Introduction

Bone and calcium metabolism abnormalities, mainly resulting in osteopenia and osteoporosis, have long been recognized as a complication of untreated coeliac disease (CD). It has also been reported that, in adults, skeletal disorders may be the only presenting feature of a gluten-sensitive enteropathy\(^{(1-3)}\). The recent availability of simple and reliable biohumoral and instrumental investigations, such as the measurement of bone density by x-ray absorptiometry (DEXA), has allowed to evaluate the frequency and extent of osteopenia and/or osteoporosis in coeliac patients during all stages of the disease, from diagnosis to follow-up\(^{(4)}\). Although bone turnover is also affected by genetic and environmental factors\(^{(5)}\), the majority of authors agree that the introduction of a gluten-free diet (GFD) in CD patients results in a significant clinical and metabolic improvement and, in some cases, avoids the progressive deterioration of existing bone alterations\(^{(6,7,8)}\). Therefore, it can be easily understood that identification of subjects with untreated CD might play a preventing role, since bone demineralization is associated with a higher risk of fractures.

Definition

Osteoporosis is a systemic skeletal disorder, characterized by bone mass reduction and deterioration of the bone tissue microarchitecture, with a consequent increased susceptibility to fractures\(^{(6)}\). From a quantitative point of view, the most widely accepted definition of osteoporosis is based on the evaluation of bone mineral density (BMD), as the World Health Organization (WHO) recently suggested. Osteoporosis is, therefore, defined as a BMD higher than 2.5 standard deviations (SD) below the young adult mean, i.e. when the T-score (the deviation of the individual value from the normal mean value of young adults of the same sex) is lower than 2.5. When the T-score is between 1 and 2.5, an osteopenia condition sets in, a problem requiring an early diagnosis and a suitable treatment, in order to avoid the development of osteoporosis\(^{(10)}\).

Epidemiology

A considerable reduction in bone mineral content and density have been reported by several studies in both adults and children with untreated CD, irrespective of the symptomatology at onset\(^{(11-15)}\). Although these studies are not homogeneous in terms of selection of patients and controls, and despite some small differences in the adopted research methods, most of them agree that treatment of CD with a strict GFD can improve bone alterations by reducing osteopenia and osteoporosis, halting their progression and decreasing the risk of fractures\(^{(13,15)}\). The extent of bone involvement at the time of CD diagnosis and the metabolic response to the GFD vary considerably among individuals. This mainly depends upon the subject’s clinical and metabolic conditions, age, duration and compliance to GFD. Other additional aspects include genetic and environmental factors (e.g. sex, race, daily calcium intake, level of physical exercise, steroid treatment and, in female subjects, lack of estrogens)\(^{(5,6)}\).

As all these factors usually overlap, it is quite difficult to estimate the real prevalence of bone disorders in subjects with gluten-sensitive enteropathy. Therefore, in order to evaluate whether untreated CD is a risk factor for the development of osteopenia and/or osteoporosis, the prevalence of this condition should be estimated in patients in whom no other cause of the above bone disorders has been found.
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During a U.S. study, 840 subjects were examined - 266 with and 574 without osteoporosis - and subjected to serological screening for CD. The subjects that were positive to either anti-tTG or anti-endomysium antibodies were submitted to intestinal biopsy with the aim of confirming the CD diagnosis. The subjects with CD confirmed by biopsy received gluten-free nutrition and were followed up to evaluate the improvements in bone mineral density. 4.5% of the 266 subjects with osteoporosis and 1% of the 574 subjects without osteoporosis were positive to the serological screening; of these, 9 osteoporotic patients and 1 non-osteoporotic patient had a positive biopsy. The prevalence of CD confirmed by biopsy was 3.4% among the population with osteoporosis and only 0.2% among subjects without osteoporosis. Anti-tTG levels were correlated with osteoporosis severity, measured by the T-score. Evidence was provided that the more severe the coeliac disease, the more severe the osteoporotic damage. The treatment of coeliac patients with GFD brought about considerable improvements in the T-score. The results of the study have also shown that CD incidence in osteoporosis is sufficiently high to recommend a serological screening for coeliac disease to all patients affected by osteoporosis.

Pathogenesis

Active calcium absorption mainly takes place in the duodenum and the upper jejunum. It is therefore possible to hypothesize that the reduced absorptive surface due to gluten-sensitive enteropathy (villous atrophy, increased intraepithelial lymphocytes and crypt hyperplasia) can also negatively affect the calcium balance. The low serum calcium levels usually found in subjects with untreated CD are, therefore, the main event, subsequently followed by some complex metabolic abnormalities, such as the increased serum parathormone (PTH) concentration.

Not only does PTH hypersecretion promote bone resorption, but it also contributes to determining alterations in vitamin D metabolism. Moreover, the reduced serum calcium levels lead, either directly or through PTH hypersecretion, to an increased activity of 1-a-hydroxylase, the enzyme responsible for the conversion of 1,25-dihydroxycholecalciferol (1.25-OH2-D3) into 25-dihydroxycholecalciferol (25-OH-D3). However, in untreated CD this compensatory effort is totally ineffective because of the target organ inability to respond to the enzyme adequately. This lack of sensitivity is mainly due to a deficiency of proteins involved in the active transport of calcium (e.g., calbindin) and leads to an increase of serum 1.25-OH2-D3 levels. Thus, calcium intestinal malabsorption becomes persistent and so does hypocalcaemia which, in its turn, maintains bone resorption. The rapid disappearance of 25-OH-D3 from coeliac patients' plasma may also suggest an increase in the conversion of this active metabolite into 1.25-OH2-D3, an event mediated by PTH or at least by hypocalcaemia.

In addition to enteropathy, other pathophysiological mechanisms may also contribute to the negative calcium balance usually found in coeliac patients, such as:

1. **Low calcium intake with food.**
   - In CD subjects, the dietary calcium intake is often inadequate, owing to both some degree of anorexia (especially when CD onset occurs in the first years of life), and the typically associated lactose intolerance condition (which can be justified by the mucosal alterations described above). Both factors induce the subject to reduce or even discontinue the intake of milk and its derivatives which, as it is known, are the main calcium source;

2. **Low calcium absorption.**
   - By a feedback mechanism in the parathyroid glands, this event leads to secondary hyperparathyroidism and osteoclast activation, with subsequent calcium resorption from bone tissue in order to maintain the serum calcium levels within normal range;

3. **Increased faecal excretion of endogenous calcium**
   - due to increased intestinal secretion and/or reduced resorption and precipitation of ingested calcium in the intestinal lumen in the form of soaps;

4. **Magnesium deficiency.**
   - The results of some studies suggest that GFD supplementation with magnesium results in increased BMD.

5. **Low serum levels of Insulin-like Growth Factor 1 (IGF-1).**
   - IGF-1 is a substance which mediates the anabolic effects of the growth hormone by acting directly on the bone tissue and whose concentrations are directly correlated with the subject's nutritional condition. In both adults and children with active CD, a reduction in the IGF-1 serum levels has been observed. A further hypothesis is that a zinc deficiency, which is frequently observed in coeliac subjects, can play a decisive role in this reduction. Only a total restoration of the intestinal mucosa, after a long treatment based on GFD, can bring the above-mentioned parameters to the normal range.

6. **Cytokine production.**
   - Several studies have demonstrated that, during the active phase of the disease, there is a production of...
proinflammatory cytokines, such as IL-1, IL-6, TNF-alpha which also seem to carry out a major role in the bone resorption process by stimulating osteoclast differentiation and activity. Moreover, bone loss can be caused by an imbalance in cytokine production: low blood levels of inhibitory cytokines such as IL-12 and IL-18 are evidence of a failed inhibitory effect on the osteoclastogenesis process\(^{(22,25)}\).

Finally, bone resorption can be induced through the activation of the RANKL/OPG cytokine system\(^{(26,27)}\). This system, playing a key role in osteoclast biology and bone remodelling, is based on RANKL, a cytokine belonging to the TNF-ligand family, expressed both on the membrane surface of stromal/osteoblastic cells and in soluble form. The system is bound to its RANK receptor, expressed on cells of the osteoclastic line, and stimulates osteoclast differentiation and activation, while inhibiting apoptosis. Osteoprotegerin (OPG) is a further component of this system. It is a soluble cytokine belonging to the TNF-receptor family, expressed by stromal/osteoblastic cells, which acts as a “trap” receptor, with a high affinity for RANKL, to which it binds, thereby preventing RANKL/RANK from binding. An increase in RANKL/OPG has been observed in untreated coeliac subjects.

**Clinical and bio-chemical aspects**

Bone tissue involvement, commonly found in both children and adults with CD, may occur as one of the presenting clinical and metabolic features but, especially in adults, it can be the only clinical manifestation, even in the absence of any gastrointestinal complaint or before either this or other symptoms become evident. The extent of bone tissue alterations does not appear to be correlated with the presence of pain in the affected skeletal segments or with the severity of intestinal disorders\(^{(5)}\). This is the main reason why the bone involvement evaluation requires not only a clinical assessment, but also a series of biochemical investigations (calcemia, phosphoraemia, serum alkaline phosphatase, PTH, vitamin D and bone remodelling markers) and instrumental tests (bone densitometry).

The presence of bone and calcium disturbances in subjects with CD, both at diagnosis and after a variable period of time (8 months – 17 years) from the introduction of GFD, has been evaluated in several studies\(^{(5)}\). The majority of these investigations agree in reporting that subjects with untreated CD show the following laboratory and metabolic abnormalities\(^{(5,7)}\):

1. **Hypocalcaemia**, increased PTH secretion, decreased serum 25-OHD3 levels with enhanced 1,25-OH2-D3 concentrations and increase of bone remodelling markers. According to some Authors, in adult coeliac patients there is a positive correlation between serum calcium levels and the CD clinical features at onset. Although in all patients with active CD the serum calcium levels are lower than in controls, these values are “better” in subjects affected by subclinical or silent CD than those in subjects presenting with the typical clinical pattern. This relationship has also been observed when the increased bone turnover markers have been taken into consideration. Conversely, the signs and/or symptoms at CD onset do not affect the serum PTH levels\(^{(28)}\). Hypovitaminosis D and secondary hyperparathyroidism are common findings in coeliac patients at the onset and in those who do not respond to the dietary treatment, with a reported frequency of 58 - 88 % and 25 %, respectively. In patients responding to GFD, the above-mentioned alterations decrease to 25 and 19 %, respectively\(^{(16)}\);

2. **BMD reduction.**
   
   At the onset, coeliac patients show lower BMD values with a reported frequency ranging between 26 and 85%, depending on the examined bone districts and the size of the studied sample\(^{(29-31)}\).
   
   As regards the existence of a correlation between the clinical expression of CD and the degree of osteopenia, the data are rather contradictory. From the biochemical point of view, it has also been observed that children with untreated CD present with hyperphosphoraemia, mild hypocalcaemia and slightly higher serum PTH levels, with low serum calcitonin levels\(^{(32)}\). According to other studies, in paediatric patients, the age at diagnosis plays a more important role than it does in adults, i.e. there is a positive correlation between the age at the time of diagnosis and BMD. Coeliac patients receiving a delayed diagnosis have a lower BMD in comparison with patients who were diagnosed CD at an earlier stage\(^{(33)}\).

Finally, the hypothesised role played by hyperparathyroidism secondary to intestinal malabsorption is not sufficient to explain all cases of low bone mass in coeliac patients, who are often affected by severe skeletal damage, but without evident intestinal symptoms\(^{(34,16)}\). In this regard, a recent study\(^{(23)}\) carried out on a group of coeliac patients who had been on a diet for at least two years and on a second group of subjects recently diagnosed and, therefore, not yet on a diet, showed that, despite some patients presenting with a slight increase in PTH levels, secondary hyperparathyroidism does not seem to be the only cause of bone loss in this patients’ sample. The NTx and “Z-score” of bone mass measured in patients do not correlate with the PTH levels, but
correlate with a series of osteoclast-stimulating cytokines. Furthermore, the patients’ serum PTH concentration does not correlate with the cytokine levels, thereby suggesting that these are not the consequence of a response to the PTH increase. The high, but still within normal range, levels of excreted calcium, observed in patients not following a diet, induce to think that increased bone resorption can be the main cause of the low bone density. If there were an inadequate calcium resorption due to damaged enterocytes, calcium excretion would be lower in patients not complying to a diet. This generally happens in coeliac patients presenting with evident intestinal symptoms, among which low calcium levels and high PTH levels can often be observed. Altogether, therefore, the authors’ observations support the assumption that cytokine imbalance is an important factor contributing to the bone resorption increase and the subsequent bone mass loss in the studied group of patients. In vitro experiments have shown that the coeliac patients’ sera directly act on both osteoclasts and osteoblasts. In particular, the high NTx levels measured in patients seem to confirm the greater influence of coeliac patients’ serum factors on osteoclasts rather than on osteoblasts. Osteoclastogenesis being stimulated in the presence of suboptimal RANKL concentrations has led to the assumption that this regulator may have been modified in the patients under study. Furthermore, the acceleration in bone loss in coeliac patients appears to be due to a reduction of IL-12 and IL-18 levels. Both cytokines take part in the production of other osteoclastogenesis inhibiting factors\(^{(35)}\); IL-18, for instance, acts on the T-lymphocytes by stimulating them to produce and release GM-CSF\(^{(35)}\) and both cytokines collaborate to the production of IFN-\(\gamma\)\(^{(36,37)}\). GM-CSF and the members of the IFN family are known for their inhibiting effect on osteoclastogenesis. It is worth noting that the serum levels of these two cytokines can be different depending on whether coeliac patients are on a diet or not. As a consequence, the contribution made by each cytokine to osteoclastogenesis as a whole will also be different.

**Bone & calcium metabolism and gluten-free diet**

The development of skeletal disorders (especially osteoporosis) is associated with an increased risk of fractures, with evident consequences in terms of population morbidity and mortality. It is, therefore, important to evaluate whether in coeliac patients the presence of a bone demineralization process can be corrected or, at least, stopped in its evolution by starting a GFD. The main purpose of the dietary treatment is to restore a normal intestinal mucosa, so that calcium absorption may take place normally.

**Adult age**

In various studies on the evaluation of bone metabolism in adults, high levels were found for both bone formation and resorption: these results have been ascribed to an increment of bone metabolism following hyperparathyroidism secondary to malabsorption and, as already said, not only that. After the gluten-free diet is introduced, a reduction of both markers can be observed, probably due to the normalisation of PTH secretion\(^{(15)}\). It is by now known that treatment of CD by complying with a strict gluten-free diet can improve bone alterations by reducing the levels of osteopenia and osteoporosis, halting progression and reducing the risk of fractures\(^{(13,15)}\). However, the data obtained in adults are contradictory: most Authors have reported that in treated coeliac adults the bone mineral content remains below normal average even years after beginning the diet\(^{(38)}\). Conversely, other studies have found normal values of bone mineral content in adults who had been on a diet for many years\(^{(39)}\). These discrepancies could also be ascribed to the physiological changes of the bone mass throughout the patient’s life. These depend on the bone mass peak reached in adolescence and the following losses which are the main causes of osteoporosis\(^{(41)}\).

**Paediatric age**

Studies focusing on the evaluation of bone metabolism alterations in untreated coeliac children have reached different results from studies on adults. In coeliac children, the bone apposition indicators are low, which is evidence of a limited osteoblast activity associated with an increment in bone resorption activity signalled by high catabolism markers. A diet introduced at paediatric age leads to an increase in bone formation markers as early as a few months later (3 months). These markers even exceed normal values. However, diet introduction does not bring about changes in bone resorption indicators, not even in the long term\(^{(15)}\). Thus, the result is higher bone metabolism. The pathogenesis of osteopenia in the untreated paediatric coeliac patients appears to be different from that of adults: studies conducted on children, for example, have not recorded an increment of parathormone, which is indeed present in adults. The existence of a not very well identified element altering the normal regulation of bone modelling has been hypothesized\(^{(15)}\). There are data suggesting a possible role for interleukins (IL-1 and IL-6) and anti-bone autoantibodies which in children appear to have a more important role than nutritional alterations in the genesis of CD-correlated osteopenia\(^{(42,43)}\). The studies concerning bone mineral content in children with CD have shown a regression to normal values within one year from the introduction of GFD, with a subsequent increase of height and weight\(^{(11,12,15)}\). Furthermore, a gluten-free diet has proved to be sufficient by itself, without calcium and vitamin D supplementation per os, to reach a normal mineral...
content and, as a result, an adequate bone mass peak.

**Conclusions**

The duodeno-jejunal mucosa abnormalities which are typically found in subjects with gluten-sensitive enteropathy make patients at risk of developing skeletal disorders and/or calcium metabolism disturbances which, in some cases, can lead to a progressive deterioration of the bone tissue resulting in osteopenia and/or osteoporosis. The recent availability of laboratory and instrumental tests has allowed to demonstrate that these metabolic problems are common in patients with CD, not only as part of the whole metabolic abnormalities but also, especially in adults, as the only presenting clinical feature of an untreated CD. The introduction of GFD, by allowing a complete recovery of the small intestinal mucosa, leads to the improvement of the bone metabolic and densitometric indexes and, in some cases, to the full normalization. These findings, together with the existence of an inverse correlation between bone density and risk of fractures, emphasise the preventive role of identifying subjects with untreated CD. Nevertheless, further epidemiological studies involving wider groups of patients with osteopenia and/or osteoporosis are needed, in order to better define whether undiagnosed CD is a significant risk factor for skeletal disease development.

**Essential bibliography**

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