Calcium metabolism and vitamin D in the extreme longevity

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Abstract

Skeletal remodelling is a continuous process during life and is still active also in extreme senescence. In the elderly, bone resorption often prevails over bone formation, causing bone loss and fragility. Elderly subjects are exposed to the risk of fractures, and loss of self-sufficiency, if considering that the proximal femur is the most frequently involved site. Bone remodelling can maintain circulating calcium within physiological ranges, at the expense of a substantial loss of this ion from the skeleton, particularly during senescence. Calcium metabolism is regulated at cellular/molecular level by a network of cytokines, growth factors, systemic hormones that act on bone in paracrine/autocrine/systemic fashion. Among the molecules involved in bone metabolism, parathyroid hormone (PTH) and vitamin D present some peculiar aspects during senescence. The osteometabolic features in a consistent group of centenarians have been evaluated. It results that a severe hypovitaminosis D was present in 99 out of 104 centenarians (25-OH vitamin D below 5 nmol/L), and that it plays an important role as a factor inducing a vicious circle involving hypocalcemia, secondary hyperparathyroidism, together with biochemical features indicating a consistent bone loss. Serum C-terminal cross-linking telopeptide, a specific marker of bone resorption was elevated in 92% of these subjects. Moreover, it has been found that several femoral fractures had occurred after 90 years of age. These data offer a rational for the possible prevention of elevated bone turnover, bone loss and consequently the reduction of osteoporotic fractures and fractures-induced disability, in the oldest olds, through the simple supplementation with calcium and vitamin D.

Keywords

1. Introduction

Considering the fast increase of life expectancy worldwide and in the developing countries in particular, the maintenance of self-sufficiency is of extreme importance in the oldest olds. Among those able to reach the extreme limits of life, centenarians represent an example of successful aging, due probably to their genotypes and to peculiar neuro-immuno-endocrine mechanisms (Mariotti et al., 1992; Sansoni et al., 1993; Franceschi et al., 1995; Mari et al.,

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[1]For IMUSCE see Appendix 1.

1996; De Benedictis et al., 1997; Baggio et al., 1998; Deiana et al., 1999; Fagnoni et al., 2000).

It is true that prevention of bone fragility fractures is of pivotal importance at all ages, but this is even more important in the oldest olds, since a fracture, especially of the hip, often means the permanent loss of walking ability, and of self-sufficiency (Passeri, 1991; Mariotti et al., 1992; Johnell and Kanis, 2006). Elderly subjects are at risk of fractures for several reasons: falls are of primary importance, but a relevant role is also played by loss of bone mass with subsequent reduction of bone strength (osteoporosis). Insufficient dietary intake of calcium, low physical activity, systemic diseases and medical treatments affecting bone, hormonal status, and finally, low circulating levels of vitamin D are all co-morbidity factors (Cooper et al., 1992; Rosen and Kiel, 1999; Martini and Passeri, 2000; Heaney, 2004; Sambrook and Cooper, 2006). The skeletal remodelling, consisting of a continuous process of bone resorption of the pre-existing bone followed by bone formation, remains active even after 80 years of age. However, it is evident a disequilibrium of these two processes, with a prevalence of bone resorption, especially in the elderly (Passeri, 1991; Marcus et al., 1995; Martini and Passeri, 2000; Sambrook et al., 2006). It has been demonstrated, above the age of 60 years, a clear prevalence of bone resorption, while bone formation is of varying extent, even if several metabolic parameters (e.g., serum osteocalcin) are also increased (Ross and Knowlton, 1998). Some changes characterizing aging at the cellular level, are due to a reduced numbers of fully functional cells, accompanied by impaired matrix production, cellular composition, and bone micro-environments, and altered responses to the environment (Carrington, 2005).

Nevertheless, bone remodelling is closely correlated with calcium exchange, and is strongly influenced by the actions of vitamin D (Burckhardt, 2002). As it is well known, calcium is an ion of fundamental importance for all living systems, and it has a great number of functions at both intra- and extra-cellular levels (e.g., blood coagulation, function of adhesive molecules, muscular contraction, second messenger functions in the cells, etc.) (Bringhurst, 1995). In bone, calcium is of basic importance for skeletal physical resistance and as a deposit from which the organism can mobilize calcium when needed. The extra-cellular concentration of Ca^{2+} ions is in the order of 10^{-3} M, while in the cellular cytoplasm is only around 10^{-6} M. Free cytoplasmatic Ca^{2+} may quickly and largely changes its concentration (up to 100 times), as a consequence of the binding of primary extra cellular messengers to the respective receptors on the membrane of the target cells. Among the fractions of the circulating calcium (bound to proteins: about 40%; complexed mainly with citrate and phosphate ions: about 10%; and the rest is ionized) (Bringhurst, 1995), the ionized fraction is the most important from a physiological point of view and it is maintained in a very narrow range by the combined effects of different hormones, in particular PTH, $1,25(OH)_2D_3$ and calcitonin (Parfitt, 1993).

These are the main factors able to control the global balance of calcium in the body, by regulating the bi-directional calcium fluxes between the gastrointestinal system, kidneys, and bones, toward the extra cellular calcium pool (Bringhurst, 1995). Nevertheless, other hormonal factors, whose secretion is not directly regulated by calcium circulating levels, have a considerably importance in calcium exchanges and, eventually, for bone health: sexual steroids, thyroid hormones, glycocorticoids and growth hormone (GH) are the most important (Marcus et al., 1995; Raisz, 2005).

2. Calcium metabolism

Intestinal calcium absorption is determined by the amount of calcium present in the food and by the absorptive capabilities of the intestine itself, at the level of duodenum and upper part of the jejunum. When the amount of calcium introduced with diet is less than 200 mg/day, the net intestinal absorption is practically zero, since approximately the same quantity is daily

secreted into gastrointestinal lumen, and lost with the feces. When dietary calcium is above 200 mg/day, the absorbed amount will vary between 15% and 40%, through both passively and actively regulated transport mechanisms. The latter are mostly regulated by the circulating levels of $1,25(OH)_2D_3$ (Sheikh et al., 1988).

The most important regulatory site of calcium excretion is the kidney. Serum Ca^{2+} is filtered at the glomerular level (about 10 g/day), and then is almost completely reabsorbed (about 9.85 g/day), and only about 1–3% of filtered calcium is excreted with the urine. This mechanism is regulated by PTH (Suki and Rouse, 1996). Calcium is bound in the bone to the connective tissue fibres, (the osteoid protein matrix), and is released from this site as the effect of bone remodelling. In a healthy adult there is equilibrium between the bi-directional calcium fluxes from and to the bone. This equilibrium is unbalanced either towards bone resorption when circulating ionized calcium levels are low, or towards deposition of calcium in the bone. Therefore, this means that the skeleton is the natural, dynamic reserve of calcium for the organism (Passeri, 1991; Marcus et al., 1995).

3. The role of the vitamin D

The action of the vitamin D, especially the active form $1,25(OH)_2D_3$, is of fundamental importance in most of the bone metabolic equilibrium processes. Vitamin D is a secosteroid synthesized in the skin starting from 7-dehydro-cholesterol: the UV light catalyzes this reaction, or it can also be introduced in the body orally or parenterally (Lund and DeLuca, 1969; Holick, 1995). Several factors may interfere with the skin-production of vitamin D: an increase in the melanin in the skin, insufficient sun exposure and even the angle of incidence of sun light on the skin surface. This last factor may indeed is important at latitudes far from the equator: especially during winter, sunlight has been demonstrated to induce a very low cutaneous production of vitamin D (Holick, 1995).

Vitamin D_3 may be absorbed from the intestine, by consuming food rich in the compound such as fat fishes (salmon and mackerel), oil of fat fishes, i.e. cod-liver oil, or yolk. Vitamin D_2, has a metabolic behaviour identical with vitamin D_3, is a plant derivative. In many countries, due to an insufficient dietary intake, vitamin D is added to milk, cereals, bread and dairy products. The daily requirement of vitamin D varies according to the age and also to particular conditions such as gestation or lactation or elderly. For an adult subject, in total absence of solar exposition, doses of 600–800 IU per day are needed (Marcus et al., 1995). Vitamin D from the diet, due to its lipo-solubility, will be incorporated in chylomicrons and absorbed through the lymphatic system. Subsequently, it is bound to a vector protein, independently from its origin, and is transported to the liver, where the initial phase of activation takes place, through the formation of 25-OH-cholecalciferol (25-OH vitamin D, or calcifediol). Then, this metabolite enters the circulation, where it is transported by a globulin. 25-OH vitamin D has a half-life of 2–3 weeks, and its serum levels may be utilized as an indicator of the vitamin D status of the subjects, since it reflects the cumulative effects of the vitamin intake and production by the sunlight (Lund and DeLuca, 1969; Holick, 1994, 1995).

25-OH vitamin D is transported to the kidney, where it undergoes a second hydroxylation resulting in the final active metabolite $1,25(OH)_2$ vitamin D_3 or calcitriol, that is 500–1000 folds more active than its precursor 25-OH vitamin D. Calcitriol is not often considered as marker of vitamin D status, since is light sensitive, easily degradated, and the method for evaluation is rather complicated as compared to measuring 25-OH vitamin D.

The mechanism of action of calcitriol is similar to other steroid hormones, on different types of target tissues (most of tissues of the organism), that have a specific receptor for the vitamin D (VDR) with an affinity 1000 times higher for the $1,25(OH)_2D_3$ than for the other mono-hydroxylated metabolites of this vitamin (Haussler et al., 1998).

$1,25(OH)_2D_3$ regulates the synthesis of calbindine, a protein that binds calcium and has also a "non genomic" action, based on the interaction of $1,25(OH)_2D_3$ with a cell membrane protein different from the VDR, causing the opening of the calcium-channels. It is assumed that these mechanisms are responsible for at least one-third of the total calcium absorbed daily (Kinyamu et al., 1997).

At the bone level, calcitriol is able to stimulate normal bone growth, and it is of fundamental importance in the mineralization processes. Probably, it mainly increases the intestinal absorbance of calcium and phosphates, allowing these components for the deposition of crystals onto the collagen fibres of the osteoid proteic matrix. There are also receptors for $1,25(OH)_2D_3$ on osteoblastic cells, able to stimulate their activity (Suda et al., 1992).

4. Vitamin D insufficiency

Hypovitaminosis D always causes a reduction of calcium intestinal absorption that induces a negative calcium balance. Hypocalcemia due to insufficient calcium absorption, induces an increment of PTH with also an increase of the synthesis of $1,25(OH)_2D_3$. This induces resorption of calcium from bone, in order to maintain serum calcium within normal levels.

Obviously, the grade and duration of the vitamin D insufficiency are important. In the past, the lower limit for 25-OH vitamin D was considered 25–37.5 nmol/L, more recently most laboratory reference ranges have extend the lower limit up to 100 nmol/L (Heaney, 2004). Serum 25-OH vitamin D concentration below 20 nmol/L is associated with clinical osteomalacia in adults and elderly people (Allain and Dhesi, 2003), with defects of mineralization of the newly formed osteoid matrix. The consequence of a moderate insufficiency defined as a serum level of 25-OHD between 37.5 and 75 nmol/L, can be considered as facilitating factor for the development of osteoporosis, while the range between 20 and 40 nmol/L have been called vitamin D insufficiency, in recognition of its inadequacy for optimal functioning of vitamin D and calcium metabolism (Heaney, 2003, 2004).

An increased fractional calcium absorption has been documented with increasing levels of 25-OH vitamin D up to 80 nmol/L, as well as an inverse association between low serum concentration of 25-OH vitamin D and high level of PTH has been reported (Heaney et al., 2003). Recently, it has been shown that calcium malabsorption and increased fracture risk are present at 25-OH vitamin D concentrations below 80 nmol/L (Trivedi et al., 2003). Especially in the elderly, low bone mass in the presence of vitamin D insufficiency have been defined as osteoporo-malacia (Ringe, 1998).

5. Bone metabolism during aging

Compared to the considerable amount of knowledge regarding bone metabolism, calcium exchange and vitamin D metabolism in adults and children, little is known regarding the situation in extreme longevity. It has been demonstrated that aging is accompanied by low dietary calcium intake, due to reduced intestinal absorption, altered renal re-absorption, and increment of both bone remodelling and intracellular calcium. Vitamin D levels tend to decrease, and circulating PTH levels are increased. All these events favour a negative calcium balance and a progressive loss of calcium from the skeleton in elderly subjects. This trend influences bone, the main site of calcium deposition. Bone mass is decreases and consequently, skeletal fragility increases with a significantly higher risk of fractures, especially at the hip, that may lead to a partial or total loss of self-sufficiency (Passeri, 1991; Wilkins and Birged, 2005).

The daily calcium requirement, in elderly subjects, has been estimated around 1500 mg/day, and very often this goal is not achieved. It is well known that daily calcium intake in the elderly is often below 600–800 mg/day (Nieves, 2003).

It has also to be considered the progressive decline of efficiency in the intestinal calcium absorption, starting after the age of 60–65 years. This trend is due to the decreased production of gastric acid, leading to a lower amount of intra-luminal ionized calcium, the changes of duodenal mucosa, and the impairment in vitamin D levels (Marcus et al., 1995).

The decreased absorption of calcium and the age-dependent decline in serum proteins, could result in a trend to a lower level of serum calcium. This is, however, compensated by the increased circulating level of PTH (Marcus et al., 1984; Forero et al., 1987). In this regard, McKane et al. (1996) have clearly demonstrated that elderly women with secondary hyperparathyroidism started to return toward normal values (PTH levels similar to those of the younger adults) after 3 years treatment with a calcium-rich diet. This means that the aging process is associated with deep changes in calcium homeostasis at both intra- and extra-cellular levels. From a quantitative point of view, the most relevant aspect is an accelerated bone remodelling, mainly in resorption, resulting in a loss of calcium from bone. In elderly people, intestinal and urinary losses of calcium are not reduced, in spite of the decreased calcium intake and increased PTH secretion, therefore is not possible to maintain the skeletal calcium content (McKane et al., 1996).

The theory of "shift of calcium" during aging has been proposed, for the first time, approximately 50 years ago. It was suggested that the progressive loss of bone calcium and its deposition in the soft tissues could be the leading cause for the increased presence of osteoporosis, hypertension, and arteriosclerosis in the elderly subjects (Elkeles, 1957). This old and over simplified idea should be kept, anyway, into some consideration.

As a matter of fact, a global increase in bone remodelling has been documented in elderly subjects with the prevalence of resorption over formation of bone tissue, resulting in weakening of bone mass, and progressive loss of total calcium content of the body (Eastell et al., 1988). In contrast to the trend of progressive loss of bone mass, increased turn-over, and decreased total calcium content of the body, observed during aging, a contemporary and gradual accumulation of calcium deposited in a non-exchangeable form has been demonstrated: in vessel walls, in extra bone calcifications and also in different intracellular compartments (Fujita, 1986). The atherosclerosis is, at least in part, correlated with an altered calcium homeostasis in endothelial cells of vascular system. The increased flux of calcium in towards cells is associated, and perhaps also induced, by the presence of plasma lipoproteins like LDL, with a consequent production of growth factors and cytokines. This induces the entrance of lymphocytes and macrophages into vessel walls, as well as, the proliferation of smooth muscle cells, inducing the formation of the atheromasic lesions (Orimo and Ouchi, 1990).

In the past few years, it has been demonstrated that a protein of the Tumor Necrosis Factor superfamily, receptor activator of nuclear factor kB ligand (RANK-L), its receptor (RANK, receptor activator of nuclear factor kB) and the decoy receptor osteoprotegerin (OPG), are key regulators of bone resorption in vitro and in vivo. RANK-L is expressed by osteoblasts, bone marrow stromal cells and activated T lymphocytes cells. RANK is present as transmembrane receptor on cells of osteoclastic lineage, and OPG is produced by osteoblasts (Lacey et al., 1998; Yasuda et al., 1998). Studies on OPG/RANK/RANK-L network has provided new insights regarding bone remodelling as well as vascular diseases (Khosla et al., 2002; Schett et al., 2004; Hofbauer and Schopper, 2004; Kiechl et al., 2006). PTH and vitamin D are involved in OPG/RANK-L expression and regulation (Suda et al., 2003), and it has been shown that OPG is increasing after 70 years of age and in centenarians, as a possible, although insufficient

mechanism able to limit bone loss (Pulsatelli et al., 2004; Passeri et al., 2005), or as marker of inflammation and cardiovascular risk (Mazziotti et al., 2006).

Circulating PTH is often increased in the elderly, and induces the entrance of Ca^{2+} from the extra-cellular to the intra-cellular compartments, i.e., acting as an ionophore. With advancing age, a gradual vitamin D deficiency becomes evident (Chapuy et al., 1983; Boonen et al., 1996; Chapuy et al., 1997; Boonen et al., 2006). This is due, to a reduction of the concentration of 7-dehydrocholesterol in the epidermis, typical during aging, and to a consequent decrease of synthesis under UV irradiation (Holick, 1994, 1995). On the other hand, a decreased exposition to UV light is frequent in the elderly people, especially when they are unable to exit from their residences, or disable and recovered in protected care-giving facilities. In addition, a low nutritional intake of vitamin D is present. These conditions are particularly frequent: 60–80% of the institutionalized elderly population and about 50% of the non-institutionalized women present a primary deficit of vitamin D, especially during winter season (Lorè et al., 1986; Rossini et al., 1990; Eastell et al., 1991; Chapuy et al., 1992; Romagnoli et al., 1999; Isaia et al., 2003).

The reduced efficiency of the renal 1α-hydroxylase present during aging, and the progressive decline of renal functions, may explain the low serum levels of active $1,25(OH)_2D_3$. This situation is further influenced by a decreased cellular response to PTH, and in women, by the hypo-estrogenism following menopause (Tsai et al., 1984; Gennari et al., 1990; Eastell et al., 1991; Nuti and Martini, 1994; Ooms et al., 1995). Therefore, the intestinal calcium absorption is reduced to an extent similar to malabsorptive syndromes (i.e. Celiac disease and Chron disease), where hypovitaminosis D is also present (Jahnsen et al., 2002).

It seems clear that elderly subjects often present $1,25(OH)_2D_3$ deficiency, that reduce calcium absorption, and this is responsible for the secondary hyperparathyroidism that eventually is leading to an increased bone loss.

A mechanism able to explain the reduced biological activity of $1,25(OH)_2D_3$ in the elderly has also been suggested, consisting in an increased resistance of the target organs to the action of vitamin D (Lund et al., 1982). Such a resistance could be explained by a reduced number of intestinal VDRs (Clemens et al., 1986; Ebeling et al., 1992; Chen et al., 1997), associated with estrogens deficiency, as it was shown after ovariectomy, or by some post-receptorial defects (Parfitt, 1993; Duque et al., 2002).

Considered all the above, it can be considered that hypovitaminosis D has multi-factorial origin in elderly. This deficiency may play a role not only in Osteomalacia, characterized by defective mineralization of bone, but also in Osteoporosis, the most prevalent bone disease.

6. Bone metabolism in centenarians

Some of the peculiarities of aging regarding calcium metabolism and vitamin D status have been confirmed in extreme senescence.

To this aim, we studied 104 subjects (90 females and 14 males) ranging between 98 and 105 years of age. They were residents in the areas of Parma and Mantova (Italy, 44° parallel north), and were living either at home or in nursing facilities. A detailed history, and general physical examinations were performed, in order to document whether any acute disease was present. Fifty-eight of these centenarians presented signs of dementia, as revealed by using the mini-mental state examination (MMSE) (Folstein et al., 1975; Guralnik et al., 1994). General hematochemical parameters, as well as specific ones related to the bone turnover, were also studied.

We found that half of them (50/104) were able to walk, 19 without any help. Nine of these 19 centenarians demonstrated stability, when this function was specifically considered, using performance-based measures as the EPESE short battery. This battery integrates the following tests: rising from a chair, timed walk over a 4.5 m course, and capacity to maintain balance in progressively more challenging standing position (balance tests) (Guralnik et al., 1994).

The medical history revealed that the subjects sustained 55 falls during the year preceding the study. It was also established that 40 centenarians sustained at least one fracture mostly due to minimal traumas or spontaneously. One-third of them was able to walk with sticks or other supporting aids, and more then 60% of this group have had at least one bone fragility fracture, while 12 of the 19 patients able to walk without help, have never suffered any fractures.

Bone densitometry by DXA analysis was not performed because of the difficulties of take most of these frail subjects to the Centre for Metabolic Bone Diseases of the Department of Internal Medicine and Biomedical Sciences of the University of Parma.

Therefore, we studied several characteristics of the bone tissue by ultra-sonography (Glüer, 1997) using two portable instruments. The Osteosonographer DBM Sonic (IGEA, Carpi, Italy) was used to study 60 centenarians, evaluating the amplitude-dependent speed of sound (AD-SoS) on the proximal phalanges of the non-dominant hand, a bone site formed for approximately 70% by the cortical bone (Glüer, 1997). The instrument forwards a calculated value of the risk of bone fractures called ultrasound bone profile index (UBPI). This method has been validated in 10,000 women of various ages including the centenarians (Bauer et al., 1997; Wüster et al., 2000; Alexandersen et al., 2005).

We used also the Sahara Ultrasonographer (Hologic, Walton-MA, USA) for the evaluation of other 28 subjects. This instrument measures both Broadband Ultrasound Attenuation (BUA) and Speed of Sound (SoS). The numeric combination of these two parameters supplies an index called Quantitative Ultrasound Index (QUI), assumed to reflect elasticity and stiffness of bone (Ensrud et al., 1995). In 16 centenarians ultra-sonographic evaluation was not possible, because of articular retraction of the feet, or due to arthritic deformations of the hands.

In agreement with data from the literature (Ensrud et al., 1995; Krieg et al., 2006), using both devices very low values were detected (Table 1A), without any significant difference between fractured and non-fractured centenarians (Table 1B).

The routine laboratory findings were within the normal limits. It should be underlined that serum creatinine was on average below 1.5 mg/dl, while the value of the calculated creatinine clearance was almost 30 ml/min, using the Cockcroft-Gault formula. The levels of creatinine clearance found in centenarians, although quite low, were not correlated with an increased serum PTH, suggesting that the secondary hyperparathyroidism found, was not due to chronic renal insufficiency. Serum phosphate was in the lowest quartile of the normal range, and calcium was, on average, close to the lower limit of normality, and below 9 mg/dl in 74% of the subjects.

The most significant results, among the specific markers of bone resorption, were serum PTH and 25-OH vitamin D levels (Table 2). The average PTH was twice the normal value, and above the upper limit in 65% of these centenarians. The increased bone resorption was demonstrated by increased levels of serum C-terminal cross-linking telopeptide (S-CTX), above the normal limits in 92% of these subjects, and significantly correlated with PTH ($r = 0.37$, $p < 0.001$), in agreement with data from the literature (Garnero et al., 1996). Mean bone alkaline phosphatase values were in the upper limit of the normal range, and it was significantly elevated in 32 subjects indicating an increased bone turnover (Table 2). The above listed evidences of an intense bone resorption were particularly evident when the individual markers

of turnover (PTH, S-CTX, IL-6) and the serum calcium levels were considered together. In addition, correlations were found between PTH and calcium ($r = -0.33$, $p < 0.001$), IL-6 and S-CTX ($r = 0.37$, $p < 0.001$), IL-6 and calcium ($r = -0.35$, $p < 0.001$), PTH and IL-6 ($r = 0.24$, $p = 0.019$). There was no correlation between the creatinine clearance and PTH ($r = -0.167$, $p = $ not significant) (Passeri et al., 2003).

There was also a significant correlation between markers of bone turnover and ultrasonographic parameters both at the phalanges and at the calcaneous: PTH and S-CTX versus phalangeal UBPI ($r = -0.38$, $p = 0.032$; $r = -0.28$, $p = 0.036$, respectively), S-CTX versus calcaneal SoS ($r = -0.4$, $p = 0.05$) (Passeri et al., 2003).

An even more evident finding was the severe hypovitaminosis D. The 25-OH vitamin D, measured by RIA (Nichols Institute Diagnostics,CA,USA), was measurable only in 5 subjects, and one of these has had therapeutic supplementation of this vitamin. The values of the other 99 centenarians were below the limit of sensibility of the method used (5 nmol/L).

When considering all these data, they indicate that a considerably large part of the centenarians studied presented an active bone turnover, accompanied by an important loss of bone mass, known from the literature in "younger" elderly people (Garnero et al., 1996; Bollen et al., 1997).

It should be emphasized that 100 of the 104 subjects have lived all their lives in an area of Italy where a particular cheese, the "Parmigiano-Reggiano", the highest in calcium content world wide, is produced, and part of the daily diet. The calcium intake of these subjects was, on average, at least 800 mg/day as revealed by the data obtained using specifically prepared questionnaires. This quantity of dietary calcium is certainly larger than what found in any other regions of Italy among elderly subjects.

Hypovitaminosis D in these centenarians seems to be of great relevance, especially when considering that blood sampling was performed in 60% of them between April and September, when sun exposition is possible. These low levels of 25-OH vitamin D, found in 99 of our 104 centenarians, can explain, on one hand, the high level of serum PTH, and, on the other hand, are certainly not caused by decreased renal functions, as shown by creatinine levels. The vitamin D deficiency and the increased parathyroid function are inducing an active bone turnover, with a tendency for continuous bone loss, present also in the oldest olds. At the same time, these data suggest the need of vitamin D supplementation to the subjects of the oldest old age group. As defined by the Food and Nutrition Board, the adequate intake of vitamin D is between 200 and 600 IU/d (FNB, 1997). 600 IU/d, equivalent to 15 μg/d, is the amount suggested after 70 y of age, as recognition that the contribution of cutaneous sources decreases with age. Several studies have demonstrated that an amount above 1000 IU/d (35 μg/d) are necessary to reach and maintain a circulating concentration of 80 nmol/L, and this can certainly be considered true for the frail centenarians. This supplement may also be beneficial not only for the protection of the skeleton, but also for improving muscle functions that responds positively to the actions of vitamin D (Glerup et al., 2000; Trivedi et al., 2003; Heaney, 2003, 2004).

Appendix A

The Italian Multicenter Study on Centenarians (IMUSCE)

Coordinators: M. Motta, C. Franceschi, L. Motta

List of participants of IMUSCE:

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Table 1

Parameter	Mean ± SD
(A) *The ultrasonographic data in centenarians*	
Phalangeal measurements (60 subjects)	
UBPI[a]	0.22 ± 0.18
T score AD-SoS	−3.7 ± 1.8
Calcaneal measurements (28 subjects)	
QUI[b]	56.7 ± 23.0
T score QUI	−2.8 ± 1.3

(B) *Phalangeal osteosonographic and calcaneal ultrasonographic differences according to fractures*

	No fractures (39 cent.)	Fractures (21 cent.)	*p*
T score AD-SoS phalanxes	−3.6 ± 1.7	3.9 ± 1.4	n.s.
	No fractures (21 cent.)	Fractures (7 cent.)	*p*
T score QUI calcaneus	−2.8 ± 1.1	3.0 ± 1.4	n.s.

Notes: The abbreviations are explained in the text.

[a]UBPI = 1 means the minimum probability, and UBPI = 0 is the maximum probability of bone fracture.

[b]QUI = numeric combination of SoS and BUA; it is referred to bone elasticity and stiffness.

Table 2

Osteometabolic parameters found in centenarians (104 subjetcs)

Parameter (units)	Normal ranges	Centenarians (mean ± SD)
25-OH vitamin D[a] (nmol/L)	37.5–125	7.1 ± 7.0 (only 5 subjects)
Phosphates (mg/ml)	2.7–4.5	3.1 ± 0.5
Calcium (mg/ml)	8.5–10.5	8.6 ± 0.7
PTH (pg/ml)	5–65	123.2 ± 108.5
S-CTX (pmole/L)	1000–2500	6335 ± 3673
IL-6 in serum (pg/ml)	0.68–1.85	8.5 ± 7.8
Bone Alc. phosphatase (U/L)	14.2–42.7	44.1 ± 39.0
Creatinine (mg/dl)	0.4–1.4	1.1 ± 0.5
Creatinine clearance (ml/min)		28.1 ± 10.5

Notes: These data represent mean ± standard deviation of the values found in 104 centenarians, except for 25-OH vitamin D.

[a]25-OH vitamin D levels were below the detectability of the method (5 nmol/L) in 99 of the 104 centenarians, therefore this parameter represents the mean ± SD of only 5 centenarians.