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Osteoporosis prevention in postmenopausal female workers: beneficial effects of silicon dietary supplementation on oxidative status. A pilot study

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Summary. In the last years, the employment of ageing women is increased, and the well-being of these workers, together with the prevention of chronic disabling diseases, is an issue of great importance. Moreover, as postmenopausal ageing is associated with the loss of bone density and consequent increased fracture risk, promoting bone health in these women could be the best strategy for avoiding osteoporotic fractures. We aimed to evaluate the effects of 3-month supplementation with a commercial antioxidant product containing Silica on oxidative status and bone markers in a sample of Italian female workers. Subjects were menopausal and osteopenic women (N=29, age 59.34±6.37, mean BMI 26.19±4.01 kg/m^2). At baseline (T_0) and after three-month treatment (T_1) bone mineral density (BMD) was evaluated by phalangeal osteosonogrammetry. Haematological, serum biochemical parameters, reactive oxygen species (ROS), total antioxidant capacity (TAC), oxydated low-density lipoproteins (oxLDL) and urinary cross-links pyridinoline (PYD) and deoxypyridinoline (DPD) were assessed. Parametric or non-parametric tests were performed at T_0 and T_1. To analyse the possible association between two variables a linear correlation test was performed. At T_0, slightly high levels of ROS (86% of subjects), oxLDL (59%), Total Cholesterol (T-Chol) (90%) and LDL-Chol (59%) were observed, together with suboptimal or deficient 25-OH vitamin D (98%) concentrations. At T_1, oxLDL levels and the ratio oxLDL/LDL-Chol significantly decreased ($p<0.01$). At T_0 significant negative correlations between BMD T-score and cross-links were observed (DPD/Crea: r=-0.57, p=0.001; PYD/Crea: r=-0.45, p=0.01). At T_1, a significant reduction (p=0.03) was observed only for DPD (μg/L) but not for cross-links normalized by creatinine amounts. In conclusion 3-months Silica supplementation improves significantly oxidative status and bone resorption markers in most postmenopausal female workers, representing a complementary treatment for early phases of BMD reduction.

Keywords: osteoporosis, osteoporosis prevention, silica supplementation, female workers.

Abbreviations

Alkaline Phosphatase: ALP, creatinine: Crea, deoxypyridinoline: DPD, HDL cholesterol: HDL-Chol, LDL cholesterol: LDL-Chol, oxidized LDL: oxLDL, pyridinoline: PYD, reactive oxygen species: ROS, total antioxidant capacity: TAC, total cholesterol: T-Chol, total homocysteine: tHcy, triglycerides: TGs, 25 hydroxy vitamin D: 25-OH vitamin D.

Introduction

In many European countries, the employment of women aged 57-64 is increased, and the well-being of these workers, together with the prevention of chronic disabling diseases, has become a topic of great interest. Moreover, as postmenopausal age is associated with the loss of bone density due to a reduction in the estrogenic tone, promoting bone health in these women could be the best strategy for avoiding osteoporotic fractures.

Bone tissue undergoes constant renewal, and this process depends on the coordinated action of osteoclasts, osteoblasts and osteocytes, together with different mediators such as hormones, growth factors and cytokines (1).

Osteoblasts have oestrogen-specific membrane-receptors that act as inhibitors of interleukins and tumour necrosis factor (known to cause oxidative stress). When oestrogenic levels decrease in menopause, bone reabsorption increases, as no longer counterbalanced by bone deposition (2).

While osteoporosis is due to the imbalance between bone resorption and bone formation, osteopenia is characterized by an unbalanced metabolic-nutritional-oxidative status (3,4). The former is preceded by the latter.

Also, osteopenia and osteoporosis are related with oxidative stress, defined as imbalance between Reactive Oxygen Species (ROS) and Total Antioxidant Capacity (TAC).

As bone loss occurs insidiously and is initially asymptomatic, osteoporosis is often diagnosed after the first clinical fracture has occurred. Consequently, an early assessment of the individual risk for osteoporosis is important to prevent the first fracture, and the supplementation of calcium salts, vitamin D and antioxidants are suggested as preventive measures (5,6).

Moreover, the intake of bioavailable silicon (Si), an oligo-element positively associated with BMD in men and pre-menopausal women (7,8,9), might have a beneficial role on bone, and a protective role in atherosclerosis development, due to its effects on collagen-like molecules of blood vessels, by preserving integrity and stability of arterial walls (10,11).

The aim of the present pilot study was to evaluate the possible beneficial effects of a commercial antioxidant suspension containing Si on both BMD and oxidative status on a sample of osteopenic postmenopausal female workers. Data were evaluated at baseline (T_0) and after 3-month supplementation (T_1).

Materials and Methods

Subjects

This study was conducted on a sample of postmenopausal women attending their annual health surveillance visit as part of a workplace health promotion campaign on active ageing, organized by the Occupational Medicine Department, Occupational Unit of Clinica del Lavoro "L. Devoto", Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan (Italy) (12).

The eligibility criteria were: menopause, defined after12 months of amenorrhea following the final menstrual period, according to the Stages of Reproductive Aging Workshop (13), and osteopenia, defined according to the BMD T-scores (as defined in the Instrumental section).

Subjects with BMI>30 Kg/m^2, history of current chronic or neoplastic disease, use of anticoagulant, estroprogestinic or osteoporosis therapies, regular use of antioxidant or other dietary supplements, where considered not eligible for the study.

The selected group comes from the sample of a broader observational cross-sectional study that recruited 385 (291 females and 94 males, age range 18-69 years) consecutive participants, and enrolled them into a periodic occupational examination program in order to test, among other parameters, the seasonal variation of vitamin D status throughout the year in several occupational areas, according to official European ATECO classification (14).

Our study sample was a heterogeneous sample representative of indoor workers from several occupational areas: 52% administration, 32% trading and industry, 4% education, 10% healthcare, and 2% services area.

Among the whole sample, 29 women met the eligibility criteria and were enrolled in the present pilot study.

During the baseline clinical examination, all subjects were asked to fill in a questionnaire on general health, habitual dietary intake and lifestyle (15) (i.e.

smoking history, alcohol consumption, occupation, educational level and socioeconomic status), and anthropometric parameters (age, height, weight and body mass index, BMI) were recorded.

All participants were asked to maintain their habitual lifestyle and dietary habits for the entire period of the supplementation. Compliance with the study protocol was determined by review of patient diaries and returned remaining supplementation at the end of the 3rd month.

No adverse events were observed in any participant throughout the whole study period.

A written informed consent was signed by each participant. The study was conducted in conformity with the Declaration of Helsinki, in accordance to the Good Clinical Practice guidelines and was approved by the Human Ethic Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy (Registration number: 852).

Supplementation and intervention

Participants were provided with Cellfood® Silica Plus (NuScience Corporation, Lancaster, CA, USA), a dietary supplement (118 mL per bottle) containing a mixture of 17 amino acids, 34 enzymes and 78 trace minerals suspended in aqueous solution of deuterium sulphate (D_2SO_4) together with silicon dioxide (SiO_2) (6,6 g silicon/100g Cellfood®). The dietary supplement has been offered free of charge to the subjects by the study group.

All women were instructed to take fifteen drops of the supplement in a glass of low-mineral water two times a day (about 4 mL per day, equal to 60 mg silicon/day), at least thirty minutes before breakfast in the morning and before dinner in the evening, for the three months of the study period.

Due to the high variety of mineral waters in Italy, the study protocol recommended to use a commercial low –mineral water (fixed residue < 200 mg/L).

Subjects' self-reported compliance to the whole study period was more than 95%.

Instrumental

BMD was evaluated by phalangeal quantitative ultrasound (QUS) method at baseline (T_0) and after the three-month supplementation (T_1). As reported by several studies, QUS method (in this protocol, assessed by the portable device DBM Sonic, IGEA, Carpi, Italy) is a relatively recent non-invasive method of estimating bone mineral status in some peripheral skeletal sites (i.e. phalanges of the hand). QUS technique is safe, easy to use, radiation-free (16). The quantitative ultrasound measurement was performed at the distal meta-diaphyseal region of the proximal phalanges of fingers 2 to 5 of the hand, and the results were expressed as T-Score and Z-Score values. Osteopenia was defined for T-score value ranges from -1 to -3.2 (16-18). The device was landed for free to check its feasibility for a preventive occupational campaign.

Blood Samples

At T_0 and T_1 blood specimens from fasting subjects were collected in test tubes, either without additives for serum lipid panel, glucose, calcium (Ca), phosphate (P) and total alkaline phosphatase (ALP), vitamin D, oxidative panel and creatinine (Crea), or with EDTA to prevent coagulation for complete blood count (CBC) and plasma total homocysteine (tHcy). A specimen of whole blood was immediately centrifuged for Hcy assay. T_0 and T_1 serum and plasma samples were frozen and stored at – 80°C for batch analysis at the end of the study.

Urinary Samples

To avoid the influences of circadian rhythm, fasting 2-h morning urine samples were collected from each subject to assess urinary cross-links (PYD and DPD) concentrations.

Analytical and Biochemical Analysis

T_0 and T_1 CBC was performed as routine samples on XE 2100 analyzer (Dasit, Cornaredo, Italy) at the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan (Italy) laboratory. Routine biochemical parameters (serum glucose, complete lipid panel, creatinine, Ca, P, ALP and plasma tHcy) were measured by commercial assays by Modular P (Roche Diagnostics International Ltd, Swiss). Serum oxLDL concentrations were measured by a commercial enzyme-linked immunoabsorbent assay (oxLDL ELISA, Mercodia, Uppsala, Sweden) on the EASIA reader (Medgenix Diagnostics, Fleurus, Belgium) (19,20).

Serum ROS levels and TAC were measured by spectrophotometric method using a commercial kit (dROMs test and OXY-Adsorbent test, respectively, Diacron International, Grosseto, Italy) on F.R.E.E. analyzer (Diacron) as previously described (21,22).

Urinary cross-links (PYD and DPD) concentrations were determined by HPLC method followed by fluorescent detection using commercially available kit (PYD and DPD, Chromsystems Instruments & Chemicals, Munich, Germany) as previously reported (23). The cross-links levels are reported both as normalized by creatinine (pmol/μmol), and as absolute concentration (μg/L). As the cut-off values in μg/L are not provided by the commercially available kit, we referred to those published by using a validated HPLC procedure using a synthesized internal standard on urine samples of 30 healthy, not menopausal women (aged 36±7.1) resulting 31.68 ±9.62 (μg/L±SD) and 190.49±49.63 (μg/L±SD) for DPD and PYD, respectively (24). Vitamin D status was evaluated measuring concentrations of the circulating form 25 hydroxy (OH) vitamin D, using DiaSorin 25-OH Vitamin D TOTAL competitive chemiluminescence immunoassay on an automated LIASON instrument (Saluggia, Vercelli, Italy). Hypovitaminosis D was defined according to the 2011 Clinical Practice Guidelines of the Endocrine Society, according to which 30 ng/ml is the minimum sufficient vitamin D level (25).

Statistical Analysis

Based on samples distribution, Student's t-test for paired data (parametric distribution) or Wilcoxon test (non-parametric distribution) were performed to compare data before and after treatment. The possible association between two variables was analysed by a linear correlation test.

Significance was set for a p-value <0.05. Data, expressed as mean ± standard deviation (SD), were analysed by using GraphPad PRISM (version 6.3) and MedCalc software (26).

Results

Of the sample of 29 subjects, mean age was 59.34 ± 6.37 years old, mean BMI 26.19 ± 4.01 kg/m^2, and mean menopausal age was 51.72± 2.53 years old. 22 women were never-smokers, 2 smokers and 5 former-smokers (who quit smoking for 15 years).

At T_0, based on the results of the standard CBC panel, all women had a normal haematological profile.

Serum 25-OH vitamin D levels (measured only at T_0), showed a hypovitaminosys D in 98% of subjects (mean 25-OH vitamin D 14.6 mcg/L, range 8.9-23,3).

Finger BMD showed a mean T-score -2.01± 0.65.

Table 1 shows the haematological and biochemical parameters of the 29 women at T_0 and T_1.

At baseline, all subjects' glycaemic profile (glucose and insulin concentrations) were within their reference range.

As far as the lipid panel is concerned, T-Chol and LDL-Chol levels were elevated in 90% and 59% of women, respectively. HDL-Chol concentrations were reduced in 38% of them, while TGs levels were within the reference range in most of the subjects. The lipoprotein ratio oxLDL/LDL-Chol was elevated in 55% of the subjects.

As regards the oxidative status, TAC was elevated in 93% of subjects; similarly, elevated ROS concentrations were observed in the majority of the participants. Ox-LDL levels exceeded the cut-off value in 59% of them. About 50% of women showed a slight hyperhomocysteinemia and in the 62% of them creatinine concentrations were slightly elevated.

At T_1, after a 3-month Cellfood® Silica Plus supplementation, as shown in Figure 1, no significant change was observed for anthropometric parameters, CBC nor BMD T-Score mean values (data not shown). Lipid profile did not show any change, except the ratio oxLDL/LDL-Chol, that decreased significantly ($p<0.01$) in most of women. Among all the other parameters, only oxLDL levels decreased significantly ($p<0.01$).

Table 2 shows the bone metabolism profile, where the cross-links values are reported both

normalized by creatinine (pmol/μmol) and as their concentration (μg/L). At T_0, the overall urinary cross-link levels were within their reference range, while PYD/Crea and DPD/Crea resulted altered in 14% and 1% of women, respectively.

A significant negative correlation between BMD T-score and cross-links was observed (DPD/Crea: r=-

Table 1. Subjects' haematological and biochemical parameters evaluated at baseline and after 3-month supplementation.

Analytes (Reference Interval or Cut-off)	T_0 Mean±SD (%)•	T_1 Mean±SD (%) •	p-value
Hb (12-16 g/dL)	13.41 ± 0.65 (0)	13.20 ± 0.72 (0)	N.S. *
HCT (%)	39.69 ± 1.88 (0)	38.98 ± 1.97 (0)	N.S. *
MCV (78-99 fL)	84.18 ± 3.70 (0)	83.77 ± 3.78 (0)	N.S. *
WBC (4-10x10^9/L)	6.25 ± 1.13 (0)	6.26 ± 1.23 (0)	N.S. *
Glucose (70-110 mg/dL)	81.87 ± 6.11 (0)	83.79 ± 7.51 (0)	N.S. *
Insulin (2.6-25 μIU/L)	8.69 ± 3.12 (0)	9.73 ± 4.50 (0)	N.S.
T-Chol (<200 mg/dL)	228.09±29.25 (90)	227.03±32.02 (79)	N.S. *
LDL-Chol (<130 mg/dL)	146.13±29.35 (59)	144.84±35.15 (62)	N.S. *
HDL-Chol (>65mg/dL)	68.25±16.22 (38)	66.13±15.20 (48)	N.S. *
TGs (<170 mg/dL)	99.62±41.09 (7)	95.28±39.32 (3)	N.S. *
TAC (>350μmolHClO/mL)	402±54.36 (7)	397.48±56.83 (17)	N.S. °
ROS (<300 U. Carr)	409.37±69.9 (90)	414.36±48.08 (97)	N.S. °
oxLDL (<70 U/L)	77.15±30.25 (59)	65.19±22.88 (28)	0.01 °
oxLDL/LDL-Chol (<0.50)	0.54±0.20 (55)	0.45±0.10 (16)	0.01 °
tHcy <10.5μmol/L	11.65±1.90 (51)	12.33±1.96 (49)	N.S. °
Creatinine (0.5-1.0 mg/dL	1.08±0.55 (62)	0.83±0.41 (24)	0.03°

In brackets: % of subjects with values out of reference interval or cut off. (* t-test, ° Wilcoxon test).
Abbreviations: WBC: white blood cells, RBC: red blood cells, Hb: hemoglobin, MCV: mean corpuscolar volume, tHCy: total homocysteine.

0.57, p=0.001; PYD/Crea: r=- 0.45, p=0.01). However, when PYD and DPD values were reported as concentrations (μg/L), both PYD and DPD levels exceeded the cut-off. Moreover, both Calcium and ALP concentrations were elevated in 14% of the subjects.
Only DPD concentration (μg/L) showed a significant reduction (p<003), whereas no changes were observed for cross-links normalized by creatinine amounts. At T_1, phosphorous level was lightly increased (p=0.05), whereas no change was observed for both Ca and ALP concentrations.

Discussion

The present pilot study evaluated the potential beneficial effect of a commercially available antioxidant nutritional supplement on bone metabolism and nutritional-oxidative status in 29 Italian osteopenic menopausal female workers, who attended a periodic occupational examination program.

The findings of our study show that the supplementation, enriched with organic Silicon in colloidal form (more bioavailable and easily assimilable), can influence the oxidative stress condition, represented by

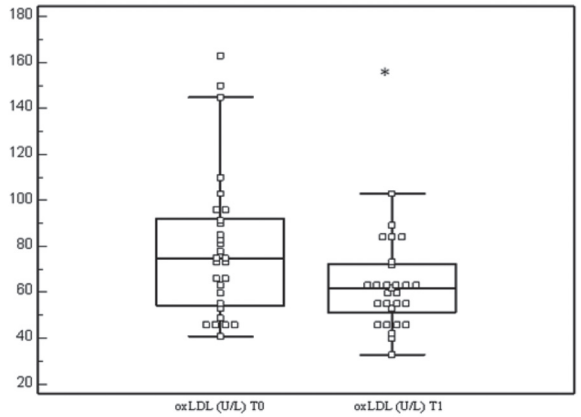

Figure 1. The box plot describes the reduction (p=0.01) in ox-LDL levels (U/L) after treatment, compared to baseline.

a significant reduction of both serum oxLDL levels, but also bone metabolism, as showed by urinary DPD reduction in most of the women.

These preliminary results point out the possible utility of the Silica supplement (containing silicon and several trace minerals as iron, copper and zinc), in the prevention of female workers osteopenia and in buffering their oxidative status condition, a risk factor for various non-communicable chronic diseases.

In the course of the whole life, bone continuously undergoes a process of remodeling, characterized by a fine balance between bone reabsorption by osteoclasts, and deposition by osteoblasts. When a state of imbalance between the two activities occurs, the resulting bone loss may lead to the pathological condition known as osteoporosis. This condition is characterized by reduced bone mass and density and increased fracture risk, and represents the most prevalent metabolic bone disease.

In the last few years, the importance of workers' well-being and the prevention of chronic disabling diseases as Public Health strategy issues, is increasing. In the European countries, the workforce is aging rapidly in all sectors and physically demanding jobs becomes increasingly difficult, especially for female postmenopausal workers (27). Workers' ageing will be accompanied by a progressive reduction in their aerobic fitness, muscle strength and bone density, which means a reduction of life quality (28). In the setting of Occupational Medicine, even a simple preventive intervention (i.e. a nutrient supplementation) in postmenopausal female workers can led to beneficial occupational health perspectives.

Bone matrix consists of an inorganic component (mostly hydroxyapatite), which provides stiffness, and an organic component, the collagen secreted by osteoblast, which supplies tensile strength, ductility and toughness. The collagen structural unit is stabilized by the formation of intra- and inter-molecular pyridinium collagen cross-links such as pyridinoline (PYD) and deoxypyridinoline (DPD), thus allowing aggregation in fibres (29,30).

Table 2. Women' urinary and serum markers of bone metabolism evaluated at T_0 and T_1.

Analytes (Reference Interval or Cut-off)	**T_0 Mean±SD (%)•**	**T1 Mean±SD (%)•**	**p-value**
PYD/Crea (25-83 pmol/μmol)	54.64±25.08 (14)	55.10±16.32 (7)	N.S. °
DPD/Crea (6-23 pmol/μmol)	11,71±5,23 (1)	11,71±3,64 (0)	N.S. °
PYD (190.49±49.63 μg/L)	229.18±129.04 (23)	179.10±128.81 (18)	N.S. °
DPD (31.68 ±9.62 μg/L)	46.21±26.73 (22)	35.61±24.38 (12)	0.03 °
Ca (8.40-10.20 mg/dL)	9.65±0.36 (14)	9.66±0.39 (10)	N.S. *
P (2.7-4.5 mg/dL)	3.62±0.43 (0)	3.76±0.57 (3)	0.05 *
ALP(35-104 U/L)	77.90±25.81 (14)	76.28±21.00 (14)	N.S. °

In brackets: % of subjects with values out of reference interval or out of cut off. (* t-test, ° Wilcoxon test).
Abbreviations: Crea: creatinine, PYD: pyridinoline, DPD: deoxypyridinoline, Ca.calcium, P: phosphate, ALP: bone alkaline phosphatase.

Although bone mineral density is the routine indicator of bone strength in clinical practice, many others biochemical markers of bone turnover could better reflect the status of bone metabolism (31,32). The pyridinium cross-links PYD and DPD are considered good markers to identify an increase in bone resorption during metabolic bone disorders. In particular, PYD is the major cross-link in all connective tissues, whereas DPD is found in high amounts in mineralized tissues. For this reason, DPD is considered more specific for bone collagen degradation than PYD (31-34).

PYD and DPD levels are usually expressed as the ratio of pyridinium crosslinks to urinary creatinine excretion (pmol/μmol). However, as creatinine is produced from the catabolism of muscle proteins, its levels can be affected by various factors (i.e. lean body mass, derangement in muscle metabolism, high or reduced dietary proteins intake, physical activities) (35). Thus, in the present study the urinary excreted cross-links were evaluated both as normalized by creatinine and as their concentrations (μg/L) (as reported in Table 2).

Notably, after Silicon supplementation, a significant decrease in the excreted DPD levels was observed, whereas no significant differences were observed when the crosslinks were related to creatinine. This finding confirms the positive effect of the supplementation on bone. We did not observe a reduction in urinary PYD excretion; this is possibly due to the poor specificity of this crosslink for bone tissue, as it is distributed also in cartilage and synovium (31-33).

The improvement in urinary markers can be justified by the reported action of silicon on collagen synthesis and reduction of bone resorption. *In vitro* studies have shown that physiological concentration of silicon stimulated collagen type I synthesis, and osteoblastic differentiation in human osteoblast-like cells (10,11).

The present findings show that the supplementation with Cellfood® Silica Plus could act on bone metabolism reducing bone resorption.

The significant association between BMD T-score and urinary cross-links observed at T_0, but lost after treatment, might be due to the faster changes in the levels of bone resorption markers than to the T-score modifications (probably occurring after a longer treatment).

In addition, it is possible that the supplementation period was too short. It appears, however, that there was a partial reduction in some of the damaging effects of osteoporosis related to the significant improvement in oxidative alterations, thus suggesting the potential use of nutraceutical treatment to reduce working-related complications.

As expected, the slight increase in ROS remained approximately unchanged. In physiological conditions, ROS are produced in our body (approximately 90% at mitochondrial level) as the result of cellular metabolism and are counterbalanced by a system of antioxidant enzymes and scavenger molecules, able to prevent and stop the chain propagation of radical reactions. The balance between the ROS endogenously produced and their neutralisation by antioxidant defence mechanisms is known as "oxidative status". When ROS concentration is higher than the physiological amount, and TAC is insufficient to neutralise them, a condition known as "oxidative stress" is produced. This condition gives rise to cellular functional and structural alterations that are potentially responsible for various diseases. Oxidative stress can be counteracted by exogenous antioxidant compounds able to reduce the osteopenic and/or osteoporosis risk (19,36).

Also, oxidative stress is considered to be closely associated with osteoporosis. Under physiological conditions, ROS production by osteoclasts is involved in bone remodeling. Post-menopausal reduction in skeletal mass seems to be associated with excessive osteoclastic activity together with decreased osteoblastic action; this is partially due to lower stimulatory effect of estrogens (molecules with antioxidant properties) or to an unbalanced oxidative status (ROS/TAC imbalance).

Moreover, Maziere C. et al. (37) reported a role for oxLDL in bone remodelling by impairing the Receptor Activator of Nuclear factor k B Ligand (RANKL), a cytokine involved in osteoclasts differentiation by preventing the effect of the inorganic phosphate (P) released by bone resorption (38).

Brodeur *et al.* (39) reported that low oxLDL concentrations induced proliferation of osteoblasts whereas high levels were cytotoxic. Thus, the noteworthy findings of the present pilot study suggest that a supplementation with antioxidant could help reduce oxidative stress and subsequently prevent bone loss in postmenopausal women.

Our previous studies in different clinical settings, highlighted that elevated oxLDL concentrations can be a consequence of oxidative stress (19,40). Oxidized LDL are formed by the reaction among LDL with the terminal compounds deriving from the free oxygen radical attach on poly-unsaturated fatty acids.

Another noteworthy point, after the three-month Silicon supplementation, was that the lipid panel remained unaltered, except for the significant decrease in the oxLDL concentrations and in the oxLDL/LDL-Chol ratio.

Oxidized Low Density Lipoprotein cholesterol (oxLDL), is able to generate ROS. As well known, oxLDL play a major role in the formation and progression of the atherosclerotic plaque, and atherosclerosis is often accompanied by osteoporosis (41).

The decrease in oxLDL concentration (Table 1, Figure 1) might be explained by different hypotheses due to the silicon presence in the supplementation, as reported by some authors: i.e. a direct scavenger effect of silicon on free oxygen radicals or its collaboration with enzymes and/or minerals or its inhibiting action on the lipoxygenase activity (42), or its interaction on superoxide dismutase activity (43,44), but this was not confirmed by other authors.

According to Pawlak *et al.* (45), the lipoprotein ratio confirms the beneficial effects of antioxidant in improving the risk of cardiovascular diseases. The oxLDL/LDL-Chol ratio can help predict the degree of clinical benefit in lowering such risk (12,40). Moreover, these markers can be associated with good and balanced antioxidant conditions (TAC).

The oxidative stress condition affects both bone and skeletal muscle structure, thus the beneficial antioxidant effect of the silicon dietary supplementation could explain the significant creatinine decrease (Table 1) (36).

As Silicon and Carbon have the same chemical affinity, we might hypothesize for SiO_2 a reaction between SiO_2 and ROS, as that reported for carbon dioxide (CO_2) (35,42), which contrasts the formation of superoxyde anion-radicals ($O_2^{\cdot-}$) by inhibiting the activity of NADPH-oxydase, the enzyme producing $O_2^{\cdot-}$ (46).

The several trace minerals, essential cofactors for enzymes involved in the synthesis of bone matrix constituents, and present in Cellfood® Silica Plus, could explain the significant increase in phosphorus (P) levels observed after supplementation (as seen in Table 2). The intake of bioavailable Silicon, an oligoelement, is positively associated with bone mineral density in men and pre-menopausal women, even after adjustment for confounding factors (7-9), and the role of silicon in bone formation, mainly due to its promotion of collagen synthesis contributing to prolyl-hydroxylase activity, has been reported elsewhere (10,11).

Therefore, Silicon supplementation could be useful as a preventive or therapeutic agent against osteopenia and/or osteoporosis and, in addition, might have a protective role in the atherosclerosis development, due to its effects on collagen-like molecules of the vasculature by preserving the integrity and stability of arterial walls (17).

In order to better define the women's bone metabolism status, vitamin D levels were measured only at baseline because the supplementation does not contain this vitamin. The fat-soluble Vitamin D is needed for Calcium absorption and bone health. In 98% of the population sample, serum 25-OH vitamin D levels were low and at the end of the study, the subjects diagnosed with low vitamin D levels were supplemented with individual dose of vitamin according to EFSA protocol.

Several findings reported that vitamin D insufficiency is more prevalent than previously thought, particularly among the elderly, among people living in northern latitudes and individuals with poor nutrition. At least a half of the women of the general population present vitamin D deficiency in their midlife, and our findings totally agree with these figures. Consequently, vitamin D deficiency could contribute to the increased risk of osteoporosis, accelerating bone loss.

In conclusion, the present study highlighted that three-months' Cellfood® Silica Plus supplementation improves the oxidative status. These promising results are confirmed by the significant decrease in oxLDL levels and oxLDL/LDL-Chol ratio (a new and more powerful biomarker than the standard lipid assessment). Notably, according to our findings, most of the women who took the supplement, showed a significant decrease in the bone resorption markers and in oxidative parameters of lipid peroxidation over the

supplement period, even if it was for a limited time. Moreover, considering its rapid clearance, silicon may be a potential benefit of long-term intake without excessive retention and accumulation in the body. Therefore, a longer silicon supplementation period could be suggested to obtain a greater effect and could represent a complementary treatment for the early phases of BMD reduction.

The present study has some limitations to be considered. First, it has to be mentioned the reduced number of participants. Indeed, only 29 women met the T-Score criteria of osteopenia and were enrolled. It must be stressed, however, that this is a pilot study, and the promising findings should be confirmed on a larger population. Second, the evaluation of BMD by routine measurement of phalangeal osteosonogrammetry (QUS). In fact, belonging this study to the wider frame of a nutritional promotion program, it was necessary to identify efficient, precise, reliable, cheap and simple indexes applicable in clinical practice and public economic management. Dual energy x-ray absorptiometry (DXA) is the most commonly used technique for bone mineral assessment worldwide but the subject is exposed to ionized radiation. However, taking measurement by DXA at femur and lumbar column as "gold standard", Omodei et al.'s study (47) reported that DXA and QUS showed an agreement in 90% of cases. This means that the agreement between the two methodologies is very high. Moreover, Albanese et al.'s contribution (48) suggested that QUS at the phalanges may represent an index of bone tissue condition usable to evaluate the degree of bone mineralization after estrogen decreases in early postmenopause.

Thus, allowing Occupational and Preventive Medicine and Governance stakeholders to plan strategic preventive measures in order to promote female workers' health and to prevent disabling chronic diseases.

Achieving the goal of extended health-span in the setting of occupational medicine will depend on elucidating and exploiting successful interactions among biological, psychosocial and environmental factors.

Promoting bone health in postmenopausal female workers can be considered part of Public Health primary and secondary life-style preventive strategies, as it can lead to beneficial working health perspective, thus preventing morbidity and improving survival by avoiding osteoporotic fractures.

The observed beneficial effects of intervention with nutraceutical formulations could encourage further investigations.

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