Parmigiano Reggiano cheese and bone health

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Summary

Osteoporosis is a multifactorial disease characterized by loss of bone mass and microarchitectural deterioration of bone tissue, which leads to a consequent increase in the risk of skeletal fractures. Diet awakes a critical interest in osteoporosis, because it is one of the few determinants that can be safely modified. A healthy well balanced nutrition can play an important role in prevention and pathogenesis of osteoporosis, but also in support of a pharmacological therapy. Numerous evidences have already established that dietary calcium, proteins and vitamin D are essential nutrients for achieved peak bone mass and maintaining skeletal health.

Dairy products, by providing both calcium and proteins, represent the optimal source of highly bioavailable nutrients for bone health. Among dairy foods in particular cheese results one of the major source of calcium in the adults western diet and also in the Italian adults diet.

Parmigiano Reggiano cheese is an homemade Italian food whose denomination "Protected Designation of Origin" is linked to an artisanal manufacturing process in limited geographic area of Northern Italy and is an optimal source of essential nutrients for acquisition and maintenance of bone health. Parmigiano Reggiano is a cheese easy digested, for the presence of ready to use proteins and lipids, lactose free, rich in calcium, with possible prebiotic and probiotic effect. On the basis of its nutritional characteristics and of its easy digestibility Parmigiano Reggiano cheese is recommended in all feeding age groups.

KEY WORDS: Parmigiano Reggiano; bone health; nutrition; osteoporosis prevention; calcium intake.

Importance of nutrition in bone health

Nutrition plays an important role at all age of life in maintaining bone health, protecting against osteoporosis both by its direct involvement in development and maintenance of bone mass and by maintaining normal postural reflexes and soft tissue mass (1). Maintaining optimal nutrition and weight bearing activities have been shown to reduce the risk of osteoporosis by as much as 50% (2). Osteoporosis is a condition of skeletal fragility characterized by decreased bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in risk of fracture (1). Bone mass increases steadily until age 20-30 years, and during the first two decades of life when peak bone mass (PBM) is acquired (2). PBM is the amount of bony tissue present in the skeleton at the end of the skeletal maturation. The achievement of genetically programmed PBM is a fundamental condition for reduction of the risk of osteoporosis later in life and nutrition is a critical factor to obtain the optimal PBM (3).

Calcium along with vitamin D and proteins, are the three essential nutrients that influence the acquisition and maintenance of bone structure. Several reviews on those nutrients intakes have underscored their importance in the prevention of bone loss and, thereby in reducing the risk of fragility fracture in elderly (4). Dairy products, by providing both calcium and proteins, can be expected to play a positive role on bone health. They are polyvalent foods containing many essential nutrients and their effects on bone health may result to be more than the sum of the single elements. As calcium is often deficient in the diets of industrialized countries, milk and dairy products could represent an ideal source of this mineral (1). Many Authors observed that optimizing calcium intake in children and adolescents, either through dairy products or supplements, BMD increases by 4–8%, depending on the study and the skeletal site measured (5, 6).

Dietary protein, including that from dairy products, influences physiological calcium balance. An adequate protein intake is important for supporting bone growth in children and maintaining bone mass in older adults (7).

High dietetic protein intake has long been considered a risk factor for osteoporosis indeed increasing protein intake may increase acid production, renal acid excretion and consequently increase calcium losses by urinary calcium excretion (8).

Interestingly as reported by Rizzoli (9), all studies assessing a possible association between bone mass measured at various skeletal sites and protein intakes, have found a positive and not a negative relationship in children or adolescents, premenopausal or postmenopausal women, as well as in men. Even in relation to increased risk of fractures, a prospective study carried out on more than 40,000 women in Iowa, shows that higher protein intake was associated with a reduced risk of hip fracture. A low (0.7 g/kg body weight) but not a high protein intake (2.1 g/kg) was associated with an increase in biochemical markers of bone turnover compared to a standard diet containing 1.0 g/kg of proteins. As reported in the review of Jesudason and Clifton, dietary protein has been shown to be more effective on bone health later in life. There are important differences between how elderly and younger individuals metabolize protein. The anabolic effect of dietary protein is reduced in the elderly, so that the amount of protein needed for anabolism is greater and would exceed the 0.8 g/kg/day that is the recom-

mended daily intake. Recommended Dietary Allowance (RDA) of dietary protein be increased to 1.0–1.2 g/kg day in the elderly to maximize its anabolic effect on muscle and bone (10).
As postulated by Rizzoli (9) at higher protein intake the role of calcium in maintaining bone mass becomes less significant, suggesting that calcium requirements for optimal bone growth could be lower at high levels of dietary protein.
The World Health Organization's recommendations for preventing osteoporosis, published in 2003, recommend a minimum intake of 400 to 500 mg/day of calcium for people who live in countries with a highest incidence of fractures and over 50 years (7).
In Italy the average RDA of calcium by LARN levels is about 900 mg/day (from 800 to 1000 mg/day) for children and adults, rising to 1200 mg/day for adolescents and the elderly (11).
These daily recommended amounts are safety levels designed to maintain bone health in adults and to ensure the achievement of an optimal peak bone mass genetically programmed in children and adolescents, essential condition for delaying the onset of osteoporosis or its complications.
The INRAN (Research Institute for Foods and Nutrition) data report on Food Consumption Survey 2005-06, shows that children aged 10-17 years (n=108) consume about 170 g/day of milk, and 13.5 g/day of yogurt, which represent 10-12% of total diet (12).
As reported by Lombardi-Boccia et al. (13), in the Italian diet the average of Ca daily intake is about 738 mg/day and milk-and-dairy foods make the greatest contribution.
In a balanced western-style diet, about 60% of dietary calcium should come from milk and dairy products, 20% from fresh vegetables and dried fruits and the remaining part from drinking water or other discrete sources. There are several studies that showed the importance of phosphorus, proteins (especially casein), potassium, magnesium, zinc, vitamin D, vitamin A, and vitamin C, in influencing the calcium physiological balance and milk and dairy products are the main food sources for most of these nutrients (1, 14-16).
Vitamin D plays a fundamental role in the calcium homeostasis, in particular being essential for the absorption of calcium and phosphate and far enhancing bone mineralization (17). It's a key hormone for bone growth and bone mineralization throughout life, in fact, a Vitamin D deficiency can lead to rickets in children or osteomalacia in adults. In adults, during the summer months, exposure to sunlight for 10 to 15 minutes a day, leads to production between 10 000 and 20 000 IU of vitamin D.
Recent estimates suggested that the prevalence of vitamin D deficiency among infants, children, and adolescents is between 12 and 24 percent, with vitamin D nutritional intake often being below the recommended daily intakes (5-15 µg) (11). In this contest an optimal vitamin D intakes reached with nutrition and/or use of supplements during all age of life become necessary (18).

Bioavailability of calcium

The bioavailability of calcium may be defined as the fraction of dietary calcium that is potentially absorbable by the intestine and can be used for physiological functions, particularly bone mineralization, or to limit bone loss (19).
Before it can be absorbed calcium must be dissolved in the acid medium of the stomach, usually in the ionized form (Ca^{++}) or bound to a soluble organic molecule to cross the gut wall. Total calcium absorption is the result of two processes, active transport and passive transport. The active transport, which occurs across cells mainly in the duodenum, is saturable, regulated by dietary intake, and influenced by Vitamin D hormone in several steps. The passive diffusion occurs via intercellular junctions, or spaces, primarily in the ileum and very little in the large intestine, and following an electrochemical gradient. These mechanism is not saturable and increases with dietary intake, provided that the calcium in the intestines is in an absorbable form. It is independent of vitamin D and age.
Several dietetic molecules make calcium soluble or keep it in solution within the ileum, particularly milk proteins, like phosphopeptides derived from casein, and amino acids like L-lysine and L-arginine. Indeed all molecules that increase the osmolarity of the liquid in the ileum are likely to stimulate the passive diffusion of calcium (19).
Calcium retention in the organism can also be conditioned by other factors in addition to mechanisms of absorption. These can be of two kinds: individual and physiological factors or extrinsic and dietary factors (20). In fact, the potential absorbability of calcium depends on the type of food, while the real calcium absorption depends primarily on the absorptive capacity of the intestines, which is affected by physiological factors such as calcium reserves, hormonal regulation or previous dietary calcium supply.
The concept of "availability of calcium" for absorption by the intestines, is often used as a synonym for bioavailability, however, it is no more than the first step towards bioavailability. So, calcium bioavailability depends on absorbability and deposition of calcium into bone (19). Calcium intake, turnover, absorption and excretion rates determine the availability of calcium for bone growth and development. During infancy and adolescence, the need for calcium and the rate of absorption are higher than during other ages, mainly due to the need to achieve the optimal peak bone mass (21).
Some types of foods increase the likelihood that calcium is adsorbed on bone, instead for others the result is that calcium is mainly excreted in the urine. For instance, calcium absorption is influenced mainly by calcium intake, vitamin D, dietary fibres, phytates, oxalates, fats, bioactive peptides and lactose (22).
The effects of small changes in the diet on the net calcium balance, have been emphasized by several studies. Thus, certain anions, such as sulphate and chloride, organic ligands (chelators) and protein or sodium excess, all increase the loss of calcium in the urine and, thus, hinder its incorporation into bone. Conversely, the incorporation of absorbed calcium in bone is stimulated by phosphorus (19).
Rich-fiber foods can affect calcium bioavailability, having a negative effects on its absorption. As reported by Camara-Martos et al. (20) an *in vitro* study with polysaccharides, classified as soluble fibre, shows that the polysaccharides with a low content in sulphate and carboxyl groups, presented a low response for binding cations, and the response was greater in polysaccharides with a high content of sulphate or carboxyl groups. Pectins bind many mineral which are gradually released in the colon when fibres are degraded by bacterial fermentation. This fermentation of dietary fibres results in an increase of calcium and magnesium absorption. On the contrary, the inhibitory effect on mineral absorption performed by phytates, present in seeds, cereals, legumes and some vegetables, is due to the formation of insoluble complexes with them and can be prevented by adding oligosaccharides to the diet. Indeed oligosaccharides have a prebiotic effect that not only increased the absorption of calcium and other minerals, but also inhibited the negative effects of phytic acid. The dietetic oligosaccharides are not degraded in the small intestine and arrive intact to the colon where are fermented by the bacterial flora. The gases and organic acids developed by fermentation decrease the pH and make the calcium more soluble. Moreover the organic acids improve the absorption of calcium in the colon, as shown in studies on rats, by forming low-charged mineral complexes, thus facilitating the transport by passive diffusion of ions like calcium (20).

The importance of caseinophosphopeptides (CPPs) in dairy products

Calcium physiological balance is known to present a close complex relation with dietary protein. A variation in the bioavailability of calcium in milk and dairy products, in relation to their protein composition, has been demonstrated in several studies on the effects of casein and some phosphopeptides formed during the digestion of casein.

In cow's milk, calcium is principally associated with casein that forms large micelles and contains calcium phosphate in a colloidal state. Probably, the type of micelle formed affects the digestibility and precipitation of calcium in the gastrointestinal tract and, subsequently, its bioavailability (20). The high calcium bioavailability in cow's milk probably is the result of the high calcium content as well as its binding with milk casein. Similarly in cheese and other dairy foods the presence of casein and its derived phosphopeptides enhance calcium absorption and bioavailability.

The caseinophosphopeptides (CPPs) are a mixture of different molecular weight peptides released by enzymatic hydrolysis of milk casein in the gastrointestinal tract and are partially responsible for the high Ca bioavailability of milk and dairy products. Several studies show that CPPs inhibit calcium phosphate precipitation in the intestine and enhance calcium absorption because, by binding with the mineral keep it in a soluble form, thus making it bioavailable and promoting its passive absorption (22). This passive transportation system allows primary absorption of calcium that is required for the calcification of bones. CPPs form the organophosphate salts combined with trace elements important for bone health, such as Fe, Mn, Cu and Se (23).

First studies on CPPs were conducted by Mellander and colleagues in the early '50s when have been used bioactive casein phosphopeptides for treatment of rickets in children. The authors showed that, also in the absence of vitamin D, the CPPs derived from the intestinal digestion of casein, enhanced bone calcification in rachitic children (24). More recently Bennet et al. (25) showed that Ca absorption was enhanced by high-casein meals (i.e. 500 g/kg diet) in rats, also if the mechanism remains unclear.

In 2001 Erba et al. (26) studied, in an animal model, if there was a variation in intestinal calcium absorption by different sources testing two calcium complexes: Ca-caseinphosphopetides and $CaCl_2$, in the absence and presence of phosphate. The authors concluded that the positive effect of caseinophosphopeptides could not only be the result of the enhancement of calcium solubility in the intestinal lumen, but also to the possible protector effect of caseinophosphopeptides against antagonist interactions between calcium and other mineral elements (20).

Still in 2002 the same authors (22) evaluated the optimal CPP/Ca ratio that maximizes passive Ca absorption. The CPP/Ca ratio of 15 (w/w) was identified as the optimal ratio for increasing Ca transport across the distal intestinal tract. The results showed that Ca absorption increases independently of the Ca concentration when the CPP/Ca ratios ranged from 5 to 15. Conversely, when CPP/Ca ratio exceeded 15, an opposite effect occurred. The excess CPPs might have formed larger complexes that hid the Ca ion and, hence, impaired mineral release and decreased its availability.

Nutritional characteristics of Parmigiano Reggiano: a functional food for bone health?

In the primary prevention of osteoporosis, an adequate nutrition and a daily physical activity are important determinants for bone health throughout life. Several intervention trials have demonstrated a favourable influence of dairy products on skeletal health and growth during childhood and adolescence (6, 9, 16, 27), in particular cheese is an optimal source of all essential nutrients for acquisition and maintenance of bone health and is the major source of calcium in the adults western diet.

Among cheeses, Parmigiano Reggiano (P-R) stands out for its high nutritional value. P-R cheese is a homemade Italian food, made from raw cows' milk produced in a limited geographic area in Northern Italy (provinces of Parma, Modena, Reggio-Emilia, some areas of Bologna and Mantua) (28). It is a hard-textured, cooked and long-ripened cheese for at least 12 months and rarely less than 15-18 months before consumption. The milk comes from cows whose diet is mainly composed of green fodder and hay in the area of origin, without the use of silage.

The transformation of milk in P-R is still based on craft technology. The raw material is obtained by commingling (approximately 1:1) the partially skimmed evening milk and the full-cream morning milk. The key features of the cheesemaking process are: the maturation of the evening milk, the use of natural whey starter cultures, the use of calf rennet, a ripening period from 12 to 24 months and the fact that the use of additives is strictly forbidden (29, www.parmigiano-reggiano.it) (30).

The reduced water content of the cheese (30% approximately) and the presence of as many as 70% of nutrients, first of all protein and fat, can explain the high energy value, equal to 388 kcal per 100 grams of product.

Protein fraction, on the average of 33% of the total component, represents the most important component of P-R cheese it is characterized by its own high quality in essential amino acids and its easy assimilation. The amino acid composition is ideal for the absorption due to the profound changes that the protein fraction undergoes during the long maturation period, which contributes to the separation of the milk casein into compounds of molecular weight smaller and smaller and finally into free amino acids (about 25% of total nitrogen) (31). It is very important to emphasize the role of bioactive peptides, the casein-phosphopeptides (CPP), which are released during the proteolytic maturation from the milk casein and show a key role in stimulating the intestinal absorption of calcium, primary action to maintain bone health.

P-R contains many minerals (calcium, phosphorus, sodium, chloride) and trace elements (in particular zinc and selenium). P-R has a very high content of calcium, equal to 1159 mg/100 g of product (with a calcium/phosphorus ratio around 1:7) highly bioavailable due to the presence of mineral in the form of lactate, both, as seen above, for the synergy with casein-phosphopeptides. From this point of view, the P-R is an excellent food supplement for bone health, we know that 50 gr of P-R cover about 75% of the calcium requirement for adults, up to 60% of the recommended amount for children and 45% of the amount recommended for women over 50 years of age.

The fat content, on average 28%, linked to the use of semi-skimmed milk, is in line with the commodity definition of half-fat cheese and the fatty acid composition reflects the composition of milk fat, with a ratio of saturated fatty acids and unsaturated 3:1. Also the contents of cholesterol is relatively modest, amounting to an average of 83-91 mg per 100 grams of cheese (31). The total lipoid components and triglycerides fraction is represented by a relatively high concentration of medium and short chain fatty acids, which are directly absorbed in the intestine and rapidly usable as a significant source of energy. During the maturation of the cheese, the lipoid component undergoes significant modifications caused by a partial lipolysis which produce a fatty acids fraction available in the free form, facilitating their absorption (32).

Another important characteristic of P-R is the total absence of lactose, the principal carbohydrate present in milk. The lactose disappears during the first hours after the cheesemaking process. On the basis of the standards of the European Commission (April 2003) P-R cheese can be defined as "lactose-free" product, because it contains 0.10 mg/100 kcal of lactose (32). Lactose intolerance is a very common disease in Western populations and causes a calcium intake below requirements, due to the elimination from the diet of milk and cheese. Several lines of evidence suggest that individuals with lactase deficiency can achieve adequate calcium intake with consumption of P-R cheese, thus enhancing bone health and preventing osteoporosis (31).

With regard to the carbohydrate fraction, is very important to note the presence of some oligosaccharides that derive not only from those normally present in cow's milk, but also from the action of the complex fermentative digestive processes that take place during the cheesemaking process. These oligosaccharides are non-digestible short-chain carbohydrates, with a possible prebiotic function that may have the ability to stimulate growth and/or activities of one or more bacteria in the colon by exerting beneficial effects on health. Prebiotics act as selective substrates for fermentation and influence microbic activity and the absorption of minerals in the intestine, as well as promoting immune response (32).
In 1989 Fuller defined the "probiotic food" as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance" (33). Pancaldi M. *et al.* (32) also underline the probiotic nature of the P-R cheese and its possible use in the intestinal and extraintestinal pathologies at all age, from newborn to elderly.

Conclusion

In conclusion Parmigiano Reggiano cheese can be considered a "functional food" because of some nutritional characteristics that be able to produce beneficial effects on health and consumer welfare. In particular we can be noted that P-R is an easy digested cheese for the presence of "ready to use" proteins and lipids, lactose free, calcium rich, with prebiotic and probiotic effect. For its high nutrient content and high digestibility Parmigiano Reggiano is a cheese recommended in all feeding age groups. Moreover P-R is a very important food for skeleton health: it is an optimal source of many essential nutrients for the acquisition and maintenance of bone health such as proteins, minerals and vitamins. The presence of high biological value protein and calcium highly bioavailable, make the Parmesan cheese a "functional food" for bone health and osteoporosis prevention.

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