 doi:10.20944/preprints202007.0531.v2

Title: Dietary calcium intake in a cohort of individuals evaluated for low bone mineral density: a multicenter Italian study

Authors: *Elisa Cairoli[1,2], *Carmen Aresta[1], Luca Giovanelli[1,3], Cristina Eller-Vainicher[4], Silvia Migliaccio[5], Sandro Giannini[6], Andrea Giusti[7], Claudio Marcocci[8], Stefano Gonnelli[9], Gian Carlo Isaia[10], Maurizio Rossini[11], **Iacopo Chiodini[1,3], Marco Di Stefano[10] on behalf of the Italian Society for Osteoporosis, Mineral Metabolism and Skeletal Diseases (SIOMMMS)

*equally contributed; **corresponding author;

Affiliations: [1]Istituto Auxologico Italiano IRCCS, Unit for Bone Metabolism Disease and Diabetes and Lab of Endocrine and Metabolic Research, Milan, Italy; [2]Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy; [3]Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy; [4]Fondazione IRCCS Ospedale Maggiore Policlinico, Unit of Endocrinology, Milan, Italy; [5]Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome, Italy; [6]Department of Medicine, Medical Clinic 1, University of Padua, Padua, Italy; [7]Unit of Rheumatology, ASL3 - Liguria Region, Genoa, Italy; [8]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy; [9]Department of Medicine, Surgery and Neuroscience, Le Scotte University Hospital, University of Siena, Siena, Italy; [10]Department of Medical Science, Section of Gerontology and Bone Metabolic Disease, University of Turin, Turin, Italy; [11]Department of Medicine, Rheumatology Unit, University of Verona, Verona, Italy.

Corresponding author: Iacopo Chiodini; Unit for Bone Metabolism Diseases and Diabetes & Lab of Endocrine and Metabolic Research, Istituto Auxologico Italiano, IRCCS, Via Magnasco 2, 20149 Milan, Italy; Tel +39 02 61911 2738; email: iacopo.chiodini@unimi.it; i.chiodini@auxologico.it

Keywords: dietary calcium intake, osteoporosis, fractures, bone mineral density.

 doi:10.20944/preprints202007.0531.v2

ABSTRACT

A low calcium intake is associated with an increased fracture risk. We assessed the dietary calcium intake in a cohort of Italian individuals evaluated for low bone mineral density (BMD).

A 7-day food-frequency questionnaire was administered to 1793 individuals consecutively referred at a Centre of the Italian Society for Osteoporosis, Mineral Metabolism and Skeletal Diseases for low BMD.

In 30.3% (544/1793) and 20.9% (374/1793) of subjects the calcium intake was inadequate (<700 mg/day) and adequate (>1200 mg/day), respectively. Patients with calcium intake <700 mg/day showed a higher prevalence of diabetes mellitus, idiopathic hypercalciuria and food allergy/intolerance (8.1%, 5.1%, 7.2%, respectively) than patients with calcium intake >700 mg/day (5.3%, 3.0%, 4.1%, respectively, $p<0.04$ for all comparisons), also after adjusting for age, gender and BMI. In 30.3% of fractured subjects the calcium intake was <700 mg/day.

In Italy, a low calcium intake is highly prevalent in individuals at risk for low BMD. Importantly, an inadequate calcium intake is highly prevalent even in patients with history of fragility fractures. Only about a fifth of patients at risk for low BMD reported an adequate calcium intake.

 doi:10.20944/preprints202007.0531.v2

INTRODUCTION

Calcium intake is a well-known factor influencing the achievement of an adequate peak bone mass [1] and, subsequently, the maintenance of bone mass later in life. Indeed, in the presence of inadequate calcium intake a negative calcium balance can develop, frequently leading to metabolic alterations, such as secondary hyperparathyroidism, increased bone turnover and, eventually, increased fracture risk [2].

The calcium intake largely differs among countries according to age, sex, ethnics, cultures and socioeconomic status [3] and the national recommendations on calcium intake vary worldwide. In the adult population, the Recommended Dietary Allowance (RDA) of calcium is between 1000 and 1300 mg/day according to the US National Institutes of Health [4] and between 700 and 1000 mg/day according to the UK National Osteoporosis Society [5]. Both the Italian Society for Osteoporosis, Mineral Metabolism and Skeletal Diseases (SIOMMMS) and the Italian Society of Human Nutrition recommends a calcium intake above 1200 mg/day in postmenopausal women not on hormone replacement therapy and in men older than 60-65 years of age [6,7].

The adequate daily calcium requirement is influenced by several other factors, such as age, comorbidities, and vitamin D levels, the latter being fundamental for a proper intestinal calcium absorption [8,9]. Even due to these variables, the threshold of calcium intake below which the use of calcium supplements is indicated, is still debated [9].

Despite these unsolved issues, a recent systematic review found that dietary calcium intakes fall below the recommended levels in many areas of the world [10], including Italy [11]. This is a matter of concern for bone health, especially if already threatened as in a population at risk for low bone mineral density (BMD), in whom an adequate calcium intake represents one of the first non-pharmacological interventions [7]. However, there is general agreement that, in the clinical practice a dietary calcium intake below 700 mg/day requires calcium supplementation, while, in patients with low BMD, a calcium intake above 1200 mg/day is considered adequate [7,8,12].

This multicenter national cross-sectional observational study was aimed to assess in patients referred for evaluation for possible low BMD in Italy: i) the overall calcium intake and its relation with their clinical characteristics; ii) the prevalence and characteristics of the patients with a calcium

 doi:10.20944/preprints202007.0531.v2

intake so low that normally requires supplementation (i.e. <700 mg/day); iii) the prevalence and characteristics of patients with an adequate calcium intake (i.e. >1200 mg/day).

Therefore, between November 2015 and June 2016, 1793 consecutive subjects referred in one SIOMMMS referral Centre for Osteoporosis and Metabolic Bone Diseases by their General Practitioners, that agreed in participating in this study, were recruited. We excluded subjects reporting the intake of calcium supplements and/or bone active drugs because these treatments inevitably imply some kind of previous medical counselling about osteoporosis and the related importance of an adequate calcium intake and then estimated dietary calcium intake would have not reflected their usual dietary habits. The inclusion protocol is reported in Figure 1**.**

RESULTS

Overall calcium intake and its relation with the clinical characteristics of the patients

The clinical characteristics of the whole cohort and the comparisons among individuals grouped according tertiles of dietary calcium intake are reported in Table 1. In the entire cohort the mean calcium intake was 874.9 mg/day, The 30.3% of the enrolled subjects showed a clearly inadequate calcium intake and in only 20.9% of subjects the calcium intake was adequate.

The enrolled subjects were mainly females and were comparable among the different tertiles as far as gender, BMI and prevalence of the main comorbidities. Subjects in the lowest and intermediate tertiles were younger and showed a lower prevalence of low BMD and major fragility fractures than those in the highest tertile. Moreover, the prevalence of premenopausal females, idiopathic hypercalciuria and adverse reaction to food was higher in subjects in the lowest tertile than in those in the highest tertile of daily dietary calcium intake.

Three hundred sixty-eight subjects reported a previous major fragility fracture, whereas the remaining 1425 did not, thus respecting the sample size calculation requested to guarantee the adequate power of the study.

The presence of a major fragility fracture was associated with calcium intake (odds ratio (OR): 1.16; 95% confidence interval (CI): 1.01-1.33; p=0.048), gender (OR: 1.56; 95%CI: 1.01-3.77; p=0.037) and menopausal status (OR: 4.51; 95%CI: 1.81-11.23; p=0.001), regardless of BMI (OR:

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2020 doi:10.20944/preprints202007.0531.v2

1.00; 95%CI: 0.98-1.04; p=0.491) and presence of type 2 diabetes (OR: 1.19; 95%CI: 0.75-1.91; p=0.461).

Prevalence and characteristics of the patients with a calcium intake <700 mg/day, the threshold below which a supplementation is considered mandatory

The Table 2 illustrates the comparisons between subjects with calcium intake <700 mg/day and >700 mg/day.

The patients with calcium intake <700 mg/day showed an increased prevalence of idiopathic hypercalciuria, diabetes mellitus and of a personal history of adverse reaction to food compared to patients with calcium intake >700 mg/day. Age, BMI, prevalence of low BMD, fragility fractures and other comorbidities (RA, endogenous or exogenous hypercortisolism, PHPT, nephrolithiasis, IBD and COPD) were comparable between the two groups.

Interestingly, about one third of subjects with low BMD (236/778) and one third of subjects with fragility fractures (113/368) had a calcium intake <700 mg/day (Figure 2).

The Logistic regression analysis showed that a calcium intake <700 mg/day was independently associated with the female gender (OR: 1.58; 95%CI: 1.01-2.47; p=0.047), a history of diabetes mellitus (OR: 1.61; 95%CI: 1.07-2.42; p=0.023), food intolerance/allergy (OR: 1.82; 95%CI: 1.18-2.82; p=0.007) or a previous diagnosis of idiopathic hypercalciuria (OR: 1.73; 95%CI: 1.04-2.89; p=0.035), regardless of age, BMI, the presence of low BMD and of major fragility fractures.

Prevalence and characteristics of patients with a calcium intake >1200 mg/day, the threshold for defining an adequate calcium intake as suggested by SIOMMMS

The comparison between individuals with calcium intake <1200 mg/day or >1200 mg/day is reported in Table 3.

Patients with calcium intake >1200 mg/day showed a lower prevalence of nephrolithiasis, but a higher prevalence of major fragility fractures and of history of previous PHPT compared to the patients with calcium intake <1200 mg/day. Age, gender, BMI and prevalence of prior evidence of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2020 doi:10.20944/preprints202007.0531.v2

low BMD and other assessed comorbidities (RA, endogenous or exogenous hypercortisolism, IBD, food intolerance/allergy, COPD and diabetes) were comparable between the two groups. It is worth underlying that only 22.8% and 27.4% of low BMD and fractured subjects, respectively, had an adequate daily calcium intake (Figure 2). A calcium intake >1200 mg/day was inversely associated with BMI (OR: 1.04; 95%CI: 1.01-1.06; p=0.016) and directly associated with a history of major fragility fractures (OR: 1.55; 95%CI: 1.16-2.07; p=0.003), a previous diagnosis of PHPT (OR: 2.66; 95%CI: 1.23-5.75; p=0.013) and the absence of nephrolithiasis (OR: 1.86; 95%CI: 1.10-3.14; p=0.020), regardless of age, gender and the presence of low BMD.

Calcium intake stratified for gender and presence of major fragility fracture and/or low BMD

The table 4 shows the comparison of the clinical variables between males and females subjects and between fractured and not fractured patients.

Male patients had older age and more frequently low BMD, major fragility fractures, RA, endogenous or exogenous hypercortisolism and COPD than female patients.

As compared with patients without major fragility fractures, patients with major fragility fractures were older and more often male and had more often an calcium intake >1200 mg/day, low BMD and fragility fractures even at sites different from spine and femur.

Individuals with low BMD and/or fractures showed a higher calcium intake (949.9±417.3 mg/day), were older (67.7±10.3 years) and less frequently premenopausal (1.9%) than those without low BMD and fractures (907.5±382.9 mg/day, p=0.025; 61.3±10.8 years, p<0.0001; 8.7%, p<0.0001, respectively.

Finally, even excluding premenopausal females from the analyses, the results did not change. Indeed, as compared with premenopausal females, post-menopausal females had higher prevalence of low BMD and major fragility fractures and lower prevalence of calcium intake <700 mg/day and adverse reaction to food, while BMI, prevalence of calcium intake >1200 mg/day and of other fragility fractures, diabetes, hypercortisolism, RA, idiopathic hypercalciuria, PHPT, nephrolithiasis, IBD and COPD were comparable between the two groups (data not shown).

 doi:10.20944/preprints202007.0531.v2

DISCUSSION

The recommended daily calcium intake in the general adult population varies across countries and according to the different guidelines. Overall, a daily calcium intake of at least 1000-1200 mg/day is usually considered adequate [4,6,7,13,14]. Importantly, a quite recent systematic review shows that mean dietary calcium intakes fall below the recommended levels in many areas of the world [10], including Italy, where a survey on this topic performed in 2005-2006 revealed an average calcium intake in the adult population of 765 mg/day [11].

Although conducted in a different population (i.e. mainly postmenopausal women), the present study shows that, 15 years after that survey, the mean calcium intake in a cohort of Italian individuals referred for the evaluation of low BMD is still lower than recommended. Indeed, the mean daily calcium intake in the present study (about 875 mg) is better than before, but still insufficient, especially considering that these data were collected in mainly postmenopausal females and in general in a population at risk of low BMD. Indeed, it is well-established that an adequate calcium and vitamin D intake is essential for bone health and that a low calcium intake is associated with an increased fracture risk [13,15]. Our results are in agreement with those of previous studies showing a mean calcium intake lower than the recommended thresholds in osteoporotic populations, both in Europe [16] and in Italy [17].

It is worth noting that within our cohort almost one third of individuals with low BMD and/or fractures had an estimated calcium intake lower than 700 mg/day, the threshold below which the dietary calcium intake is considered to be associated with an increased risk of fracture and osteoporosis and below which a supplementation is considered mandatory [13] and less than a quarter of individuals with low BMD and/or fractures had an adequate daily calcium intake. Despite these findings, indicating an insufficient awareness of the importance of this nutritional issue for bone health, the prevalence of low BMD and previous major fragility fractures were higher in subjects of the third tertile than in those of the first and second tertiles of daily dietary calcium intake. This finding might be explained by the possibility that individuals with low BMD and/or fractures are more prone to increase the daily calcium intake. In keeping, in the present study individuals with low BMD and/or fractures were older than those without low BMD and/or fractures and the dietary

 doi:10.20944/preprints202007.0531.v2

calcium intake was directly associated with age. Accordingly, premenopausal females were more represented in the lowest tertile of daily calcium intake and had more frequently an inadequate calcium intake and less frequently an adequate calcium intake than post-menopausal women. Therefore, even if entirely speculative, this may suggest that the awareness of a low BMD and/or of a fragility fracture could have positively influenced the nutritional habits.

Moreover, individuals in the lowest tertile of daily calcium intake showed a higher prevalence of idiopathic hypercalciuria and of adverse food reactions (mainly lactose intolerance, to a lesser extent food allergy or autoimmune intolerance) than patients in the highest tertile. This result was also confirmed when we compared patients with calcium intake <700 mg/day and >700 mg/day and, in particular, these conditions were associated with an inadequate calcium intake independent of possible confounders, including the presence of low BMD and prevalent fragility fractures. These findings suggest that adverse food reactions and the presence of hypercalciuria may have contributed to decrease the calcium intake. Indeed, despite the availability of lactose-reduced or lactose-free dairy products, lactose-intolerant individuals frequently avoid milk and derivatives which represent the main sources of dietary calcium and so are at risk of calcium inadequacy [18]. Likewise, although entirely speculative, it is conceivable that hypercalciuric subjects, possibly not appropriately informed, could have been worried of worsening the urinary calcium excretion by consuming dairy products. Conversely, it is known that an adequate dietary calcium intake is important even in hypercalciuric individuals in order to counterbalance the increased urinary loss and avoid a negative calcium balance [19]. Unfortunately, data regarding the type of hypercalciuria and the urinary calcium-to-creatinine/ratio are not available.

An inadequate calcium intake was also independently associated with the female gender and with the presence of diabetes mellitus. The first result, despite being consistent with previous data [10], acquires even more value considering that calcium requirements in women are generally higher than in men of the same age [14]. A history of diabetes mellitus could have negatively influenced calcium intake due to the need to follow not only a hypoglucidic but also a cholesterol-lowering diet, which is a known factor risk for reduced calcium intake and low BMD [20]. On the other hand, it is not possible to exclude that the association between low calcium intake and prevalence of diabetes

 doi:10.20944/preprints202007.0531.v2

mellitus simply reflects a multi-morbidity condition and/or the socioeconomic status. However, in the present cohort, the BMI and the presence of diabetes did not influence the association between the presence of major fragility fracture and the calcium intake.

The comparison between patients with calcium intake <700 mg/day and >1200 mg/day showed that an adequate daily calcium intake was associated with a lower prevalence of nephrolithiasis, and a higher history of major fragility fractures and of a previous diagnosis of PHPT. The lower rate of nephrolithiasis in patients with adequate calcium intake is in keeping with the amount of data about the protective role against the lithogenic risk of the normocalcic diet [21].

We found an unexpected inverse correlation between calcium intake and prevalence of low BMD and of major fragility fractures (table 1). Indeed, the prevalence of low BMD and of major fragility fractures were higher in patients included the I and II tertiles of calcium intake than in those in the III tertile (i.e. with the highest calcium intake). These differences are no longer present when comparing patients with calcium intake <700 mg/day with the remaining subjects. This is explained by the fact that in the group of individuals with a calcium intake >700 mg/day were comprised all subjects belonging to both the II tertile and the III tertile of calcium intake. In keeping, patients with adequate calcium intake (i.e. >1200 mg/day) unexpectedly showed an increased prevalence of major fragility fractures. Similarly, the finding of an increased frequency of previous PHPT in patients with adequate calcium intake (i.e. >1200 mg/day) as compared to those with a calcium intake <1200 mg/day could be considered unexpected. In our opinion, these findings of a higher frequency of major fragility fractures and of a previous diagnosis of PHPT in patients with adequate calcium intake have a plausible explanation. Indeed, as discussed above, a previous fragility fracture and/or the finding of low BMD have probably encouraged subjects to increase their daily calcium intake. Likewis a past diagnosis of PHPT and the subsequent post-surgical transient hypocalcemia may have contributed to increase the awareness of the importance of an adequate calcium intake in our patients.

Anyway, even though fractured patients have more likely an adequate calcium intake as compared to the not fractured ones, the proportion of fractured subjects with an adequate calcium intake does not exceed the 28%, thus indicating that over two-thirds of fractured patients had an inadequate calcium intake. Finally, the fact that hypercortisolism was not associated with a higher

 doi:10.20944/preprints202007.0531.v2

prevalence of adequate calcium intake confirms the still insufficient awareness in Italy of the importance of and adequate calcium intake for maintaining the skeletal health [22].

The current study has several limitations. Firstly, the cross-sectional design permits to find associations but not to demonstrate a link of causality. Indeed, the recruitment of patients in referral centers for osteoporosis can have introduced a selection bias. Indeed, in some individuals the calcium intake assessed in this study reflects the individual habits before the diagnosis of osteoporosis and/or the occurrence of a fragility fracture. However, many subjects have probably been referred to these centers after the diagnosis of osteoporosis was made by their General Practitioners. Thus, it is not possible to discriminate whether these data reflect the attitude of the Italian General Practitioners in suggesting an adequate calcium intake in patients with at risk for fractures or the individual dietary habits. Secondly, we lack good tools for assessing dietary history and the food frequency questionnaires may not be accurate for estimating dietary intakes. In addition, the questionnaire assessed the dietary calcium intake at the moment of the study enrolment, and, therefore, could not have been informative of the dietary calcium intake in the past. Thirdly, the cohort is rather heterogeneous as it includes not only post-menopausal women, but even a small number of premenopausal women and men (5% and 6.6% of the entire cohort, respectively). As shown, male patients were older and affected by a more severe form of osteoporosis or by a more frequent secondary form than female patients and premenopausal female had more frequently a calcium intake <700 mg/day and adverse reaction to food than postmenopausal ones. These differences should be taken into account when looking to the present data. It should be observed, however, that the inclusion of gender and the menopausal status in the logistic regression model did not influence the association between fractures and calcium intake. In addition, even excluding premenopausal females the results were confirmed.

It is clear, however, that the inclusion of men and premenopausal females in such a study can be questionable. Nonetheless, we still believe that a “real life” study in all patients with a possible low BMD in Italy should be conducted on all subjects referred to our centers. This study design could have consented to obtain some data on the calcium intake in men and premenopausal women, that, nowadays, are lacking. However, we are aware that, given the low prevalence of men and

 doi:10.20944/preprints202007.0531.v2

premenopausal women, even the present study cannot give information regarding these populations. This is why in the present study the menopausal status and gender have be considered as possible covariates rather than objective of the study. Therefore, being over 90% of the subjects females and nearly 95% of them in postmenopausal status, this is essentially an analysis of postmenopausal females rather than a representative sample of the entire community. Given this large imbalance in the sample population, the results of this study cannot be generalized to the entire Italian population. Finally, we have to acknowledge also the following additional limitations of the study: i) the lack of data on vitamin D status in our sample which could have been useful in the interpretation of the results; ii) the fact that DXA machines for bone mineral density were not calibrated across participating canters; however, as these latter were all centers endorsed by SIOMMMS, the quality of their BMD assessment by DXA could be assumed as satisfactory; iii) the lack of data regarding the presence of morphometric vertebral fractures, that could have led to possible incorrect categorization of patients with vertebral fractures.

The strengths of our study are related firstly to the large sample of patients included and to the "real life" design that consented us to describe a real picture of the current calcium intake in a population at risk for osteoporosis. Moreover, the exhaustive information obtained from participants gave us the possibility to describe particular populations at risk of inadequate calcium intake (i.e. patients with diabetes, idiopathic hypercalciuria and adverse reaction to food).

METHODS

Methods

In all individuals height and weight were measured and body mass index (BMI) was calculated.

The dietary calcium intake, expressed as mg/day, was assessed using a specific questionnaire. In particular, usual calcium intake coming from some selected calcium-rich foods was estimated by a 7-day food frequency questionnaire derived for the International Osteoporosis Foundation (IOF) Calcium Calculator [23] after simplification according to the ordinary Italian alimentary habits. Portion sizes were quantified by means of household measures (slices, cups, glasses). The time interval between the questionnaire and BMD measurement was ±3 months. The questionnaire has

 doi:10.20944/preprints202007.0531.v2

been always administrated by investigators blinded to the clinical and BMD data of the patients. Furthermore, in 200 consecutive patients the questionnaire has been administrated simultaneously by two different investigators and the interrater reliability between the two investigators was satisfactory ($\kappa = 0.85$). The history of clinical fragility fractures (i.e. caused by low energy trauma, such as falling from a standing height or less) was investigated at consultation. Hip and vertebral fractures (major fragility fractures) were considered apart from the others (e.g. wrist, ribs and proximal humerus) and were verified by consulting medical records. At variance, the presence of previous fragility fractures other than hip and vertebral fractures was ascertained by self-report and no additional validation of this information was conducted. No spinal radiograph was performed for assessing the presence of morphometric vertebral fractures. If nephrolithiasis was reported by the patients, the medical records (ultrasound and/or abdominal radiograph) were reviewed.

Information about bone mineral density (BMD), measured by performed Dual-energy X-ray Absorptiometry (DXA) using reliable densitometers at lumbar spine, total femur and femoral neck and expressed as standard deviation units in relation to the young (T-score) and age-matched (Z-score) reference healthy population, were collected. In all patients DXA was performed within 12 months before the enrolment and only one BMD determination has been considered. The DXA machines have not been calibrated across participating center and DXA scans had been carried out according to the Italian Ministry of Health recommendations [24]. As our sample included postmenopausal females, premenopausal females and men younger than 50, we used the term “low BMD” in the presence of T-score at any site ≤ -2.5 for postmenopausal women and men older than 50 [25] or in the presence of Z-score at any site < -2.0 for premenopausal women and men younger than 50 [26].

Demographic and clinical data were collected anonymously regarding the following comorbidities: diabetes mellitus, endogenous or iatrogenic hypercortisolism, rheumatoid arthritis (AR), idiopathic hypercalciuria, primary hyperparathyroidism (PHPT), nephrolithiasis, adverse reaction to food (including food allergy or intolerance) [27], inflammatory bowel diseases (IBD) and chronic obstructive pulmonary disease (COPD). Clinical data were confirmed by the review of medical reports. No blood or additional instrumental tests were performed.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2020 doi:10.20944/preprints202007.0531.v2

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved (November 15th, 2015) by the Ethical Committee of each SIOMMMS center and all subjects gave their written informed consent before participating in the study.

Study design and statistical Analysis

Following the available literature [28] we considered a calcium intake <700 mg/day as the threshold below which the dietary calcium intake is clearly inadequate and a supplementation is generally considered mandatory. On the contrary, we considered a calcium intake >1200 mg/day as the threshold for defining an adequate calcium intake, although this threshold is suggested by SIOMMMS [6] specifically in men or premenopausal women. We decided to use this threshold for the sake of consistency in the data analysis, since even men and premenopausal females had been however referred for low BMD and/or fragility fracture and since they represented a minority of the entire cohort (n=119 and n=88, respectively).

Statistical analysis was performed by SPSS version 21.0 statistical package (SPSS Inc, Chicago, IL). In a previous study reporting the daily calcium intake in subjects with and without vertebral fracture [28] the response within each subject group was normally distributed (standard deviation 300) and the difference in daily calcium intake between fractured and not fractured patients was 60 mg/day. On the basis of these data we needed to include 329 fractured subjects and 1316 not fractured (power 90%, type I error 5%) for the study to be adequately powered.

For each continuous variables the normality of distribution was tested by the Kolmogorov–Smirnov test). Data were expressed as median (range) for non-normally distributed continuous variables or as mean±standard deviation for normally distributed variables, and as absolute and relative frequencies for categorical variables. Continuous variables were compared using one-way Student t test or Mann–Whitney U test, as appropriate. Comparison of continuous variables among groups was performed using one-way ANOVA and Bonferroni post-hoc analysis as appropriate. Categorical variables were compared using $\chi 2$ or Fisher's Exact test, as appropriate.

The multivariate logistic regression analysis was performed in order to assess the association between inadequate or adequate calcium intake (categorical dependent variable) and relevant clinical

 doi:10.20944/preprints202007.0531.v2

characteristics (categorical independent variables), after adjusting for possible confounders. The same test was used to assess the association between the present of major fragility fracture (categorical dependent variable) and inadequate or adequate calcium intake, gender, BMI, menopausal status and presence of diabetes (independent variables).

P-values <0.05 were considered significant.

CONCLUSIONS

In conclusion, our data suggest that, to date, an inadequate calcium intake is still highly prevalent in a population with low BMD or at risk for this condition. Educational campaigns should be encouraged to correct the lack of knowledge about the safety and the benefits of an adequate calcium intake and, conversely, about the risks associated with a low calcium intake, particularly in osteoporotic or already fractured subjects

Author Contributions: Conceptualization I.C., C.E-V., S.M., S.G., C.M., S.G., GC. I, M.DS.; Methodology, I.C., C.E-V., S.M., S.G., C.M., GC.I., M.DS; Software, I.C, M.DS.; Validation M.R., I.C., A.G.; Formal Analysis, I.C., C.A., L.G., C.E-V.; Investigation, C.A., L.G., C.E-V., A.G.; Resources, M.A., C.M., S.G.; Data Curation, C.A., L.G., C.E-V. A.G.; Writing – Original Draft Preparation, I.C., E-V.C., M.DS; Writing – Review & Editing, M.R, C.M., S.G., A.G.; Visualization, M.R., C.M., S.G., GC.I.; Supervision, M.R.; Project Administration, M.R.

Conflicts of Interest: The authors declare no conflict of interest

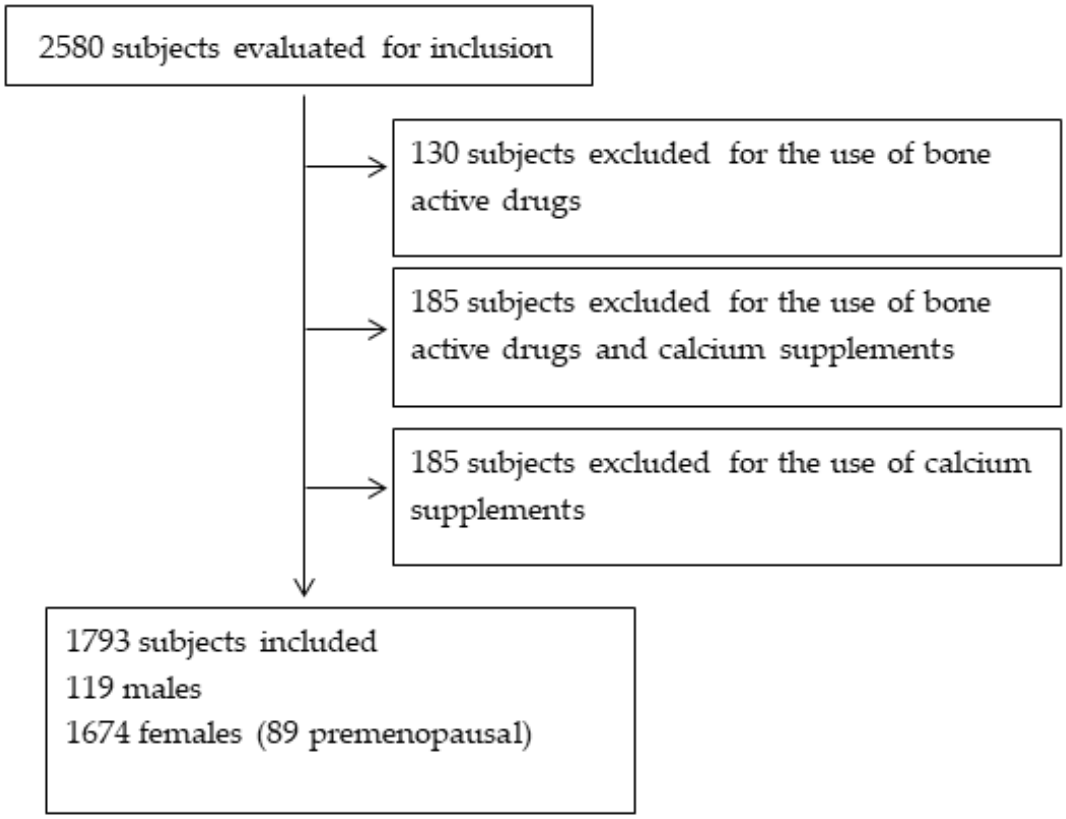
Funding: This research received no external funding

Acknowledgments: the following SIOMMMS colleagues actively participated in the subjects' recruitment:

1. Valter Galmarini, Unit of Orthopedic and Traumatology, Ospedale Fatebenefratelli-Sacco, Milan
2. Giovanni Passeri, Department of Clinical and Experimental Medicine, Center for Metabolic Bone Diseases, University of Parma
3. Fabio Di Salvo, Sanicam Palermo
4. Giangiacomo Osella, Medina Interna, AOU San Luigi Gonzaga, Orbassano, Turin
5. Francesco Tripodi, Center for the Prevention, Diagnosis and Cure of Osteoporosis and Osteoarticular Disorders, Ospedale Fucito di Mercato Ss. A.O.U.Salerno
6. Roberto Valenti, Center for Metabolic Bone Diseases, Unit of Internal Medicine - AOU Senese "S. Maria alle Scotte", Siena
7. Gloria Bonaccorsi, Department of Morphology, Surgery and Experimental Medicine, University of Ferrara
8. Gilberta Giacchetti, Endocrinology Clinic- Azienda Ospedaliero-Universitaria Ospedali Riuniti Ancona
9. Flavia Pugliese, Unit of Endocrinology, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia
10. Andrea Casabella. Dept of Internal Medicine and Medical Specialties, Policlinico San Martino, Genova
11. Bruno Seriolo, Rheumatology, University of Genova
12. Antonio Giovanni Emilio Masala and Pileri Piera Veronica, Istituto Patologia Medica AOU Sassari
13. Letizia Maninetti, Unit of Internal Medicine, ASST-Cremona
14. Alessio De Santis, Casa di Cura Villa Serena, Catanzaro
15. Alfredo Bardoscia, IRCCS Bari
16. Alice Parma, Rheumatology, University of Pisa
17. Paolo Caso and Monica Mazza, University of Rome.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2020 doi:10.20944/preprints202007.0531.v2

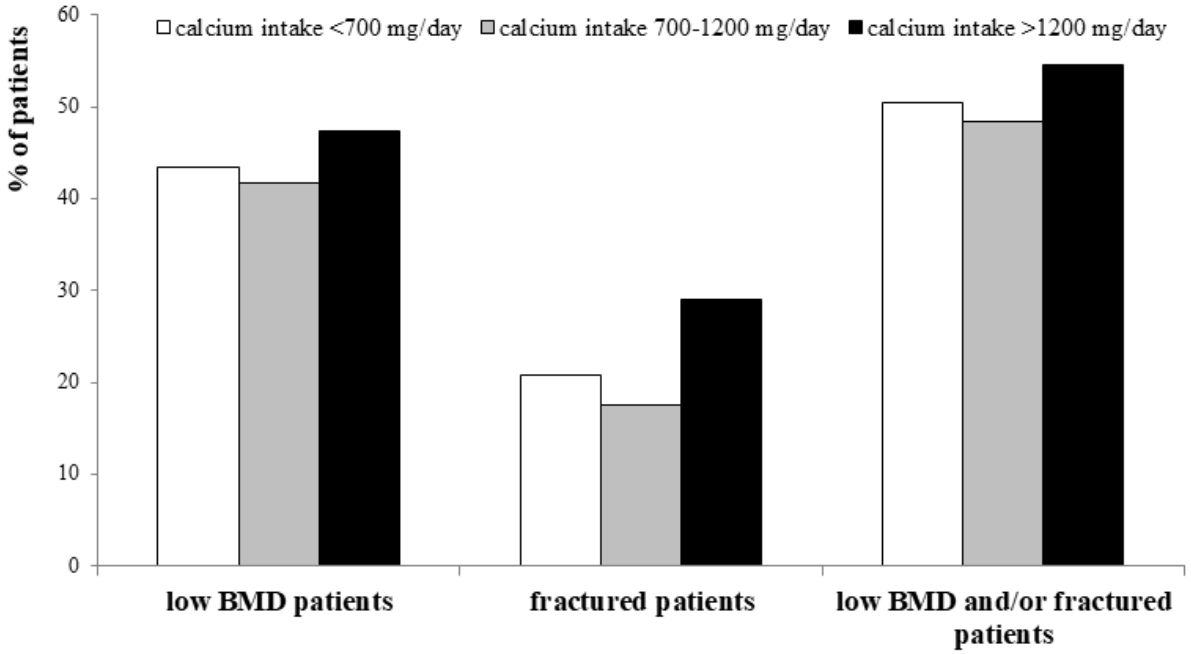
Figure 1: The inclusion protocol



Between November 2015 and June 2016, 1793 consecutive subjects referred in one SIOMMMS referral Centre for Osteoporosis and Metabolic Bone Diseases by their General Practitioners, that agreed in participating in this study, were recruited. We excluded subjects reporting the intake of calcium supplements and/or bone active drugs because these treatments inevitably imply some kind of previous medical counselling about osteoporosis and the related importance of an adequate calcium intake and then estimated dietary calcium intake would have not reflected their usual dietary habits.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2020 doi:10.20944/preprints202007.0531.v2

Figure 2: Prevalence of adequate, intermediate and inadequate dietary calcium intake (>1200 mg/day, 700-1200 mg/day and <700 mg/day, respectively), in 1793 individuals referred to outpatients clinics for osteoporosis of the Italian Society for Osteoporosis, Mineral Metabolism and Skeletal Diseases stratified on the basis of the presence of densitometric low bone mineral density (BMD) or fragility fractures or both.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2020 doi:10.20944/preprints202007.0531.v2

Table 1: clinical characteristics of the whole cohort and the comparisons among individuals grouped according tertiles of dietary calcium intake

	All subjects (n=1793)	**I tertile** (n=598)	**II tertile** (n=598)	**III tertile** (n=597)
Daily calcium intake (mg/day)	874.9	<723	723-1043	>1043
Sex (females)	1674 (93.4)	567 (94.8)	556 (93.0)	551 (92.3)
Premenopausal females	88 (5.3)	41 (7.2)˜	28 (5.0)	19 (3.4)
Age (years)	65.0 (25-97)	64.5 (25-97)˜	65.0 (28-90) ˜	66.0 (27-94)
BMI (kg/m^2)	24.6 (14.2-48.6)	25.1 (14.3-48.6)	24.2 (14.2-43.9)	24.3 (15.1-44.9)
Low BMD	778 (43.4)	253 (42.3)˜	242 (40.5)˜	283 (47.3)
Prevalence of calcium intake <700 mg/day	544 (30.3)	544 (100)	0 (0.0)	0 (0.0)
Prevalence of calcium intake >1200 mg/day	374 (20.9)	0 (0.0)	0 (0.0)	374 (100)
Major fragility fractures	368 (20.5)	119 (19.9)˜	99 (16.6)*	150 (25.1)
Other fragility fractures	334 (18.6)	102 (17.1)	119 (19.9)	113 (18.9)
Low BMD and/or major fragility fractures	901 (503)	297 (49.7)˜	279 (46.7)*	325 (54.4)
Diabetes Mellitus	110 (6.1)	47 (7.9)	32 (5.4)	31 (5.2)
Hypercortisolism (endogenous or exogenous)	137 (7.6)	44 (7.3)	48 (8.0)	45 (7.5)
RA	269 (15.0)	87 (14.5)	93 (15.6)	89 (14.9)
Idiopathic hypercalciuria	65 (3.6)	31 (5.2)˜	18 (3.0)	16 (2.7)
PHPT	30 (1.7)	7 (1.2)	11 (1.8)	12 (2.0)
Nephrolitiasis	132 (7.4)	47 (7.9)	49 (8.2)	36 (6.0)
Adverse reaction to food	90 (5.0)	41 (6.9)˜ç	25 (4.2)	24 (4.0)
IBD	65 (3.6)	27 (4.5)	16 (2.7)	22 (3.7)
COPD	71 (4.0)	22 (3.7)	24 (4.0)	25 (4.2)

Data are expressed as median values (range) or absolute number (percentage). I tertile: 68.3-723.7 mg/day; II tertile 725.0-1042,9 mg/day; III tertile 1043.1-3534.4 mg/day; *p<0.005 vs tertile III; ˜p<0.05 vs tertile III; çp<0.05 vs tertile II. BMI: body mass index. RA: rheumatoid arthritis. PHPT: primary hyperparathyroidism. IBD: inflammatory bowel disease. COPD: chronic obstructive pulmonary disease. Low BMD: T-score at any site ≤−2.5 for postmenopausal women and men older than 50 or in the presence of Z-score at any site <−2.0 for premenopausal women and men <50 years.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2020 doi:10.20944/preprints202007.0531.v2

Table 2: Comparisons between subjects with calcium intake <700 mg/day and >700 mg/day.

	Calcium intake <700 mg/day (n=544)	**Calcium intake >700 mg/day (n=1249)**	***p***
Sex (females)	517 (95)	1157 (92.6)	0.060
Age (years)	65 (25-97)	65 (27-94)	0.170
BMI (kg/m^2)	25.0 (17.3-48.6)	24.4 (14.2-44.9)	0.190
Low BMD osteoporosis	236 (43.4)	542 (43.4)	0.990
Major fragility fractures	113 (20.8)	255 (20.4)	0.860
Other fragility fractures	92 (16.9)	242 (19.4)	0.220
Low BMD and/or major fragility fractures	274 (50.4)	627 (50.2)	0.948
Diabetes Mellitus	44 (8.1)	66 (5.3)	**0.020**
Hypercortisolism (endogenous or exogenous)	40 (7.4)	97 (7.7)	0.720
RA	76 (14.0)	193 (15.5)	0.420
Idiopathic hypercalciuria	28 (5.1)	37 (3.0)	**0.020**
PHPT	6 (1.1)	24 (1.9)	0.210
Nephrolithiasis	45 (8.3)	87 (7.0)	0.330
Adverse reaction to food	39 (7.2)	51 (4.1)	**0.006**
IBD	24 (4.4)	41 (3.3)	0.240
COPD	19 (3.5)	52 (4.2)	0.500

Data are expressed as median values (range) or absolute number (percentage). BMI: body mass index; RA: rheumatoid arthritis; PHPT: primary hyperparathyroidism; IBD: inflammatory bowel disease; COPD: chronic obstructive pulmonary disease. BMD: Bone mineral density. Low BMD: T-score at any site ≤ -2.5 for postmenopausal women and men older than 50 or in the presence of Z-score at any site <-2.0 for premenopausal women and men younger than 50.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2020 doi:10.20944/preprints202007.0531.v2

Table 3: Comparison between individuals with calcium intake <1200 mg/day or >1200 mg/day

	Calcium intake <1200 mg/day (n=1419)	**Calcium intake >1200 mg/day (n=374)**	***p***
Sex (females)	1329 (93.7)	345 (92.2)	0.330
Age (years)	65 (25-97)	65 (37-94)	0.073
BMI (kg/m^2)	24.7 (14.2-48.6)	24.2 (15.1-38.0)	0.063
Low BMD	601 (42.4)	177 (47.3)	0.080
Major fragility fractures	267 (18.8)	101 (27.0)	**0.001**
Other fragility fractures	268 (18.9)	66 (17.6)	0.580
Low BMD and/or major fragility fractures	697 (49.1)	204 (54.5)	0.062
Diabetes Mellitus	94 (6.6)	16 (4.3)	0.090
Hypercortisolism (endogenous or exogenous)	105 (7.4)	32 (8.6)	0.520
RA	219 (15.4)	50 (13.4)	0.320
Idiopathic hypercalciuria	53 (3.7)	12 (3.2)	0.630
PHPT	19 (1.3)	11 (2.9)	**0.032**
Nephrolithiasis	114 (8.0)	18 (4.8)	**0.034**
Adverse reaction to food	78 (5.5)	12 (3.2)	0.070
IBD	56 (3.9%)	9 (2.4)	0.160
COPD	54 (3.8)	17 (4.5)	0.510

Data are expressed as median values (range) or absolute number (percentage).

BMI: body mass index. RA: rheumatoid arthritis. PHPT: primary hyperparathyroidism. IBD: inflammatory bowel disease. COPD: chronic obstructive pulmonary disease. BMD: bone mineral density. Low BMD: T-score at any site ≤ -2.5 for postmenopausal women and men older than 50 or in the presence of Z-score at any site < -2.0 for premenopausal women and men younger than 50.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 14 December 2020 doi:10.20944/preprints202007.0531.v2

Table 4: Comparison of the clinical variables between males and females subjects and between fractured and not fractured patients.

	Females N= 1674	Males N=119	Fractured N=368	Not fractured N=1425
Sex (females)	-	-	334 (90.8)	1340 (94.0)[2]
Age (years)	64 (25-97)[1]	67 (27-89)[1]	71 (27-94)	63 (25-97)[3]
Adequate calcium intake (i.e. > 1200 mg/day)	345 (20.6)	29 (24.4)	101 (27.4)[3]	273 (19.2)
BMI (kg/m^2)	25 (14.2-48.6)	26 (17.7-40.8)[1]	25 (16.5-48.6)	25 (14.2-48.2)
Low BMD	741 (95.2)	37 (31.1)[1]	245 (66.6)[3]	892 (37.4)
Major fragility fractures	334 (20.0)	34 (28.6)[2]	-	-
Other fragility fractures	314 (18.8)	20.0 (16.8)	105 (28.5)[3]	229 (16.1)
Diabetes Mellitus	102 (6.1)	8 (6.7)	25 (6.8)	85 (6.0)
Hypercortisolism (endogenous or exogenous)	22 (6.9)	22 (18.4)[3]	25 (6.8)	112 (7.8)
RA	234 (14.0)	35 (29.4)[3]	42 (11.4)[2]	227 (15.9)
Idiopathic hypercalciuria	60 (3.6)	5 (4.2)	16 (4.3)	49 (3.4)
PHPT	0 (0.0)	30 (1.8)	5 (1.4)	8 (1.8)
Nephrolithiasis	120 (7.2)	12 (10.1)	28 (7.6)	104 (7.3)
Adverse reaction to food	80 (4.8)	10 (8.4)	14 (3.8)	76 (5.3)
IBD	60 (3.6%)	5 (4.2)	13 (3.5)	52 (3.6)
COPD	58 (3.5)	13 (10.9)[3]	21 (5.7)	50 (3.5)

Data are expressed as median values (range) or absolute number (percentage). [1]$p<0.005$, [2]$p<0.05$, [3]$p<0.0001$ males vs females or fractured patients vs non fractured patients. BMI: body mass index. RA: rheumatoid arthritis. PHPT: primary hyperparathyroidism. IBD: inflammatory bowel disease. COPD: chronic obstructive pulmonary disease. BMD: bone mineral density. Low BMD: T-score at any site ≤ -2.5 for postmenopausal women and men older than 50 or in the presence of Z-score at any site < -2.0 for premenopausal women and men younger than 50.

References

1. Weaver, C.M.; Gordon, C.M.; Janz, K.F.; Kalkwarf, H.J.; Lappe, J.M.; Lewis, & R.; O ’karma, M.; Wallace, T.C. The National Osteoporosis Foundation’s position statement on peak bone mass development and lifestyle factors. *Osteoporos Int* **2016**.
2. Mirza F, C.E. Management of endocrine disease: Secondary osteoporosis: pathophysiology and management. *Eur. J. Endocrinol.* **2015**, *173*.
3. Burckhardt, P. Calcium revisited: part I. *Bonekey Rep.* **2013**, doi:10.1038/bonekey.2013.167.
4. National Institutes of Health Calcium fact sheet for consumers. *Off. Diet. Suppl.* **2016**.
5. Royal Osteoporosis Society. Calcium.
6. Rossini, M.; Adami, S.; Bertoldo, F.; Diacinti, D.; Gatti, D.; Giannini, S.; Giusti, A.; Malavolta, N.; Minisola, S.; Osella, G.; et al. Guidelines for the diagnosis, prevention and management of osteoporosis. *Reumatismo* **2016**, doi:10.4081/reumatismo.2016.870.
7. Società Italiana di Nutrizione Umana-SINU LARN – Livelli di assunzione di riferimento per la popolazione italiana: MINERALI. **2014**.
8. Heaney, R.P. Vitamin D and calcium interactions: Functional outcomes. *Am. J. Clin. Nutr.* 2008.
9. Chiodini, I.; Bolland, M.J. Calcium supplementation in osteoporosis: Useful or harmful? *Eur. J. Endocrinol.* **2018**, *178*, doi:10.1530/EJE-18-0113.
10. Balk, E.M.; Adam, G.P.; Langberg, V.N.; Earley, A.; Clark, P.; Ebeling, P.R.; Mithal, A.; Rizzoli, R.; Zerbini, C.A.F.; Pierroz, D.D.; et al. Global dietary calcium intake among adults: a systematic review. *Osteoporos. Int.* 2017.
11. Sette, S.; Le Donne, C.; Piccinelli, R.; Arcella, D.; Turrini, A.; Leclercq, C. The third Italian

National Food Consumption Survey, INRAN-SCAI 2005-06 - Part 1: Nutrient intakes in Italy. *Nutr. Metab. Cardiovasc. Dis.* **2011**, doi:10.1016/j.numecd.2010.03.001.

12. Cano, A.; Chedraui, P.; Goulis, D.G.; Lopes, P.; Mishra, G.; Mueck, A.; Senturk, L.M.; Simoncini, T.; Stevenson, J.C.; Stute, P.; et al. Calcium in the prevention of postmenopausal osteoporosis: EMAS clinical guide. *Maturitas* **2018**, doi:10.1016/j.maturitas.2017.10.004.

13. Eller-Vainicher, C.; Chiodini, I.; Santi, I.; Massarotti, M.; Pietrogrande, L.; Cairoli, E.; Beck-Peccoz, P.; Longhi, M.; Galmarini, V.; Gandolini, G.; et al. Recognition of morphometric vertebral fractures by artificial neural networks: Analysis from gismo Lombardia database. *PLoS One* **2011**, *6*, doi:10.1371/journal.pone.0027277.

14. IOM (Institute of Medicine) *Dietary Reference Intakes for Calcium and Vitamin D. Washington*; 2011; ISBN 9781893997820.

15. Weaver, C.M.; Alexander, D.D.; Boushey, C.J.; Dawson-Hughes, B.; Lappe, J.M.; LeBoff, M.S.; Liu, S.; Looker, A.C.; Wallace, T.C.; Wang, D.D. Calcium plus vitamin D supplementation and risk of fractures: an updated meta-analysis from the National Osteoporosis Foundation. *Osteoporos. Int.* **2016**, doi:10.1007/s00198-015-3386-5.

16. Bruyere, O.; De Cock, C.; Mottet, C.; Neuprez, A.; Malaise, O.; Reginster, J.Y. Low dietary calcium in European postmenopausal osteoporotic women. *Public Health Nutr.* **2009**, doi:10.1017/S1368980008002024.

17. Adami, S.; Giannini, S.; Giorgino, R.; Isaia, G.C.; Maggi, S.; Sinigaglia, L.; Filipponi, P.; Crepaldi, G.; Di Munno, O. The effect of age, weight, and lifestyle factors on calcaneal quantitative ultrasound: The ESOPO study. *Osteoporos. Int.* **2003**,

 doi:10.20944/preprints202007.0531.v2

doi:10.1007/s00198-002-1352-5.

18. Suchy, F.J.; Brannon, P.M.; Carpenter, T.O.; Fernandez, J.R.; Gilsanz, V.; Gould, J.B.; Hall, K.; Hui, S.L.; Lupton, J.; Mennella, J.; et al. NIH consensus development conference statement: Lactose intolerance and health. *NIH Consens. State. Sci. Statements* 2010.

19. Escribano, J.; Balaguer, A.; Roquéi Figuls, M.; Feliu, A.; Ferre, N. Dietary interventions for preventing complications in idiopathic hypercalciuria. *Cochrane Database Syst. Rev.* 2014.

20. Varenna, M.; Binelli, L.; Zucchi, F.; Ghiringhelli, D.; Sinigaglia, L. Unbalanced diet to lower serum cholesterol level is a risk factor for postmenopausal osteoporosis and distal forearm fracture. *Osteoporos. Int.* **2001**, doi:10.1007/s001980170119.

21. Prezioso, D.; Strazzullo, P.; Lotti, T.; Bianchi, G.; Borghi, L.; Caione, P.; Carini, M.; Caudarella, R.; Ferraro, P.M.; Gambaro, G.; et al. Dietary treatment of urinary risk factors for renal stone formation. A review of CLU Working Group. *Arch. Ital. di Urol. e Androl.* 2015.

22. Chiodini, I.; Falchetti, A.; Merlotti, D.; Eller-Vainicher, C.; Gennari, L. Updates in epidemiology, pathophysiology and management strategies of glucocorticoid-induced osteoporosis. *Expert Rev. Endocrinol. Metab.* 2020.

23. International Osteoporosis Foundation International Osteoporosis Foundation (IOF) Calcium Calculator. *https://www.iofbonehealth.org/calcium-calculator*.

24. Gazzetta Ufficiale Serie Generale Fattori di rischio per l'erogazione delle prestazioni di densitometria ossea.

25. Kanis, J.A.; Kanis, J.A. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: Synopsis of a WHO report. *Osteoporos. Int.* **1994**,

doi:10.1007/BF01622200.

26. Baim, S.; Binkley, N.; Bilezikian, J.P.; Kendler, D.L.; Hans, D.B.; Lewiecki, E.M.; Silverman, S. Official Positions of the International Society for Clinical Densitometry and Executive Summary of the 2007 ISCD Position Development Conference. *J. Clin. Densitom.* **2008**, doi:10.1016/j.jocd.2007.12.007.

27. Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes. *EFSA J.* **2014**, doi:10.2903/j.efsa.2014.3894.

28. Warensjö, E.; Byberg, L.; Melhus, H.; Gedeborg, R.; Mallmin, H.; Wolk, A.; Michaëlsson, K. Dietary calcium intake and risk of fracture and osteoporosis: Prospective longitudinal cohort study. *BMJ* **2011**, doi:10.1136/bmj.d1473.