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Alternative hypothesis for the origin of osteoporosis: The role of Mn

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1. ABSTRACT

Antlers represent an ideal experimental model for bone biology studies, because of their easy accessibility, and their rapid growth. Findings from our previous studies revealed that Mn plays an essential role in incorporating the circulating bone Ca to the growing antlers. Based on these findings, we hypothesize that Mn, an essential mineral for Ca fixation (or incorporation) into bones, might be released from bone, during its remodeling, to be available for prioritized function, most likely, brain function; Consequently, Ca incorporation will be dramatically affected, leading to osteoporosis, particularly in elderly people. Therefore, osteoporosis would precede brain malfunctioning diseases such as Alzheimer's or Parkinson's, and clinical data are available to support some of the predictions derived from this hypothesis.

2. INTRODUCTION

Bone has various functions that can range from serving as levers in locomotion, mainly for long bones, to acting as clubs or ballast (1-2). Bone mineral content varies significantly depending on these functions, because it is intimately linked to mechanical performance (1, 3). Among these bones, antler is of particular interest, as being the toughest bone (4-6), and is nearly unbreakable when it is wet. We have previously reviewed available data on the importance of variations in the content of minerals other than just Ca and P (the main minerals in bones) on the mechanical properties of antler bone (7). It is not clear whether the same effects found in antlers will be observed in internal bones, in contrast to what seem to happen in mature antler tissue, which undergo extensive remodeling (8-9). Nevertheless, internal bones do not have the same mechanical properties as antlers (5-6).

A previous study reported that the majority of fractured antlers observed in Spain in the 2005 occurred in an event linked to an exceptional cold whether, which led to a change in the mineral composition of plants (reviewed in our reference 10). Moreover, the authors showed that the 27% reduction found in impact energy required to break antler bone material was caused by a decrease in Mn content, rather than the variations in Si, Na, Ca or P content found in antlers (reviewed in our reference 10). During antlers growth 20% of the Ca in the skeleton is transferred to the antlers (7), leading to a transient osteoporosis (11-12). The authors suggested that the drastic reduction in antler mechanical performance and weight during the exceptionally cold winter was not caused by a deficiency in Ca, but rather a reduction in Mn that would prevent circulating Ca from being incorporated into antler tissue. Therefore, a deficiency in such neglected mineral in the diet may have a disproportionate effect on bone mechanical performance and even bone mass. These findings put together with available clinical data support our current hypothesis on the cause of osteoporosis.

Osteoporosis is a bone disease, mainly related to aging in humans (8, 13-14), but is not caused by aging (9). Nevertheless, permanent osteoporosis has also been reported in deer (15). In humans, osteoporosis is defined as a skeletal disorder characterized by compromised bone strength, predisposing affected individual to increased risk (up to 10 fold) of fracture (8-14). In the past, and still for many practitioners today, it is defined as a reduction of bone mineral density (BMD) of 2.5 standard deviations or more from that found in the young adult (8). However, recent studies have emphasized the importance of other factors such as remodeling rate (rate of resorption and formation of bone), as well as quality of bone material (13). Osteoporosis is thus a result of an imbalance between bone resorption and bone formation during remodelling (8-9); Nevertheless it is not well understood why such imbalance occurs.

Traditionally, Ca is considered the most abundant and important mineral in bones, and loss of bone has been labeled as loss of Ca; this is why Ca has attracted nearly all the attention of the researchers on osteoporosis (14). The fact that bone is lost and consequently Ca is lost, does not, however, mean that loss of Ca causes osteoporosis. Another factor (to be identified) might causes loss of Ca, ultimately leading to the development of osteoporosis. One of the most reputed researchers in osteoporosis has pointed out that "it is believed that in humans, changes in nutrition brought about by current agriculture might exert an effect on bone mechanical performance *via* changes in remodelling rate" (13). We believe, therefore, that loss of a minor mineral, most likely Mn, might be the culprit factor we are searching for. The aim of this paper is to review the literature scientific evidence to support our current hypothesis.

3. ALTERNATIVE HYPOTHESIS ON THE CAUSE OF OSTEOPOROSIS: THE ESSENTIAL MINERAL FOR Ca FIXATION HYPOTHESIS (*EMCaF*)

Our previous studies reporting generalized antler fractures induced by changes in the diet of Mn, indicate that a deficiency in Mn may have prevented incorporation of skeleton Ca, thus resulting in antler bone material which is less tough and dense, and even more porous than normal (10). This prompted one of us (TLC) to propose that some minerals, most likely Mn, are essential for incorporating Ca and P in bones. Moreover their deficiency might reduce the accretion of Ca and P, despite the appropriate amount of other minerals and nutrients, including Ca. Thus, if Mn (or the essential mineral for Ca incorporation, *EMCaF*) is absorbed with more difficultly as the individual is aging, the bone remodeling process may render internal bones less dense and more porous even when there is no problem with Ca absorption or deficiency. In addition, according to *EMCaF* hypothesis, remodeling might be a process aiming, among others, to recruit important minerals for physiological process of higher priority than the maintenance of the skeleton, particularly brain or nerve functions. Because Ca is one of the minerals, whose concentration in the blood is under a tight control (16-17), circulating Ca that cannot be fixed in the bones, as a result of reduced amounts of the *EMCaF*, will be eliminated in the urine.

Predictions derived from the EMCaF hypothesis. The most important predictions derived from the *EMCaF* hypothesis are as follows:

1) *The amount* of Mn (or *EMCaF* if it is not Mn) should decrease in human bones with aging.

2) *The amount* of Mn (or the *EMCaF*) in osteoporotic bones should be lower than in non-osteoporotic bones.

3) If Mn (or *EMCaF* if it is not Mn) is consumed through a prioritized function (brain or nerve function), brain dysfunction will occur (Alzheimer's disease, Parkinson, or others) in osteoporotic patients than in non-osteoporotic patients depending on the age and other factors.

4) Mn (or the *EMCaF*) depletion in the bones might cause brain degeneration diseases.

4. ASSESSING CLINICAL EVIDENCE TO TEST *EMCaF* PREDICTION 3

If our hypothesis suggesting that osteoporosis is caused by depletion of Mn or other mineral *EMCaF* is true, according to prediction 3, the proportion of brain dysfunction derived from degenerative diseases should be higher in osteoporotic than in non-osteoporotic patients. To test this hypothesis, we compared the proportions of patients with brain dysfunction (Alzheimer's disease, Parkinson, senile dementia or serious disorientation while in hospital) in osteoporosis and control groups. In order to avoid confounding factors (e.g. those derived from surgery, or being examined by different practitioners), we used patients that underwent surgery in the same traumatology service (patients with osteoarthritis) as a control group. A

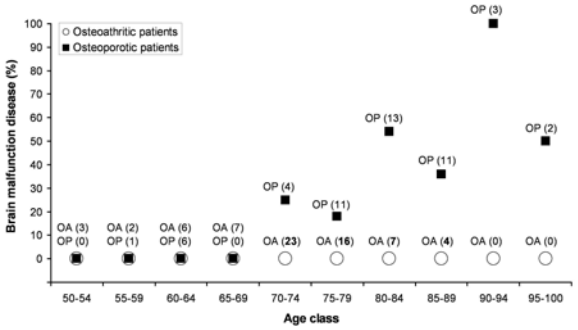

Figure 1. Percentage of patients with brain dysfunction in osteoporotic (OP) or osteoarthritic (OA) patients (number of patients in brackets).

further reason for this choice is that both operations affect mainly elderly people. Finally, osteoarthritis operations are performed because the cartilage is worn off, but bone quality is not affected. It is, therefore, rather unlikely that a patient has both osteoporosis and osteoarthritis. According to our hypothesis, the proportion of brain dysfunction should be higher for patients with osteoporosis once sex and age had been controlled for.

We have collected clinical data from digital files in the surgery services of the hospital (Hellin hospital, Albacete, Spain). The digital records included all surgery operations taking place in 2009 and 2010. As mentioned above, only osteoporotic and osteoarthritic operations were included. Clinical history data were recorded, regarding whether the patient suffered Parkinson's, Alzheimer's disease, senile dementia, strong disorientation, or being operated for hip replacement because of fracture, knee or hip replacements required for osteoarthritis. Age and sex of patients was also recorded, as well as the year when surgery took place.

4.1. Statistical analyses

Results are shown as means ± SE. One-way ANOVAs examined differences in age for osteoporotic *vs*. non-osteoporotic, or brain-malfunctioning *vs*. normal brain functioning. A chi-square test examined differences in counts between patients with brain dysfunction in osteoporotic and non-osteoporotic patients. A logistic model including sex, age, year, and osteoporosis *vs*. osteoarthritics, as well as their two-way interactions examined whether any of these factors increased the probability of brain dysfunction once controlled for the remaining factors.

5. OSTEOPOROTIC BUT NOT OSTEOARTHRITIC PATIENTS SHOW BRAIN DYSFUNCTION.

The mean age of the total number of patients (N=113) was 76.0 ± 0.9 years. The mean age for men (N=28) and woman (N=85) was 74.8 ± 2.0 years and 76.4 ± 0.9 years, respectively. The mean age for osteoporotic (N = 45) and non-osteoporotic (N = 85) patients was 81.9 ± 1.1 and 72 ± 1.0 years. Most of the patients had no brain dysfunction, but 18 of them had either Alzheimer's (N = 6), Parkinson's (N = 4), senile dementia (N = 3), and the remaining 5 had serious disorientation or some type of serious loss of memory, which were recorded by the practitioner. Chi-square test showed a strong correlation between osteoporosis and brain dysfunction: all 18 patients with brain dysfunction were osteoporotic, and none of the osteoarthritic showed any brain degenerative defect or even slight disorientation (chi-squared = 31.96, $P < 0.001$; table 1). Osteoporotic patients were older than non-osteoporotics ($F_{1, 111} = 44.305$, $P < 0.001$). Similarly, patients with brain dysfunction were older than those with normal brain function ($F_{1, 111} = 25.207$, $P < 0.001$). A logistic model including sex, age, osteoporosis and two-way interactions showed an increasing probability of brain dysfunction with increasing age for osteoporotic only, but no sex or year of operation effect (logistic regression on brain dysfunction with age, sex and osteoporosis as factors: deviance explained by the model = 44%; intercept = -9.9 ± 4.5; age effect = 0.116 ± 0.055, chi-square = 5.67, df = 1, P = 0.0173; Age of non-osteoporotics = -0.23 ± 0.52, chi-square = 18.04, df = 1, $P < 0.001$). The results are, therefore, in agreement with the *EMCaF* prediction 3 (i.e., that the proportion of patients with brain dysfunction is higher in osteoporotic than non-osteoporotic patients, once age and sex effects have been eliminated from the equation). More interestingly, the prediction that osteoporosis should precede brain dysfunction, is supported by the analysis. Depletion of Mn or another *EMCaF* mineral should show a steady increase with age (not up and down regulation). Therefore, as the age (proxy for *EMCaF* depletion from the skeleton) increases, the increased probability of brain dysfunction should occur only in patients where the *EMCaF* is being depleted (i.e., in osteoporotic but not in osteoarthritic patients). In other words, the age of patients with osteoporosis and no brain dysfunction was lower than those with both osteoporosis and brain dysfunction (79.9 ± 1.5 years *vs.* 84.9 ± 1.5, P = 0.025). Figure 1 illustrates clearly this observation of age appearing only in osteoporotic patients.

Our current data support the hypothesis that degenerative diseases linked to brain dysfunction are associated with osteoporosis and not osteoarthritis, as expected by prediction 3 of our hypothesis. Furthermore, as predicted by a corollary of this prediction, brain dysfunction should increase with age, only in the group where Mn or other *EMCaF* is depleted (osteoporotics). Evidence regarding Mn and bone accretion on one hand, and Mn and brain dysfunction on the other is described below.

6. EVIDENCE REGARDING THE ROLE OF Mn IN BONE ACCRETION OR OSTEOPOROTIC BONES

Apart from our study showing the role of Mn in Ca accretion in antlers, a study using rat model (18), showed similar effects in internal bones under artificial diet regiments. A diet depleted from Mn but not Ca produced femurs with levels lower in Mn and Ca in rats that were growing at a normal rate with no other effects of the diet. Other studies conducted in antlers, tooth or other mammal

Table 1. Number of patients with osteoporotic or osteoarthritic surgery who suffered brain diseases (Alzheimer's disease, Parkinson, etc).

	Brain malfunction	No brain malfunction	Total
Osteoporotic	18	27	45
Osteoarthritic	0	68	68
TOTAL	18	95	113

Table 2. Correlation between severity of Alzheimer disease induced by Al-treatment and contents of Mn in various organs in rats (test performed after data shown in (23))

	Days of treatment	Bone	Brain	Liver	Testes	Spleen
Bone	-0.88[1]					
Brain	-0.20	0.22				
Liver	-0.13	0.02	0.46			
Testes	0.35	-0.28	0.37	0.37		
Spleen	0.27	-0.23	0.46	0.55	0.71[2]	
Kidneys	0.08	0.22	0.76[2]	0.28	0.42	0.59

Abbreviations: [1] Significant at $P<0.01$ [2] Significant at $P<0.05$

bones, reported statistical trends showing lower Mn contents in more fragile bones, but these trends were not statistically significant (reviewed in reference10). However, a significant decrease in BMD and bending strength was observed in bones from moose living in south Sweden that was associated with significantly higher amounts of Pb in comparison with those living in other regions of the country (15). More interestingly, all other minerals examined showed non-significant trends for higher content (Ca, P, Fe and Zn), except Mn, which was the only mineral with a lower, but non-significant, content in osteoporotic.

According to prediction 2, osteoporotic bones in humans should show lower contents of Mn than normal (or osteoarthritic in our case) ones. This hypothesis will be tested by studying mechanical properties and bone porosity of human bones. In the literature there are very few papers examining mineral profiles of osteoporotic bones. One study examined differences in diabetic osteoporotic bone compared to normal bone (19), but unfortunately, Mn was not included in the list of the minerals that were examined (Ca, P, Zn, Sr, S, Fe, Cu, Pb, and Cr). In a recent and extensive review on bone changes with increasing age, including osteoporosis (9), Boskey and Coleman reported changes in crystal size, carbonate substitution, Ca/P ratio and various changes in proteins, but no change was observed in the composition of minor minerals.

7. EVIDENCE FOR THE ROLE OF Mn IN BRAIN DEGENERATIVE DISEASES AND EPILEPSY

Deregulation in the content of certain minerals appears to be linked to brain dysfunction. The best known example showed brain dysfunction caused by toxic effect rather than a deficiency in a mineral. Animals loaded with Aluminum (Al) developed both symptoms and brain lesions that are similar to those found in Alzheimer's disease (20). Although this effect is extensively reviewed in the literature, a previous study investigating the toxic effect of Al on concentrations of other minerals in the brain of young and older rats (21), showed that among a complex pattern of mineral changes, Mn was reduced with longer exposure to Al, and Mn content was lower in older rats. A more direct relationship between Mn and brain function in human epilepsy indicates that human epileptics have low whole blood Mn levels (22), reflecting low values of Mn in soft tissues, including probably brain (i.e., Mn metabolism is abnormal in epileptics); this suggests that a reduction of circulating Mn may affect brain function.

7.1. Depletion of bone Mn with increasing Alzheimer's disease

One of the best pieces of evidence supporting our hypothesis linking Mn, brain dysfunction and bone composition is a study carried out by Sanchez *et al*. (23). They assessed how mineral homeostasis in several organs was affected by Al-induced Alzheimer's disease. They examined mineral content, particularly Mn content, in various organs of young, adult and old rats at 0, 50 and 100 days post-Al treatment. According to our hypothesis 2 and 4, bone Mn should be consumed to keep the brain functioning, and when it is depleted brain dysfunction might occur. In other words, the greater the severity of Alzheimer symptoms, the lower the Mn in bones. We have not been able to test this in human bones yet, but inadvertently and without analyzing their own data in this respect, Sanchez *et al*. (23) gave evidence supporting this prediction. A correlation performed on the data they presented shows (Table 2) that Mn is significantly reduced with length of Al-treatment in bones only (i.e. the more severe the symptoms of induced Alzheimer's disease, the less Mn in bone), and not in liver, testes, spleen, kidneys or brain. The correlation is very high considering that it is based on means ($R = -0.88$). Thus, Al might be impeding Mn from reaching its target or being used. It is also possible that Al prevents fixation of Mn in bones only, but it is unclear why this would happen in this tissue only. It would be interesting to see the effect of Al treatment on mechanical properties and porosity of bones. It should increase porosity and reduce impact energy as we anticipate. In any case, it seems that, as Al-induced Alzheimer develops, Mn is depleted from bones only (23).

7.2. Role of Mn in Parkinson's disease

Mn is also associated with other major brain diseases examined in this study, including Parkinson's disease. In this case, chronic exposure to toxic levels of Mn leads to a neurological disorder known as manganism which shares many common features with Parkinson's disease (24). It seems reasonable that toxic effects appear, particularly in physiological routes where Mn is used. One

reason to explain this phenomenon is that where Mn is required, receptors and transporting proteins should be present to incorporate Mn into cells. Thus, it is more likely that organs and routes that are sensitive to Mn toxicity are also more susceptible to Mn deficiency than tissues where Mn is not used (and for instance, do not have receptors or transporters for Mn). If so, deficiency would affect the same cells that suffer Mn toxicity in the brain, including astrocytes (24), which, among other functions, support the metabolism of neurons, where Mn affects energy metabolism (25). In particular Mn is an essential component of glutamine synthetase, an astrocyte-specific enzyme (26). The study by Lee *et al*. (24) also showed an indirect evidence linking Mn and bone loss; estrogen protected against toxic effects of Mn in the brain, but as pointed by Pietschmann *et al*. (8) it is also estrogen deficiency which "is deemed to be the main cause of bone loss in early and also late postmenopausal women, and elderly men". Thus, a potential but rather speculative scenario might be that, under standard estrogen levels, Mn homeostasis would be maintained resulting in an appropriate supply of Mn to astrocytes in the brain. However, in elderly people, particularly women, estrogen reduction would impair Mn supply to astrocytes, and skeleton stores would be mobilized at a fast rate causing quick bone mass loss.

Although Mn seems to be the *EMCaF*, other minerals cannot entirely be ruled out. For instance Cu plays a role in the brain dysfunction caused by Scrapie, a disease similar to mad-cow disease in the sheep (27).

In conclusion, in light of our recent studies, we postulate that osteoporosis is caused by depletion of Mn, or possibly other minor mineral essential for incorporation of Ca in the bones. The mineral, whatever it is, is extracted from the skeleton during the process of remodeling, and used for a higher priority function, most likely brain function as we suggest here. Clinical data presented is compatible with the predictions of our hypothesis, showing brain dysfunction in patients with osteoporosis only, and not with osteoarthritis. The other predictions are partially supported by findings reported in the literature.

8. PERSPECTIVES

In the future, research examining factors that affect antler mineral composition, mechanical properties and histology might be of great importance for bone biology and medicine. Our research showed that Mn appears to be essential for fixing Ca in bones. A potential mechanism might be through chondroitin sulphate, a major constituent of cartilage that contains bound Mn (28). Our present hypothesis on osteoporosis suggests that Mn needed for brain function might be extracted from the bone. The prediction that osteoporosis patients depleting bone Mn would likely to develop Alzheimer's or Parkinson's disease was supported by 40% cases of brain dysfunction in these patients vs. 0% in osteoarthritic patients. However, osteoarthritic patients are treated with chondroitin sulphate to alleviate symptoms and improve mechanical performance (29-31). Further research is needed to clarify whether, in addition to bone depletion of Mn leading to brain dysfunction, chondroitin sulphate treatment is effective in slowing brain degenerative processes, and whether it has any effect on delaying osteoporosis.

Other studies on factors affecting antler breakage are needed to understand the mechanical performance of internal bones. An ongoing study by our group showing a large number of micro-cracks caused by lack of Mn, seems particularly promising.

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