

Alexandria University Faculty of Medicine







# Nutritional risk factors for postmenopausal osteoporosis



# Olfa Berriche, Amrouche Chiraz, Rym Ben Othman<sup>\*</sup>, Hamdi Souheila, Ines Lahmer, Chaabani Wafa, Imen Sebai, Haifa Sfar, Feten Mahjoub, Henda Jamoussi

Abstract Background: Osteoporosis is a bone disease that combines both a decrease in bone den-

Department A, National Institute of Nutrition, Tunis, Tunisia

Received 12 February 2016; revised 26 March 2016; accepted 31 March 2016 Available online 26 July 2016

# **KEYWORDS**

	sity and its internal architecture changes. Nutrition is one of the major determinants of osteoporo-
Osteoporosis; Menopause;	sis.
Risk factors;	Aim: The purpose of our study was to identify nutritional risk factors of osteoporosis of two
Nutrition	groups of osteoporotic women and witnesses.
	Methods: We conducted a comparative cross-sectional study including 60 postmenopausal women
	and screening for osteoporosis by a bone densitometry, recruited the outpatient service of Rheuma-
	tology of the Institute KASSAB.
	Results: We have identified excessive supply of saturated fatty acids (SFA) in the osteoporotic
	compared with controls (13.27% vs 10.23%, $p = 0.002$ ) and an inadequate intake of monounsat-
	urated fatty acids (MUFA) (12.6% vs 16.16%, $p = 0.012$ ).
	A low calcium intake is another factor of risk of osteoporosis (574.27 $\pm$ 336.9 mg/day vs 782.45
	$\pm$ 340.54 mg/day; $p = 0.021$ ). This is explained by the low consumption of milk and milk products
	objectified in the osteoporotic group ( $p = 0.001$ ). We also found a negative relationship between
	inadequate intakes of potassium and osteoporosis $(2241.55 \pm 1049.85 \text{ mg/day vs } 2988.17 + 1146.52 + (1 + 1) + 1146.52 + (1 + 1) + 1146.52 + (1 + 1) + 1146.52 + (1 + 1) + 1146.52 + (1 + 1) + ($
	$\pm$ 1146.52 mg/day; $p = 0.011$ ). This may be due to the low consumption in fruit and vegetables,
	sources of potassium, found in the osteoporotic group ( $p = 0.003$ ).
	We found a significant increase in the consumption of the VVPO group in the osteoporotic toward women witness ( $2.23 \pm 0.99$ number of times/day vs $1.67 \pm 0.76$ number of times/day; $p = 0.019$ ).
	A high consumption of coffee appears also as a risk factor since the osteoporotic group consume
	almost twice than controls ( $p = 0.002$ ).
	<i>Conclusion:</i> Nutritional risk factors of osteoporosis are all the most important that they are edita-
	ble and can take their place in a prevention of public health policy.
	© 2016 Alexandria University Faculty of Medicine. Production and hosting by Elsevier B.V. This is an
	open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

\* Corresponding author. Tel.: +216 52262806.

E-mail address: benothmanr@gmail.com (R.B. Othman).

Peer review under responsibility of Alexandria University Faculty of Medicine.

http://dx.doi.org/10.1016/j.ajme.2016.03.007

2090-5068 © 2016 Alexandria University Faculty of Medicine. Production and hosting by Elsevier B.V.

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

# 1. Introduction

Osteoporosis is a broadcast disease of the skeleton characterized by low bone mass and abnormal microarchitecture, responsible for an increase in bone fragility.<sup>1</sup> Osteoporosis greatly affects the health of postmenopausal women and is recognized as a major public health problem worldwide.<sup>2</sup>

The evaluation of bone mineral density (BMD) surface X-ray absorptiometry dual energy is currently used to retain the diagnosis of osteoporosis and low BMD is the main risk factor for the occurrence of fracture complications.<sup>3</sup>

The determinism of osteoporosis is multifactorial, dominated by genetic factors controlling bone metabolism. Among the exogenous factors, nutrient intakes play an important role appeared to be a target for cheaply therapeutic measures. Besides the calcium and vitamin D, widely known as key components of the metabolic bone, other nutrients may intervene in a non negligible way the changes in bone mass. Thus micronutrients and vitamins other than vitamin D, are substances essential to the success of numerous stages of bone metabolism.<sup>4</sup>

The objectives of our study were to determine nutritional risk factors for osteoporosis by comparing two groups of women with osteoporosis and controls.

#### 2. Methods

This is a comparative cross sectional study involving 60 postmenopausal women, recruited from outpatient in a rheumatology service in Tunis, over a period of three months from December 2014 to March, 2015.

Women over the age 50 and postmenopausal for at least 5 years were included in this study. Women with hormonal diseases (hyperthyroidism, hyperparathyroidism) or inflammatory rheumatic diseases (rheumatoid arthritis, ankylosing spondylitis) or heparin or in some antiepileptic or in oral anticoagulants were excluded from the study.

Our population has been screened for osteoporosis by BMD and was distributed into two groups according to the results of this review.

The "control" group consisted of 30 women with a T-score at the lumbar spine or hip to the upper 1 and group "case" consists of 30 women with osteoporosis with a T-score at the lumbar spine or hip or less equal to -2.5.

All women underwent anthropometric measurements (weight in kilograms (Kg), height in meters (m), the calculation of BMI body mass index).

A food survey was conducted using the recall method 24 h and food consumption frequency specifying the changes in eating habits over the past ten years, the overall caloric intake, the shares of major nutrients (carbohydrates, fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyun-saturated fatty acids (PUFAs), protein, animal protein (PA) and vegetable protein (PV)), mineral intake, vitamins and fiber.

We used a questionnaire, referred to the National Health and Nutrition Program (PNNS), which consists of 20 items that match the majority of foods that may be consumed, gathered in groups 6 groups: milk and dairy products, meat, poultry, fish, eggs (MPFE), Fruits and vegetables, legumes and starchy foods, drinks: coffee, tea and soft drinks, oil seeds and olive oil. $^{5}$ .

In order to convert the intake of food nutrients we performed a manual calculation using the food composition table CIQAUL 2013.

#### 3. Statistic study

We undertook a descriptive statistical analysis of both groups, and a multivariate analysis using SPSS v 17.0 software.

In all comparisons, the level of significance was set at 0.05.

# 4. Results

The average age of the population is 56 years, and it is comparable in both groups. However, the age of menopause is earlier in the osteoporotic group compared with the control group (p = 0.011) (Table 1).

Food survey found that the majority of the population (71.7%) did not change their eating habits over the past ten years, allowing to reflect the nutritional profile of our sample. Thus, we found no significant difference between the two groups (Table 2).

The average calorie intake was similar in both groups; it is  $2131.43 \pm 782.34$  kcal/day in osteoporotic group and 2097.61  $\pm$  706.49 kcal/day in control and follows the recommendations of the AFSSA 2010.<sup>6</sup> As regards protein intake, it is excessive in both groups and PA/PV ratio is greater than 1 (Table 3).

PUFA intakes reduced in osteoporotic group benefit contributions in AGS are high. However, the intake of MUFA, was significantly lower in osteoporotic group compared with controls (12% vs 16.16%, P = 0.012) (Table 3).

The dietary fiber intake was comparable in both groups and is below the recommendations of the AFSSA 2010.

The average calcium intake in osteoporotic group is significantly lower than that of controls (574.27  $\pm$  336.90 mg/day vs 782.45  $\pm$  340.54 mg/day; p = 0.021). Similarly, the average intake of potassium was significantly lower in osteoporotic women compared to controls (2241.55  $\pm$  1049.85 mg/day vs 2988.17  $\pm$  1146.522 mg/day; p = 0.011) (Table 4).

The average daily intake of copper, magnesium and phosphorus is lower in osteoporotic group than in controls, but with no significant difference.

We found a dietary insufficiency of vitamin D in two groups. However, these contributions are more important in comparison with osteoporotic witnesses, but without significant difference (Table 4).

The frequency of consumption of milk and dairy products was significantly lower in osteoporotic group than in controls (p = 0.001). However, daily consumption of meat is more

 Table 1
 General characteristics of the population.

Characteristics	Cases	Controls	р
Age (years)	$56.2 \pm 3.74$	$56~\pm~3.97$	0.8
BMI $(Kg/m^2)$	$27.3 \pm 5.27$	$28.21 \pm 5.21$	P = 0.503
Age at menopause	$43.26 \pm 3.91$	$45.66\pm3.07$	0.011
(years)			

 Table 2
 Change in eating habits before screening for osteoporosis.

			Change in eating habits		Total
			No	Yes	
Group	Controls	Effective	21	9	30
		%	70%	30%	100%
	Cases	Effective	22	8	30
		%	73.3%	26.7%	100%
Total		Effective	43	17	60
		%	71.7%	28.3%	100%

common in women with osteoporosis than in controls. Thus, the total consumption frequency of MPFE group is significantly higher in women with osteoporosis (p = 0.019). As for the consumption of fruits and vegetables, it is significantly lower in osteoporotic group compared to controls (0.002). Caffeine consumption frequency is doubled in osteoporotic group ( $1.23 \pm 0.87/0.61 \pm 0.56$  vs j/d; p = 0.002) (Table 5).

#### 5. Discussion

The intake of saturated fatty acids (SFA) was significantly higher in osteoporotic group compared to controls (13.27% vs 10.23%, P = 0.02). This value is above the recommendations of the AFSSA, which advocates limiting total intake of SFA to 12% of total fat intake.

The intake of monounsaturated fatty acids (MUFA) is otherwise significantly lowered in osteoporotic women (12.60% vs 16.16%, p = 0.012). From a qualitative standpoint, clinical and preclinical studies have demonstrated that the effect of the lipid on the bone varies depending on the degree of saturation and the length of fatty acid chains. Indeed, several studies have shown that a diet rich in saturated fatty acids resulted in a decrease in calcium absorption and decreased bone mineralization.<sup>8</sup> The literature has largely related fat intake and the inflammatory status, a key player involved in bone resorption. Presumably, Lipids have a duality of action, sometimes with the pro and sometimes anti-

	Controls $(n = 30)$ $m \pm \text{ET}$	Cases $(n = 30)$ $m \pm \text{ET}$	Р
Caloric intakes (kcal/J)	$2097.62 \pm 706.5$	2131.44 ± 782.34	0.861
Carbohydrates (g/J)	$259.683 \pm 88.992$	$253.01 \pm 94.64$	0.301
Lipids (g/J)	$82.46 \pm 38.86$	$88.65 \pm 42.60$	0.559
SFA <sup>a</sup> (g/J)	$28.12 \pm 14.23$	$39.21 \pm 22.25$	0.002
(%)	10.23	13.27	
MUFA <sup>b</sup> (g/J)	$44.44 \pm 42.15$	$37.24 \pm 23.25$	0.012
(%)	16.16	12.6	
PUFA <sup>c</sup> (g/J)	$9.89 \pm 12.56$	$12.19 \pm 10.23$	0.389
(%)	3.61	4.13	
Proteins (g/J)	$79.18 \pm 32.29$	$80.37 \pm 40.74$	0.901
Fiber (g/J)	$21.88 \pm 7.23$	$21.72 \pm 9.80$	0.942

<sup>a</sup> SFA: saturated fatty acids.

<sup>b</sup> MUFA: monounsaturated fatty acids.

<sup>c</sup> PUFA: polyunsaturated fatty acids.

	Controls $(n = 30)$ $m \pm \text{ET}$	Cases $(n = 30)$ $m \pm \text{ET}$	Р
	$m \perp \pm 1$	<i>m</i> ± E1	
Calcium (mg/J)	$782.45 \pm 340.54$	$574.27 \pm 336.90$	0.021
Copper (mg/J)	$1.49 \pm 1.51$	$1.13 \pm 0.51$	0.22
Iron (mg/J)	9.11 ± 3.09	$9.03 \pm 4.36$	0.939
Magnesium (mg/J)	$245.53 \pm 74.80$	$232.01 \pm 112.72$	0.586
Manganese (mg/J)	$2.62 \pm 1.09$	$2.79 \pm 1.58$	0.637
Phosphorus (mg/J)	$1426.14 \pm 564.69$	$1212.09 \pm 619.53$	0.167
Potassium (mg/J)	$2988.17 \pm 1146.52$	$2241.55 \pm 1049.85$	0.011
Sodium (mg/J)	$2099.36 \pm 943.61$	$1967.06 \pm 985.11$	0.597
Zinc (mg/J)	$8.19 \pm 3.28$	$7.94 \pm 3.90$	0.783
Vitamin A (ER/J)	$1230.21 \pm 880.1$	$1044.51 \pm 852.99$	0.410
Vitamin B6 (mg/J)	$1.71 \pm 0.79$	$1.48 \pm 1$	0.338
Vitamin C (mg/J)	$141.79 \pm 96.55$	$115.82 \pm 78.13$	0.257
Vitamin K (µg/J)	$15.35 \pm 18.72$	$10.29 \pm 15.29$	0.257
Vitamin D $(\mu g/J)$	$2.68 \pm 4.55$	$2.35 \pm 3.57$	0.754

food groups.					
Foods (number of	Cases	Controls	Р		
times/day)	$(m \pm \text{ET})$	$(m \pm \text{ET})$			
Milk and milk products					
Milk	$0.87\pm0.49$	$1.45~\pm~0.85$	0.002		
Fermented milk	$0.08~\pm~0.07$	$0.17~\pm~0.35$	0.229		
Yogurt	$0.22\pm0.25$	$0.43~\pm~0.31$	0.006		
Cheese	$0.23~\pm~0.14$	$0.45\pm0.32$	0.001		
Total	$1.42~\pm~0.72$	$2.51~\pm~1.04$	0.001		
MPFE <sup>a</sup>					
Eggs	$0.41 \pm 0.27$	$0.35 \pm 0.20$	0.285		
Meat and poultry	$1.32 \pm 0.77$	$0.83 \pm 0.61$	0.009		
Fishes	$0.16 \pm 0.11$	$0.17 \pm 0.11$	0.803		
Liver	$0.08~\pm~0.10$	$0.09~\pm~0.08$	0.751		
Giblets	$0.05  \pm  0.06$	$0.05~\pm~0.06$	0.945		
Total	$2.23~\pm~0.99$	$1.67~\pm~0.76$	0.019		
Fruits and vegetables					
Vegetables	$0.85 \pm 0.61$	$1.28~\pm~0.68$	0.012		
Fruits	$0.81 \pm 0.73$	$1.51 \pm 1.01$	0.003		
Total	$1.66~\pm~1.15$	$2.79~\pm~1.47$	0.002		
Starchy foods and legumes					
Bread	$1.85 \pm 0.78$	$1.53 \pm 0.63$	0.082		
Cereals, pastas	$1.03~\pm~0.59$	$0.812 \pm 0.48$	0.132		
Legumes	$0.33 \pm 0.13$	$0.28 \pm 0.14$	0.189		
Total	$3.22\pm0.98$	$2.63\ \pm\ 0.78$	0.014		
Beverages					
Soft drinks	$0.23 \pm 0.15$	$0.06~\pm~0.07$	0.001		
Café	$1.23 \pm 0.87$	$0.61~\pm~0.56$	0.002		
Tea	$0.65 \pm 0.64$	$0.77~\pm~0.67$	0.487		
Total	$2.12\pm0.86$	$1.45~\pm~0.86$	0.004		
Olive oil	$0.65\pm0.80$	$0.87\pm0.85$	0.293		

Table 5Usual daily frequency of consumption of differentfood groups.

<sup>a</sup> MPFE: meat, poultry, fish, eggs.

inflammatory effects depending on their structures and their metabolisms.

Studies reporting a correlation between the protein intake and bone metabolism showed that an excess of a protein deficiency caused an imbalance in the calcium balance. An increase in protein intake increases the acid load to be removed by the kidney and urinary calcium loss. We can estimate that a doubling of the protein intake increases calcium excretion by 50%.<sup>7</sup> Indeed, the calcium/protein is the most important determinant of bone acquisition during the third decade in women. There is an inverse correlation between a high protein intake and bone loss in women aged over 55 years.<sup>8</sup>

It should be noted however that proteins must be sufficient because a deficit is deleterious on bone metabolism, while any excess not offset by increased intake of alkalizing foods is unfavorable.

In most epidemiological studies, the increased risk of osteoporotic fractures is found in animal protein, and inversely correlated with the consumption of vegetable protein.<sup>9</sup>

The results of a recent study showed among 457 women aged 45–49 years that increased intake of dietary fiber improves bone mineral density. However, other studies have suggested that a diet rich in fiber (more than 40 g/day) may impair the absorption of calcium.<sup>7–10</sup> Meanwhile, epidemiological arguments are rather reassuring about the role of fiber in

the diet: three studies comparing women with vegetarian diets and women with an omnivorous diet showed that women in the first group showed no decrease in BMD.<sup>11-13</sup>

Several studies have examined the relationship between consumption of calcium and bone health. In 1990, a metaanalysis of all published works on the relationship of calcium intakes–bone mass found a positive relationship between the two variables.<sup>14</sup> However, some research suggests that the amount of calcium consumed is only moderately correlated with bone health of the child or the adult<sup>15</sup> because calcium is a threshold nutrient: beyond a swing value between 800 and 1200 mg/day, any additional increase in calcium consumption does not seem to have any effect on bone tissue.<sup>16</sup>

According to Lutz<sup>17</sup> Potassium can act on the bone through its buffering capacity, that is to say its ability to compensate for variations in pH, thus avoiding to request the bone calcium in the event of excess of acidity. In fact, a synthesis of the various recent studies suggests that an adequate intake of potassium promotes renal calcium retention while a potassium diet raises its urinary excretion.

Another study showed among 457 women aged 45–49 years that the potassium intake reduction is associated with an increased risk of osteoporotic fractures.<sup>7</sup>

Vitamin D facilitates active uptake of calcium in the digestive tract. This effect is more pronounced in case of low dietary calcium intakes.<sup>18</sup> According to Dawson-Hughs et al.,<sup>19</sup> vitamin D intake reduces the loss of winter bones and lowers the high levels of PTH. Indeed, in a study carried out over three years among 3270 women with an average age of 60, the extra intake of calcium (1200 mg/day) and vitamin D (20 mg/day) led to both a decrease of bone loss and fracture incidence at the end of the first year.<sup>20</sup>

Over the last thirty years, more than 250 ecological types of observational studies, case-control and prospective have established a relationship between the consumption of fruit and/or vegetables and osteoporosis. In over 80% of them, a protective effect of one or more fruit or vegetable groups was found.<sup>21</sup> A growing number of studies in women describe a protection effect against osteoporosis of fruit and vegetables at any age, recently confirmed by the DASH intervention study that showed a significant decrease in markers of bone turnover related to an inadequate intake of fruits and vegetables.<sup>22–23</sup> Similarly, the Apple intervention study was a reduction of calcium leakage in the kidneys when ingested daily serving of fruits and vegetables pass from 3.6 to 9.5.<sup>24</sup>

Fruit and vegetable consumption would by an alkalizing effect and through various polyphenols to phyto-estrogenic properties protect against osteoporosis. In addition, some green leafy vegetables are significant sources of calcium. It is clear that the available scientific data show a positive association between the consumption of fruits and vegetables and a reduced risk of the disease.<sup>21</sup>

Although the bone protective role of calcium has been widely demonstrated in several studies for decades, none of the studies that have lingered to prove a link between the consumption of dairy products and BMD could not conclude a protective effect of this group food on bone health and the preservation of bone mineral density in adulthood postmenopausal women.

The lack of positive relationships between dairy consumption and BMD could be explained by the involvement of some factors that stimulate or inhibit the absorption of dairy calcium and/or excretion; either a high protein intake, salt, fiber, sugar or caffeine, or a reduced intake of vitamin D, K, magnesium or phosphorus.

A study conducted by the University of California in 2001 found that women who got most of their protein from animal sources had three times more bone loss and almost 4 times more hip fractures than those obtained with the majority of their protein from vegetable sources.<sup>25</sup>

Among the five epidemiological studies that examined the link between red meat consumption and bone mineral density, ten of them have demonstrated the existence of a relationship between a high protein intake and decreased bone mineral density.<sup>26</sup> Most epidemiological studies have highlighted the potential risks from high consumption of meat on the development of osteoporosis. However, other studies suggest that adequate protein intake preserves greater bone mass.<sup>27</sup>

Caffeine is an active substance with important physiological effects. Consumed in adequate amounts, it is a nervous system stimulant that reduces the perception of fatigue. We find caffeine in coffee but also in tea, soft drinks and cocoa. Barger et al have shown an increase in urinary calcium excretion during caffeine ingestion.<sup>28</sup> Moreover, it was observed a positive association between caffeine consumption and risk of fracture at the hip. On the other hand, it has been demonstrated in postmenopausal women consuming about 200 mg/day of caffeine and whose dairy consumption was irregular, a significant decrease in BMD is noticed.<sup>29–30</sup> However, other epidemiological studies found a positive association between tea consumption and bone mineral density. This effect is explained by the polyphenols, phytoestrogens, and fluoride in tea.<sup>31</sup>

## 6. Conclusion

Nutrition is a major determinant of osteoporosis. Thus, awareness of the protective role played by the power saw the emergence of the concept of preventive nutrition that, given its involvement in the acquisition of bone and its subsequent preservation, it is obvious that the recommendations adapted nutrition and nutrition education program targeting the general population and particularly the population at risk would pave the way for true prevention. Indeed, any prevention strategy based on food will seek to overcome any deficiencies and advise protective nutrients. It is therefore essential to promote a diverse diet, following the major food balances, and this throughout the life.

Prevention must be early because it promotes the acquisition of peak bone mass during the growing period and determines the speed of bone loss during aging.

### **Conflict of interest**

Nothing to disclose.

#### References

- 1. WHO Study Group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Geneva: World Health Organisation; 1994.
- Healthy People. *Objectives: draft for public comment*. Washington (DC): Office of Disease Prevention and Health Promotion, Government Printing Office; 2010. p. 1998.

- Johnston CC, Melton LJ, Lindsay R, Eddy DM. Clinical indications for bone mass measurements. *J Bone Miner Res* 1989;4(Suppl. 2):1–28, 2 Jensen GF, Christiansen C, Boesen.
- Coxam Véronique, Wauquier Fabien, Darie Cédric, Spilmont Marie-Jeanne, Davicco Marie-Jeanne, Wittrant Yohann. Huile d'olive et santé osseuse. OCL 2014;21(5):D511.
- Agence française de sécurité sanitaire des aliments (Afssa). Actualisation des apports nutritionnels conseillés pour les acides gras; 2010.
- Tsutsumi R, Xie C, Wei X, Zhang M, Zhang X, Flick LM, et al. PGE2 signaling through the EP4 receptor on fibroblasts upregulates RANKL and stimulates osteolysis. *J Bone Miner Res* 2009;24:1753–62.
- Heaney RP. Bone mass, nutrition, and other lifestyle factors. Am J Med 1993;29S.
- Freudenheim JL, Johnson NE, Smith EL. Relationships between usual nutrient intake and bone mineral content of women 35–65 years of age: longitudinal and cross-sectional analysis. *Am J Clin Nutr* 1986;44:863–76.
- Frassetto Lynda A, Todd Karen M, Morris R Curtis. Worldwide Incidence of Hip Fracture in Elderly Women: Relation to Consumption of Animal and Vegetable Foods. J Gerontol Ser A: Biol Sci Med Sci 2000;55 A(10):585–92.
- Weaver CM, Heany RP, Martin BR, Fitzsimmons ML. Human calcium absorption from whole wheat product. J Nut T 1991;121:1769–75.
- Tesar R, Notelovitz M, Shim E, Kauwell G, Brown J. Axial and peripheral bone density and nutrient intakes of postmenopausal vegetarian and omnivorous women. *Am J Clin Nutr* 1992;56:699–704.
- Lwyd T, Schaeffer JM, Walker MA, Demers LM. Urinary hormonal concentrations and spinal bone densities of premenopausal vegetarian and nonvegetarian women. *Am J Clin Nutr* 1991;54:1005–10.
- Reed JA, Anderson JB, Tylavsky FA, Gallagher JR. Comparative changes in radial bone density of elderly female lactovegetarians and omnivores. *Am J Clin Nutr* 1994;59:11978–2028.
- Cummings SR, Nevitt MC, Browner WS, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. N Engl J Med 1995;332(12):767–73.
- Lanou AJ, Susan E, Berkox D. Calcium, dairy products and bone health in children and young adults: a reevaluation of the evidence; 2005.
- Matkovic V, Heaney RP. Calcium balance during human growth. Evidence for threshold behavior. Am J Clin Nutr 1992;55:992–6.
- Lutz J. Calcium balance and acid-base status of women as affected by increased protein intake and by sodium bicarbonate ingestion. *Am J Clin Nutr* 1984;**39**:281–8.
- 18. INSERM. Stratégies de prévention et de traitement; 1996.
- Dawson-Hughs B, Dallal GE, Krall EA, Harris S, Sokoll LJ, Falconer G. Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. *Ann Internal Med* 1991;115(7):505–12.
- **20.** Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, et al. Vitamin D3 and calcium to prevent hip fractures in the elderly women. *N Engl J Med* 1992 Dec 3;**327**(23):1637–42.
- Amiot-Carlin MJ, Caillavet F, Causse M, Combris P, Dallongeville J, Padilla M, et al., editors. Les fruits et legumes dans l'alimentation. Enjeux et déterminants de la consommation. Expertise scientifique collective, synthèse du rapport, INRA (France); 2007. 80p.
- Prynne CJ, Mishra GD, O'Connell MA, et al. Fruit and vegetable intakes and bone mineral status: a cross sectional study in five age and sex cohorts. *Am J Clin Nutr* 2006;83: 1420–8.
- Doyle L, Cashman KD. The DASH diet may have beneficial effects on bone health. *Nutr Rev* 2004;62:215–20.

- 24. Appel LJ, Moore TJ, Obarzanek E, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med* 1997;**336**:1117–24.
- Lecerf J-M. Fruits et prévention de l'ostéoporose. *Phytothérapie* 2008;6:103–7.
- Kerstetter JE, O'Brien KO, Insogna KL. Low protein intake: the impact on calcium and bone homeostasis in humans. J Nutr 2003;133:8558–618.
- Patureau MP, Remond D. La viande, un aliment vanté ou décrié: un point sur ses propriétés nutritionnelles et sa place dans une alimentation humaine équilibrée. CRA-W&FUSAGx- Carrefour Productions animales; 2008.
- Barger-Lux MJ, Heaney RP, Stegman MR. Effects of moderate caffeine intake on the calcium economy of pre-menopausal women. *Am J Clin Nutr* 1990;52:722–5.
- 29. Hernandez-Avila M, Colditz GA, Stampfer MJ, Rosner B, Speizer WC, Willett WC. Caffeine, moderate alcohol intake, and risk of fractures of the hip and forearm in middle-aged women. *Am J Clin Nutr* 1991;54:157–63.
- Kiel DP, Felson DT, Hannan MT, Anderson JJ, Wilson PW. Caffeine and the risk of hip fracture: the Framingham Study. *Am J Epidemiol* 1990;132:675–84.
- 31. Hegarty VM, May HM, Khaw KT. Tea drinking and bone mineral density in older women. *Am J Clin Nutr* 2000;**71**(4):1003–7.