Dual-energy X-ray Absorptiometry (DXA) to screen for osteoporosis, and to correlate the calcium intake with parameters of bone density, measured by DXA. Total daily calcium intake and daily intake from food were similar among women found to have osteoporosis, osteopenia or normal condition. The main food source was milk and dairy products, while calcium supplementation was consumed by only 14% of subjects, irrespectively from osteoporosis conditions. DXA parameters were not significantly correlated with total daily calcium intake and calcium from food. The present study highlighted no qualitative and quantitative differences in the consumption of food groups contributing to calcium intakes in women with and without osteoporosis.

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food sources; calcium intake; calcium supplementation; bone; osteoporosis; DXA

Introduction

Osteoporosis is a disease characterised by skeletal fragility and microarchitectural deterioration of bone that can lead to a significantly higher risk of fracture, mainly at spine, hip, forearm and proximal humerus. In turn, this can result in poor quality of life, loss of self-sufficiency and an increased risk of death, other than posing a burden on health services (Black and Rosen 2016). In 2010, it was estimated that 22 million women and 5.5 million men in the European Union were affected by osteoporosis, by following the diagnostic criterion of the World Health Organisation (WHO) (WHO 1994). A wide variety of techniques is available to assess Bone Mineral Density (BMD), but the most accurate are the ones based on X-ray absorptiometry of bone, particularly Dual-energy X-ray Absorptiometry (DXA), since the absorption of X-rays is very sensitive to the calcium content in the bone (Martini et al. 2018) and is still considered the "gold standard" for assessing bone density by the WHO (WHO 1994).

DXA T-scores are delta values which take into consideration the number of standard deviations of difference between the patient and the average value for young healthy individual (WHO 1994). According to WHO criteria, the definition of osteoporosis is based on the T-score for BMD and is defined as a BMD value at the femoral neck of $-$ 2.5 SD or more below the young female adult mean (T-score ≤ -2.5 SD). Nevertheless, other central sites (e.g. lumbar spine, total hip) can be used and are widely used for diagnosis of osteoporosis in clinical settings (WHO 2004). In addition, the WHO describes the condition of osteopenia as a T-score that lies between -1 and -2.5 SD.

Due to the impact of osteoporosis on health, quality of life, and maintenance of self-sufficiency in the elderly people, preventive measures are strongly recommended. Among the modifiable factors, diet has proven to be an extremely important factor affecting

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bone health with some dietary patterns that have been associated with a reduced risk of low BMD and osteoporotic-related fractures and others that are instead inversely associated (Fabiani et al. 2019). Although many dietary compounds such as protein, potassium and vitamin K have been shown to play a role on bone health (Kong et al. 2017; Shams-White et al. 2017), mostly the role of calcium and vitamin D is well-established for bone health for osteoporosis prevention. Calcium is an important structural component of bone while vitamin D is essential for efficient calcium absorption, for calcium deposition in bone and for the maintenance of normal blood levels of calcium (European Food Safety Authority (EFSA) 2009). Although diets low in calcium have been associated with a lower number of deaths and disability-adjusted life years (DALYs) compared to other regimens (e.g. diet high in sodium or low in whole grains), inadequate calcium intake causes thousands DALY at a global level (Afshin et al. 2019). Thus specific dietary choices, such as adequate calcium and vitamin D intake, may play a role in preventing osteoporosis and therefore decreasing thousands DALYs at a global level. Among calcium-rich foods, the food category of milk and dairy products is the most widely investigated for the putative role in the maintenance of bone health and in the prevention of bone fractures. This is because dairy products are among the richest in calcium content and have high availability once ingested (Guéguen and Pointillart 2000), despite contrasting results when considering different types of products and different types of studies (e.g. cross-sectional, case-control or cohort studies) (Bian et al. 2018; Fabiani et al. 2019; Malmir et al. 2019; Chakhtoura et al. 2020).

An adequate calcium intake is generally recommended together with vitamin D in all the dietary guidelines worldwide, although with some differences among countries (Dai et al. 2019). In Italy, the population reference intake for calcium is 1000 mg for both men and women (aged ≥18 years) and up to 1200 mg for postmenopausal women (aged ≥60 years), who are facing an increased mineral loss from bones, leading to an increased risk of fractures (Italian Society of Human Nutrition 2014). If possible, it is recommended to reach calcium intake through diet instead of supplements, also because the absolute safety of calcium supplements above 1 g daily and without vitamin D supplementation is still matter of debate (Rossini et al. 2016).

Among the studies surveying the calcium intake in different populations, in Italy it has been found intake lower than 800 mg, with only a ∼20% of individuals meeting the recommendations (Sette et al. 2011; Castiglione et al. 2018). In order to decrease this gap from the recommended value and the real intakes, healthcare practitioners should help individuals in including correct calcium-rich foods with adequate frequency and serving sizes into the daily diet. However, how different food groups contribute to calcium intakes with regards to prevalence of osteoporosis has been barely explored. Thus, aims of the present study were i) to investigate the intake of calcium from a variety of food groups in Italian adult women immediately before undergoing DXA screening for osteoporosis, ii) to correlate the calcium intake with specific parameters of bone density (T-score of lumbar spine, total femur and femoral neck) measured by means of DXA.

Materials and methods

Subjects

This study is part of a multicentric trial conducted in 40 Italian medical centres selected by the Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases (SIOMMMS). The study design was approved by the Ethics Committee of the A.O.U. Città della Salute e della Scienza di Torino, Turin, Italy (Protocol N°0076273) and individual agreed to informed consent documents. Subjects were enrolled in the study at their first DXA scan evaluation, while exclusion criteria were being in treatment with drugs for osteoporosis (i.e. bisphosphonates, oestrogens, teriparatide or denosumab) and having history of previous osteoporotic fractures. The present study reports the findings from consecutive women undergoing a DXA scan at the Centre for Metabolic Bone Diseases of the Parma University Hospital (Parma, Italy).

Assessment of calcium intake

Subjects were asked to fill a questionnaire devoted to investigate the calcium intake from different dietary sources. This tool was created using the same questions as in a previous food frequency questionnaire for nutritional calcium intake assessment in Italian women (Montomoli et al. 2002), except for water consumption, that was omitted in this revised version. This choice was due to the extreme difference of sources of water (both mineral and tap) available, which have a large variability in terms of calcium content (Azoulay et al. 2001), deeply higher than those among other food groups. Moreover, some food groups were revised to make them as close as possible to the local eating habits. In detail, the questionnaire investigated the habitual consumption, in terms of frequency (number/week) and serving amount (grams/millilitres), of the following foods: dairy (milk, whole and skimmed; yogurt, whole and skimmed; cheese, cream, hard and grated cheese; ice-cream; milk-based desserts), vegetables (broccoli, cabbage, green beans), dried fruits and nuts (apricots, figs, raisins, almonds, nuts, hazelnuts), fish (sardines and salmon), cereal-based foods (pasta, rice and bread), and others (pizza, lasagne, omelette with cheese). In detail, subjects were provided with specific serving sizes for each group (based on LARN 2014 (Italian Society of Human Nutrition (SINU) 2014)), expressed as grams or millilitres depending on the food group, and were asked to estimate how many servings per week they consumed.

The Food Composition Database for Epidemiological Studies in Italy (Gnagnarella et al. 2015) was used as reference to estimate the calcium content for each of the considered foods.

The daily calcium intake from food was determined by multiplying the number of servings/week by the calcium content of each serving and thus summing the calcium content of all the consumed foods divided by 7 days, in order to allow comparison with the nutrition recommendations. In addition, daily calcium intake from supplements was estimated by asking the type and the quantity of supplements taken on a daily basis. Lastly, the total daily calcium intake was calculated as the total calcium from both food and supplements, i.e. calcium carbonate or calcium citrate. Participants were assisted by a trained physician to answer all the questions and to report any confusion and/or difficulties with the questionnaire.

Measurement of lumbar and femor bone mineral density

Subjects underwent to a DXA to investigate the potential diagnosis of osteoporosis. Bone mineral density was measured by DXA with a HOLOGIC Discovery A system, using standard protocols. Lumbar spine (L1–L4), total femur and femoral neck BMD were evaluated and were expressed as T-score (Lewiecki 2010). According to the WHO criteria (WHO 1994), subjects were classified normal if spine and/or femoral BMD was above −1 SD, osteopenic if between −1 and −2.5 SD, and osteoporotic if below −2.5 SD.

Statistical analysis

G*Power software version 3.1.9.3 was used to calculate the sample size for this study. A total sample size of 126 subjects was required to obtain a 80% power and an alpha of 10% for a one-way ANOVA test to explore mean differences among three groups of women with no-osteoporosis, osteopenia or osteoporosis.

IBM SPSS statistics for Macintosh Version 26.0 (Armonk, NY: IBM Corp.) was used to perform the statistical analysis, establishing the significance at $p < .05$. Normality of data distribution was verified through the Kolmogorov-Smirnov test. A one-way between-groups ANOVA with Bonferroni *post hoc* test and a two-sample Student's *t*-test was used to compare participants' characteristics (age, weight, height, BMI) and parameters for osteoporosis diagnosis (lumbar, femur and femoral neck T-Score) respectively among subjects in the no-osteoporosis, osteopenia and osteoporosis groups and between subjects meeting or not the recommended daily calcium intake, between BMI categories (normal weight 18.5–25 kg/m^2 *vs.* overweight or obese > 25 kg/m^2), and between age groups (adult <60 *vs.* ≥60 years). Since differences were found among osteoporotic, osteopenic and normal participants for age and BMI, these two variables were used as covariates in an ANCOVA model used to explore differences in calcium daily intakes (total calcium, calcium from supplements, calcium from food, and calcium from specific food groups: dairy, nuts/dried fruit, vegetables, fish, cereals, others) and intakes of specific food items (milk and dairy products) between subjects with or without osteoporosis, after checking that there was no violation of the assumptions of normality, linearity, homogeneity of variances, homogeneity of regression slopes, and reliable measurement of the covariate. The two-sample Student's *t*-test was used to explore differences between subjects meeting or not the age-specific recommended daily calcium intake, between age groups (adult <60 *vs.* ≥60 years), and between BMI categories (normal weight 18.5–25 kg/m^2 *vs.* overweight or obese > 25 kg/m^2). Analyses referred to calcium from supplements were performed considering only supplement users ($n = 18$). Correlations between parameters for osteoporosis diagnosis (lumbar spine, total femur and femoral neck T-Scores) and calcium daily intakes (total calcium, calcium from supplements, calcium from food, and calcium from specific food groups: dairy, nuts/dried, vegetables, fish, cereals, others) were investigated through the non-parametric Spearman's Correlation test. In addition, a general

Table 1. Anthropometric characteristics and femoral and spinal T scores of participants by osteoporosis condition groups and Ca intake recommendation (PRI) groups.

	Total population ($n=126$)	No osteoporosis ($n=29$)	Osteopenia ($n=67$)	Osteoporosis ($n=30$)	p value*	Non-meeting Ca PRI (n = 97)	Meeting Ca PRI (n = 29)	p value**
Age (y)	61 ± 10	56 ± 10[a]	60 ± 10[ab]	65 ± 10[b]	.003	61 ± 11	61 ± 9	.993
Weight (kg)	65.4 ± 10.5	73.8 ± 11.3[b]	64.1 ± 9.1[a]	60.4 ± 8.3[a]	<.001	65.6 ± 10.2	65.0 ± 11.8	.785
Height (m)	1.62 ± 0.06	1.64 ± 0.06	1.61 ± 0.06	1.62 ± 0.06	.153	1.62 ± 0.06	1.61 ± 0.06	.620
BMI (kg/m^2)	24.9 ± 4.1	27.6 ± 4.5[b]	24.7 ± 3.6[a]	23.1 ± 3.4[a]	<.001	25.0 ± 3.9	25.0 ± 4.6	.960
Lombar spine T-score	−1.42 ± 1.38	0.19 ± 0.87[c]	−1.39 ± 0.73[b]	−3.04 ± 1.00[a]	<.001	−1.33 ± 1.37	−1.71 ± 1.39	.197
Total femur T-score	−0.88 ± 1.07	0.41 ± 0.76[c]	−0.98 ± 0.58[b]	−1.92 ± 0.87[a]	<.001	−0.83 ± 1.07	−1.07 ± 1.06	.280
Femural neck T-score	−1.52 ± 1.08	−0.09 ± 0.74[c]	−1.66 ± 0.60[b]	−2.61 ± 0.57[a]	<.001	−1.49 ± 1.08	−1.65 ± 1.09	.489

Values are expressed as mean ± SD. *One-way between-groups ANOVA with Bonferroni *post hoc*, $p < .05$. Different letters in the same row indicate differences among groups (a<b<c). **Two-sample Student's *t*-test, $p < .05$.

linear model including osteoporosis groups, total calcium, and the interaction of total calcium intake x osteoporosis groups was run to explore between groups differences in the relationship between calcium intake and parameters for osteoporosis diagnosis.

Results

A total of 126 women were included in the final analysis, 24% of whom were found to have osteoporosis and 53% osteopenia, while 23% met the calcium recommended daily intake (Table 1).

Mean age was 61 ± 10 years, slightly higher in subjects with osteoporosis than subjects without osteoporosis ($p = .003$). Participants with osteoporosis or osteopenia had a significantly lower body weight and BMI than subjects without osteoporosis ($p < .001$ for both parameters). As expected, the lowest levels of total femur, femoral neck and spinal T-scores were found in osteoporotic subject while the highest level in participants without osteoporosis ($p < .001$ for all parameters). No differences were found between subjects meeting or not the daily calcium recommended intake for age, anthropometrics and DXA parameters. When subjects were grouped for their BMI or age (Supplemental Table 1), total femur, femoral neck and spinal T-scores were found to be lower in normal weight than in overweight or obese subjects ($p = .045$, $p = .011$, and $p < .001$, respectively for the three parameters) and in participants older than 60 years for age than in younger ones ($p = .016$, $p = .001$ and $p = .006$, respectively for the three parameters).

Calcium intake

The total calcium was similar among subjects with osteoporosis, osteopenia and without osteoporosis, after adjusting for age and BMI (Table 2).

Calcium supplementation was taken by only 18 participants (14%) and was similar between osteopenia and osteoporosis groups.

Calcium intake from foods accounted for ∼90% of the total calcium intake and was found to be similar among osteoporosis condition groups. Likewise, intakes from different food groups were similar among osteoporotic, osteopenic and normal women. When considering the different food groups, dairy products were the main contributors, with ∼78% of dietary calcium intake, followed by cereal-based foods (7%) and vegetables (6%) (Table 2).

When subjects were split on the basis of reaching or not the recommended daily calcium intake, participants who met the recommendations had a higher total daily calcium intake ($p < .001$) and intake from food ($p < .001$) than participants not reaching the recommended amount. Among food sources, calcium intakes from milk and dairy products ($p < .001$), from vegetables ($p = .019$), and from fish ($p = .048$) were higher in the group of subjects meeting the recommendation than participants having a daily calcium intake lower than the recommended one (Table 2).

No differences were found between subjects <60 years and subjects ≥60 years or between normal weight and overweight/obese subjects for total intake, intake from supplements, or intake from food (Supplemental Table 2).

An analysis of food intake in terms of grams/day or grams/week and of number of standard servings was performed for milk and dairy products, due to the huge contribution of these products to the daily calcium intake (Table 3).

No differences in daily intakes of milk and yogurt were found among osteoporosis condition groups and their consumption was lower than the recommended intake in all groups (∼0.5 serving/day *vs.* 3 servings/ day recommended). Similarly, the weekly

Table 2. Daily total calcium intake, daily calcium intake from supplements and from food, and daily calcium intake from food groups by osteoporosis condition groups and Ca intake recommendation (PRI) groups.

Calcium	Total population (*n* = 126)	No osteoporosis (*n* = 29)	Osteopenia (*n* = 67)	Osteoporosis (*n* = 30)	*p* value*	Non-meeting Ca PRI (n = 97)	Meeting Ca PRI (n = 29)	*p* value**
Total intake (mg/day)	812.30 ± 412.70	744.48 ± 416.99	828.77 ± 471.42	843.80 ± 628.19	.804	595.34 ± 268.87	1540.82 ± 390.24	< .001
Intake from supplement*** (mg/day)	655.56 ± 161.69	–	700.00 ± 185.16	622.22 ± 148.14	.351[2]	583.33 ± 40.83	691.67 ± 188.09	.079
Intake from food (mg/day)	719.30 ± 412.70	723.79 ± 419.29	745.19 ± 407.24	657.13 ± 425.81	.598	559.26 ± 253.89	1254.61 ± 392.09	< .001
Dairy (mg/day)	599.25 ± 392.91	615.62 ± 407.74	622.40 ± 386.70	531.74 ± 397.94	.569	449.15 ± 246.08	1101.31 ± 377.43	< .001
Nuts/dried fruits (mg/day)	14.30 ± 13.89	12.30 ± 12.27	14.98 ± 14.52	14.71 ± 14.20	.895	12.83 ± 12.24	19.20 ± 17.76	.079
Vegetables (mg/day)	31.74 ± 24.74	30.23 ± 20.94	31.97 ± 26.51	32.69 ± 24.73	.964	28.06 ± 20.47	44.05 ± 33.05	.019
Fish (mg/day)	15.34 ± 38.46	6.36 ± 23.83	21.90 ± 46.14	9.39 ± 27.66	.106	10.71 ± 33.27	30.85 ± 49.84	.048
Cereal-based foods (mg/day)	39.18 ± 18.42	38.56 ± 24.49	37.08 ± 16.19	44.47 ± 15.70	.390	38.65 ± 17.42	40.97 ± 24.65	.554
Others (mg/day)	19.48 ± 17.67	20.72 ± 15.75	16.86 ± 15.60	24.14 ± 22.63	.193	19.85 ± 17.55	18.24 ± 18.32	.668

Values are expressed as mean ± SD. *One-way between-groups ANCOVA adjusted for age and BMI, $p < .05$. **Two-sample Student's *t*-test, $p < .05$. ***Only 18 participants took supplements (no osteoporosis group: $n = 1$, this group was excluded from the analysis, osteopenia group: $n = 8$, osteoporosis group: $n = 9$; non-meeting Ca recommendation group: $n = 6$, meeting Ca recommendation group: $n = 12$).

Table 3. Milk and dairies intakes (grams per day or grams per week) and number of standard portions per day (milk and yogurt) and per week (cheese) by osteoporosis condition groups and Ca intake recommendation (PRI) groups.

Food	Total population (*n* = 126)	No osteoporosis (*n* = 29)	Osteopenia (*n* = 67)	Osteoporosis (*n* = 30)	*p* value*	Non-meeting Ca PRI (*n* = 97)	Meeting Ca PRI (*n* = 29)	*p* value**
Milk (g/day)	46.71 ± 80.65	43.10 ± 72.93	48.67 ± 87.35	45.83 ± 74.25	.976	35.42 ± 62.02	84.48 ± 118.03	.039
Yogurt (g/day)	22.77 ± 37.48	24.14 ± 30.60	27.15 ± 45.42	11.67 ± 16.78	.289	19.81 ± 28.76	32.68 ± 57.46	.253
Hard cheese (g/week)	373.45 ± 251.36	403.10 ± 250.81	380.07 ± 267.97	330.00 ± 212.69	.360	296.49 ± 193.02	630.86 ± 255.32	< .001
Soft cheese (g/week)	255.56 ± 244.80	248.28 ± 204.63	256.72 ± 257.74	260.00 ± 258.11	.948	214.43 ± 198.43	393.10 ± 327.25	.009
Milk and yogurt (portion/day)	0.6 ± 0.7	0.5 ± 0.6	0.6 ± 0.7	0.5 ± 0.6	.659	0.4 ± 0.5	0.9 ± 0.9	.008
Cheese (portion/week)	10.0 ± 6.0	10.5 ± 5.7	10.2 ± 6.4	9.2 ± 5.3	.566	8.1 ± 4.1	16.6 ± 6.8	< .001

Values are expressed as mean ± SD. *One-way between-groups ANCOVA adjusted for age and BMI, $p < .05$. **Two-sample Student's *t*-test, $p < .05$.

consumption of cheese was similar among groups, but was found to be around three times higher than the recommendation (∼9–10 servings/week *vs.* 3 servings/week recommended for the Italian adult population (CREA 2020)).

Subjects meeting the daily calcium intake recommendation had a higher intake of milk ($p = .039$), hard cheese ($p < .001$) and soft cheese ($p = .009$) than subjects not reaching the recommended intake of calcium, and the number of daily servings of milk and dairy products ($p = .008$) and of the weekly servings of cheese ($p < .001$) was almost double in subjects meeting the daily calcium intake recommendation than the one of subjects in the other group (Table 3).

The intake of milk and dairy products and the number of standard portions per day (milk and yogurt) and per week (cheese) were similar between normal weight and overweight/obese subjects and between subjects <60 years and subjects ≥60 years (Supplemental Table 3).

Relationship between calcium intake and T-scores

By considering the whole population, irrespectively from the osteoporosis status, DXA parameters were not correlated to total daily calcium, calcium from food, and calcium from specific food groups (dairy, nuts/dried, vegetables, fish, cereals, others) or with calcium daily intake from supplements (considering supplement users only, data not shown).

The relationship of total daily calcium intake with parameters of osteoporosis diagnosis was explored by considering differences between groups. The interaction of osteoporosis groups *x* total calcium intake revealed no significant differences for lumbar, total femur, and femoral neck T-scores.

Discussion

The present survey investigated the calcium intake in a group of adult Italian women and aimed at correlating these values with DXA markers of osteoporosis, and how different food groups contribute to calcium intakes with regards to prevalence/risk of osteoporosis. Globally, data from the Italian Ministry of Health show rates of 23% osteoporotic women older than 40 years old (Italian Ministry of Health 2019), accounting for at least 4 million women in the Country. Despite the limited sample size, the 24% rate of osteoporotic women found in our study is in line with these data and generally with ones of literature concerning other Italian postmenopausal cohorts, ranging from 18% (Cavalli et al. 2016; Tarantino et al. 2017) to 36% (Cipriani et al. 2018) of the total population. Although women had an age in the tight range 50–70 years old, as expected, osteoporotic women were older than normal ones, underlying that bone density loss is strongly age-related (Demontiero et al. 2012). The significant higher weight and BMI values found for normal women compared to osteoporotic ones confirms data from previous studies (Premaor et al. 2010).

Concerning the daily calcium intake, the majority of participants (71%) did not reach 1000 mg/day, and only 19% had more than 1200 mg/day, which is the daily population reference intake (PRI) for the Italian adult women (Italian Society of Human Nutrition 2014). These values are in line with the ones of a recent survey conducted in Florence, Italy, on 838 women who filled a questionnaire similar to the one used in the present study (Vannucci et al. 2017). Results were similar to those found in this study for the mean intake of calcium from diet (620 mg/day), while only the 10.4% of the women exceeded the 1000 mg/day intake from diet (Vannucci et al. 2017).

Our findings show that the majority of participants did not consume enough calcium to meet recommended values, regardless of their osteoporosis status. However, we found high variability in calcium intakes from all foods and dairy food consumption within each group and among groups, even after adjusting for age and BMI. Dairy products were the main contributors to calcium intake from food and their consumption was similar among not-osteoporotic, osteopenic and osteoporotic women. Regarding dairy foods, generally a lower consumption of milk and yogurt was reported (∼0.5 serving/day) compared to the recommended 3 servings in the Italian dietary guidelines (CREA 2020). Higher calcium intake, despite lower than recommendation, was instead observed in subjects meeting calcium PRI compared to the non-meeting ones. Overall, these data are slightly lower than those observed by Leclercq and colleagues, who estimated a consumption of ∼1 serving/day in adults and older Italian women (Leclercq et al. 2009). Conversely, intake of cheese was higher than the 3 recommended servings/week (CREA 2020) in all groups, and particularly higher in subjects meeting Ca PRI. In this regard, despite cheese is likely to contribute the calcium intake, it is worth noting that it is often rich in salt and saturated fats and, as for other foods, its consumption should be compliant with dietary guidelines. In a previous Italian survey on 1771 early menopausal women, researchers found a significant positive trend of higher dairy foods intake and BMD as well as a significant increased risk of osteoporosis for the subjects in the lowest quartile of dairy foods intake compared with the highest quartile (Varenna et al. 2007). The lack of a similar trend in our study is probably attributable to the lower sample size of participants, which makes it impossible to subgrouping our population in quartiles of intakes.

The second highest source of dietary calcium were cereal-based foods, which contribution to calcium intake was found not to differ among subjects' group. To the best of our knowledge there is no evidence in literature linking higher consumption of cereal-based products with lower BMD and, in turns, osteoporosis. Surely cereal products are not considered a good source of calcium due to the presence of phytates that can decrease calcium availability at the gut level (Theobald 2005). In the frame of a healthy diet and also for subjects following particular dietary regimens, other food categories may play a role in reaching the adequate daily intake of calcium and in counteracting BMD loss. In fact, dry fruits as plums (Hooshmand et al. 2016), seeds and nuts (Suliburska and Krejpcio 2014; Agnoli et al. 2017), pulses and plants from *Brassicaceae* family (Agnoli et al. 2017) are modestly rich in calcium and not deeply affected by phytates or oxalates and their chelating effect. However, the contribution to calcium intake of these food categories did not differ among the different groups of women.

Focussing on daily intake of calcium in other countries, a survey by the International Osteoporosis Foundation Calcium Steering Committee published on 2017 found a wide variability of intake, ranging from 175 to 1233 mg/day, accounting to Nepal and Iceland, respectively (Balk et al. 2017). While Pacific Asian, African and South American citizens have the lowest intake, North American and North European ones accounts for the highest intakes. These data seem to reflect the level of consumption of the main food groups source of calcium, such as dairy ones, as reported by the Food and Agriculture Organisation

(FAO) which finely mapped pro-capita consumption of milk and its derivatives (FAO 2008).

It is worth noting that, in the present study, the calcium daily value includes only calcium from food and dietary supplements, not accounting for the calcium intake from water. This is because calcium content in water is highly variable depending on type of water consumed (e.g. tap versus mineral water) as well as by the specific brands of mineral water consumed. Moreover, subjects usually consume different types of water during the day, making a sensitive and realistic estimation of calcium intake very tricky. Linked to this, the availability of calcium from water varies among the different waters, and evidence of a significant calcium intake have been found only for mineral calcium-rich waters (Heaney 2006; Greupner et al. 2017). Considering the mean water consumption of Italian women (193 g/day of tap water and 498 g/day of bottled water for the adult women population and 268 g/d of tap water and 375 of bottled water for elderly women, (Leclercq et al. 2009)), and a rough average calcium content in drinking water of 100 mg/L (Azoulay et al. 2001; Gnagnarella et al. 2015), the daily calcium intake could be increased of around 60–70 mg/day. Considering the median intake (667 mg/day) of our population and an additional potential intake due to water consumption, our results are in line with the national data: median intake 697 mg/day for women aged 18–64.9 year and 735 mg/day for elderly women aged ≥65 years (Sette et al. 2011). Further studies should focus on the contribution of water in daily calcium intake in general and osteoporotic populations.

Lastly, there was no relationship between the total calcium intake with the three considered T-scores. These data are partially in agreement with a recent model-based meta-analysis, showing that dietary calcium has a role in preventing BMD decrease, but only over 1200 mg/day (Wu et al. 2017). However, our survey pointed out that subjects who met the calcium intake recommendations had a higher total daily calcium intake, resulted from an higher intake from food, compared to subjects not matching the recommendation for calcium intake. Among the food categories, milk and dairy products have been confirmed as first source of calcium, but different surveys showed a scarce consumption of calcium-rich foods, particularly milk-derived ones (Chapman et al. 1995; Winzenberg et al. 2005). Among the reasons, from one side many women are not conscious to introduce a not adequate amount of calcium (Chapman et al. 1995), while from the other side socio-economic factors and misleading information about unhealthiness of milk and cheese (e.g. high cholesterol or high fat foods) seem to influence women dietary choices (Chapman et al. 1995; Winzenberg et al. 2005). These aspects may be related then to the need of calcium supplementation, for which a weak inverse correlation has been found with both the considered scores in the present surveys. It is worth noting that only few subjects, and equally distributed in both women groups, declared calcium supplementation. However, these results might be explained by considering that, despite the supplementation, the process of BMD decrease might not be reversed, and other factors are involved in BMD status such as age, 25-OH vitamin D circulating levels and physical activity.

Our study shows some limitations. Firstly, the calcium intake has been evaluated based on self-reported data and not by using a biomarker of its intake, leading to a possible over/under-estimation of dietary intake. An additional source of misreporting may be due to the fact that serving sizes were provided to participants only as grams or millilitres, not using atlas or different serving sizes. Secondly, we found a lack of evidence in the relationship between daily calcium intake from diet and parameters linked to bone health maybe because of the limited sample size. Despite the sample size of the present study was similar to those used in previous investigations (Lowe et al. 2011; Kapetanović and Avdić 2012; de França et al. 2016; Ilesanmi-Oyelere et al. 2019), this lack of evidence suggests that a food frequency questionnaire requires a higher number of participants for significant results. Besides the relative small sample size, the cross-sectional design of this study may not allow generalisation and inference of causality. Further, in the present study we did not consider the frequency and consumption of mineral water, so future questionnaires should also include specific questions taking into account the variability of calcium as well as sodium intake from water and, in general, from beverages. Moreover, collecting data on total energy intake would have allowed to express results as ratio of calcium to energy, to explore its use as a putative indicator of the role of energy intake on calcium intake. Last, since the calcium-vitamin D association is well established, the inclusion of data on vitamin D intake would be surely of interest to better elucidate its role on the health outcomes under study.

Conclusion

The present study did not detect any difference in the consumption of food contributing to calcium intakes in women with and without osteoporosis. Despite the several limitations of the study, this lack of evidence may suggest that calcium intake is not the only factor affecting the development of the osteoporosis. Regardless of the absence of significant findings, data confirmed a well-known low calcium intake in Italian women. In this frame, the added value of this simple questionnaires should be found in informing the women about their dietary habits and how these ones can be improved in order to increase calcium intake and the overall diet quality, and in turn to decrease the risk of osteoporosis and fractures. To do this, nutritional education focussed on healthy diets, including the consumption of foods rich in calcium and vitamin D, is highly desirable starting from childhood and mostly in post-menopausal women and elderly subjects. Moreover, linked to the nutritional education, it would be useful to investigate their knowledge about sources of calcium and role of calcium on human health. This would allow to understand whether the low calcium intake is driven by a scarce awareness and would put the basis for the development of tailored nutrition education strategies.

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