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Bone—more than a standalone organ: a system sharing multiple connections with other tissues

by G. Karsenty, USA

For many scientists other than bone biologists, bones are viewed as a mere assembly of calcified, i.e., inert tubes whose study is not of great interest beyond their embryonic development. As usual, in biology, and life in general, there is more than meets the eyes, and only recently have we come to realize the wealth of biology surrounding bone tissue.

Indeed, bone has several peculiarities that suggested from the outset that this superficial view could not be further away from reality. For instance, bone constantly undergoes destruction followed by de novo bone formation in the context of two important physiological functions, bone modeling during childhood and bone remodeling during adulthood. To achieve this, bone is the only tissue that contains a cell type, the osteoclast, whose main—if not only—function is to destroy the host tissue. This function distinguishes osteoclasts from macrophages, monocytes, or lymphocytes, which are there to fight foreign bodies. Instead, osteoclasts are there to destroy what is not a foreign body, but our own mineralized bone extracellular matrix. Bone is also the tissue in which most of hematopoiesis occurs during adult life. On the basis of these two features alone, it was therefore likely that bone cells must be connected, in ways that remained to be defined, to many other organs in the body. If one comes to think about it, is this not the rule rather the exception in vertebrate physiology? And if it is the rule, why would skeleton, unlike any other organ, be a standalone entity not affected by, and not affecting, other organs and functions?

In the first phase of its history, biology established that bone was influenced by long-acting hormones such as parathyroid hormone or sex steroid hormones. This was a de facto demonstration that there is more to bone biology than bones themselves. Subsequent phases in the history of bone biology elucidated the molecular bases of how osteoblasts promote osteoclast differentiation and identified novel hormones regulating bone mass. Molecular biologists and geneticists are now busily identifying novel bone functions.

This issue of Medicographia, in addition to providing a much needed update on more traditional issues of bone biology, reviews some of the exciting recent advances that are changing the way in which we perceive bone, its functions, and its multiple connections with the rest of the organism. These advances involve two main areas: the first concerns the connections between the control of bone mass and hematopoiesis and various aspects of immunology; the second, the relationship between bone and diverse aspects of energy metabolism.

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Let us look first at the hematopoietic stem cell (HSC) niche. This is the anatomical location in which HSC cells reside and self-renew, and which, in addition to hematopoietic cells, also contains a host of nonhematopoietic cells, such as fibroblasts, reticular cells, endothelial cells, adipocytes, and osteoblasts. What we have learned in recent years is that the osteoblasts are a critical component of the HSC niche in that they are capable of influencing the size of the HSC pool. We have also discovered that many of the cytokines whose role was established in lymphopoiesis also affect osteoclast differentiation, while cytokines or soluble receptors promoting or inhibiting osteoclast differentiation are also involved in several aspects of the immune response. This emerging field of osteoimmunology will be expounded by Anna Teti and Nadia Rucci in the first themed article of this Medicographia monograph.

In relation with this topic, Jorge Cannata-Andía and his colleagues discuss the connection between bone remodeling and erythropoiesis, which comprises the connections between kidney and bone. This connection is well known to the clinician since it explains the emergence of a devastating disease, renal osteodystrophy, which leads to renal failure. However, the molecular and genetic bases of this relationship are not all understood.

Several chapters of this monograph touch upon another recent advance in bone biology, the emerging relationship between the control of bone mass and the regulation of energy metabolism. I postulated this relationship some 10 years ago, based on the huge energetic needs imposed on the body by bone modeling and remodeling. Energy metabolism is a broad entity encompassing food intake, appetite, energy expenditure, and glucose metabolism. As a result, it also includes many organs such as the gastrointestinal tract where food is absorbed, the brain, which controls appetite and energy expenditure, the islets of the endocrine pancreas, (which produce not only insulin, but other hormones regulating glucose metabolism), and, ultimately, all the target tissues of insulin.

The crosstalk between the regulation of bone metabolism and energy metabolism occurs at multiple levels, several of which are discussed in this monograph.

A first aspect of this crosstalk has to do with the fortuitous, but groundbreaking, discovery that serotonin, produced by the enterochromaffin cells of the gastrointestinal tract, is in fact a hormone that acts through a specific receptor on osteoblasts to inhibit their proliferation and thereby dampen bone formation. This discovery is important for several reasons, not less because it increases our understanding of the molecular regulation of bone remodeling. The fortuitous discovery that a very-well-known neurotransmitter also is such a powerful hormone illustrates how ignorant we still are about whole-organism physiology and how an all-out genetic approach to the entire organism is needed to increase our knowledge.

This discovery provided a molecular explanation for two rare human genetic diseases, osteoporosis-pseudoglioma syndrome and high-bone-mass syndrome, which are caused, respectively, by loss and gain of function due to mutations in the surface molecule Lrp5. Lrp5 acts as an inhibitor of serotonin synthesis by enterochromaffin cells. Patients with high-bone-mass syndrome have lower circulating serotonin levels, and provide an in vivo demonstration that inhibiting serotonin synthesis by enterochromaffin cells of the gut could be a means to treat osteoporosis, since these patients do not develop osteoporosis after the menopause. Thus, a direct outcome of the better understanding of the role of serotonin in bone remodeling has been the definition of a new class of bone anabolic drugs.

A second aspect of the crosstalk between the regulation of bone metabolism and energy metabolism was the identification of the genetic and molecular mechanisms that coordinate bone mass accrual and energy metabolism. Although not specifically covered in this monograph, this novel area of bone physiology permeates three of its contributions. As discussed by Vijay Yadav and colleagues, 10 years ago now we showed that the adipocyte-derived hormone leptin, which, remarkably, appears during evolution in parallel with the evolution of of bone, inhibits bone mass accrual. This led to the demonstration that bone mass accrual is regulated centrally, and is an aspect of bone biology now studied in many laboratories around the world and which is covered in Maria Luisa Brandi’s article. This aspect is also relevant to the understanding of how the skeletal manifestations of anorexia nervosa and of obesity develop (see Bernard Cortet’s article).

Looking at bone and its most closely connected “companion”—muscle—Laurence Vico shows that physical exercise—hence muscle mass—is directly related to bone mass, some sports being bone-building (eg, jogging and gymnastics), while others are far less osteogenic (cycling and swimming). She then discusses the potential osteogenic benefits of whole-body vibrations as a therapeutic means to increase bone mass.

Heike Bischoff-Ferrari looks at another connection between bone and the organism: the skin, and an old friend, vitamin D, which is produced there after exposure to the sun’s ultraviolet B light. The author discusses the benefits of vitamin D in regard to fracture reduction, related to it dual role of decreasing falls and increasing bone density.

Finally, in the last themed article, Daniel Lajeunesse and Johanne and Jean-Pierre Pelletier highlight recent advances concerning two diseases hitherto thought to be mutually exclusive, osteoporosis and osteoarthritis. It now seems increasingly likely that the mechanisms leading to these two major health burdens overlap, and are ascribable to changes affecting bone and subchondral bone tissue. This of course
has major therapeutic implications since osteoarthritis could benefit from agents inhibiting subchondral bone resorption and/or promoting bone formation.

These new lines of research are exciting in themselves and because of the insights they provide into how bone mass is regulated. They are also a clear indication that we are far from having discovered all the functions exerted by the skeleton. Since so many hormones are now known to regulate bone mass accrual, could it be that bone is only a recipient of influences, or rather that it reacts to them by determining the synthesis of these hormones? In other words, is the skeleton an endocrine organ regulating energy metabolism? And if this is the case, does the skeleton have other endocrine functions beyond those related to energy metabolism? These questions open up exciting perspectives, and it is increasingly obvious that this is the direction that modern bone biology is taking.

**Keywords:** bone metabolism; physiology; crosstalk; serotonin; energy metabolism; leptin
De récentes avancées ont mis en évidence les connexions métaboliques multiples entre les os et le reste de l'organisme : nous sommes loin d'avoir découvert toutes les fonctions exercées par le squelette. L'acquisition de la masse osseuse étant régulée par un grand nombre d'hormones, le squelette n'est-il qu'un organe passif ou réagit-il activement aux influences qui agissent sur lui en déterminant la synthèse de ces hormones ? En d'autres termes, le squelette est-il un organe endocrine régulant le métabolisme énergétique, voire d'autres fonctions endocrines ?

En effet, les os présentent plusieurs caractéristiques qui ont suggéré dès le début que cette vision superficielle ne pouvait pas être plus éloignée de la réalité. Par exemple, les os subissent en permanence une destruction suivie par une formation osseuse de novo dans le cadre de deux fonctions physiologiques importantes, le « modelage » (ou phase d'acquisition de la masse osseuse) au cours de l'enfance et le remodelage osseux au cours de l'âge adulte. Ces phénomènes sont soutenus par un type de cellule spécifique du tissu osseux, les ostéoclastes, dont la principale fonction – si ce n'est la seule – est de détruire le tissu hôte. Cette fonction distingue les ostéoclastes des macrophages, des monocytes ou des lymphocytes, dont le rôle est de combattre les corps étrangers. Au contraire, les ostéoclastes détruisent, non pas un corps étranger, mais leur propre matrice extracellulaire osseuse minéralisée. Le tissu osseux est le seul dans lequel se déroule la plus grande partie de l'hématopoïèse au cours de la vie adulte. En ne considérant que ces deux caractéristiques, il était par conséquent probable que les cellules osseuses soient connectées, par des liens restant à identifier, à de nombreux autres organes du corps humain. À la réflexion, cette situation ne constitue-t-elle pas plutôt la règle que l'exception dans la physiologie des vertébrés ? Et s'il s'agit d'une règle, pourquoi le squelette, contrairement à tout autre organe, constituerait-il une entité indépendante ne subissant ni n'exerçant aucune influence vis-à-vis d'autres d'autres organes et fonctions ?

Dans la première phase de son histoire, la biologie nous a appris que les os étaient soumis à l'influence d'hormones à longue durée d'action, notamment la parathormone et les hormones stéroïdes sexuelles. Ces découvertes ont constitué de facto une démonstration que la biologie osseuse s'étendait au-delà des os eux-mêmes. Les phases ultérieures de l'histoire de la biologie osseuse ont permis d'élucider les bases moléculaires par lesquelles les ostéoblastes favorisaient la différenciation des ostéoclastes, et d'identifier de nouvelles hormones régulant la masse osseuse. Les spécialistes de la biologie moléculaire et de la génétique continuent encore aujourd'hui à découvrir de nouvelles fonctions osseuses.

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Pour de nombreux scientifiques, à l'exception des biologistes spécialisés dans le tissu osseux, les os sont considérés comme un simple ensemble de tubes calcifiés, inertes, dont l'étude n'est pas d'un grand intérêt mis-à-part leur développement embryonnaire. Comme c'est souvent le cas en biologie, et dans les sciences de la vie en général, les apparences peuvent être trompeuses, et ce n’est que récemment que nous avons pris conscience de la richesse biologique qui émane du tissu osseux.

En effet, les os présentent plusieurs caractéristiques qui ont suggéré dès le début que cette vision superficielle ne pouvait pas être plus éloignée de la réalité. Par exemple, les os subissent en permanence une destruction suivie par une formation osseuse de novo dans le cadre de deux fonctions physiologiques importantes, le « modelage » (ou phase d’acquisition de la masse osseuse) au cours de l’enfance et le remodelage osseux au cours de l’âge adulte. Ces phénomènes sont soutenus par un type de cellule spécifique du tissu osseux, les ostéoclastes, dont la principale fonction – si ce n’est la seule – est de détruire le tissu hôte. Cette fonction distingue les ostéoclastes des macrophages, des monocytes ou des lymphocytes, dont le rôle est de combattre les corps étrangers. Au contraire, les ostéoclastes détruisent, non pas un corps étranger, mais leur propre matrice extracellulaire osseuse minéralisée. Le tissu osseux est le seul dans lequel se déroule la plus grande partie de l’hématopoïèse au cours de la vie adulte. En ne considérant que ces deux caractéristiques, il était par conséquent probable que les cellules osseuses soient connectées, par des liens restant à identifier, à de nombreux autres organes du corps humain. À la réflexion, cette situation ne constitue-t-elle pas plutôt la règle que l’exception dans la physiologie des vertébrés ? Et s’il s’agit d’une règle, pourquoi le squelette, contrairement à tout autre organe, constituerait-il une entité indépendante ne subissant ni n’exerçant aucune influence vis-à-vis d’autres d’autres organes et fonctions ?

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Plus qu’un organe « en solo » : l’os, un système partageant des connexions multiples avec d’autres tissus

par G. Karsenty, États-Unis
Ce numéro de Medicographia, outre une mise à jour très attendue sur des aspects plus traditionnels de la biologie osseuse, passe en revue les avancées passionnantes les plus récentes qui sont en train de changer la manière dont nous comprenons le tissu osseux, ses fonctions et ses connexions multiples avec le reste de l’organisme. Ces avancées portent sur deux domaines principaux : le premier concerne les connexions entre la régulation de la masse osseuse et l’hématopoïèse et différents aspects de l’immunologie ; le second, la relation entre les os et différents aspects du métabolisme énergétique.

Examinons tout d’abord la « niche hématopoïétique ». Il s’agit de la localisation anatomique dans laquelle les cellules souches hématopoïétiques (CSH) résident et s’auto-renouvellent, et qui contient, outre les CSH, un grand nombre de cellules non hématopoïétiques, notamment des fibroblastes, des cellules réticulaires, des cellules endothéliales, des adipocytes et les ostéoblastes. Nous avons appris ces dernières années que les ostéoblastes constituaient un élément essentiel de la niche hématopoïétique, dans la mesure où ils sont capables d’influencer la taille de la population des CSH. Nous avons en outre découvert que de nombreuses cytokines, dont le rôle a été établi dans la lymphopoïèse, étaient également impliquées dans la différenciation des ostéoclastes, et que certaines cytokines ou récepteurs solubles favorisant ou inhibant la différenciation des ostéoclastes participaient à divers aspects de la réponse immunitaire. Ce domaine émergeur de l’ostéo-immunologie sera exposé par Anna Teti et Nadia Rucci dans le premier article de cette monographie thématique de Medicographia.


Plusieurs chapitres de cette monographie concernent une autre avancée récente de la biologie osseuse, la relation nouvelle et découverte entre le contrôle de la masse osseuse et la régulation du métabolisme énergétique. J’avais postulé cette relation il y a environ 10 ans, sur la base des besoins énergétiques considérables imposés à l’organisme par le modelage et le remodelage osseux. Le métabolisme énergétique est un vaste concept qui recouvre l’apport alimentaire, l’appétit, la dépense d’énergie et le métabolisme glucidique. Il fait intervenir par conséquent de nombreux organes, notamment le tractus gastro-intestinal où sont assimilés les aliments, le cerveau qui contrôle l’appétit et la dépense énergétique, les îlots pancréatiques endocrines, qui produisent non seulement l’insuline, mais également d’autres hormones régulant le métabolisme glucidique, et enfin tous les tissus cibles de l’insuline.

Les interactions entre la régulation du métabolisme osseux et le métabolisme énergétique se manifestent à plusieurs niveaux, dont certains sont abordés dans cette monographie.

Un premier aspect de ces interactions concerne la découverte fortuite, mais fondamentale, ayant montré que la sérotonine, produite par les cellules entérochromaffines du tractus gastro-intestinal, est en fait une hormone qui agit par l’intermédiaire d’un récepteur spécifique situé sur les ostéoblastes afin d’inhiber leur prolifération, et par conséquent réduire la formation osseuse. Cette découverte est importante pour plusieurs raisons, en particulier parce qu’elle enrichit notre compréhension de la régulation moléculaire du remodelage osseux. La découverte fortuite que ce neurotransmetteur parfaitement connu était également une hormone particulièrement puissante illustre notre ignorance encore profonde de la physiologie générale de l’organisme, et la nécessité d’une approche génétique globale de l’organisme.

Cette découverte fournit une explication moléculaire à deux maladies génétiques rares chez l’homme, le syndrome d’ostéoporose avec pseudo-gliome et le syndrome de masse osseuse élevée, qui sont provoquées respectivement par une perte et un gain de fonction due à des mutations de la molécule de surface Lrp5. La molécule Lrp5 agit comme inhibiteur de la synthèse de la sérotonine par les cellules entérochromaffines. Les patients souffrant d’un syndrome de masse osseuse élevée présentent des concentrations circulantes de sérotonine plus faibles, et constituent une démonstration in vivo du fait que l’inhibition de la synthèse de sérotonine par les cellules entérochromaffines de l’intestin peut constituer un mode de traitement de l’ostéoporose, dans la mesure où les patientes atteintes ne développent pas d’ostéoporose après la ménopause. Par conséquent, l’un des résultats directs de la meilleure compréhension du rôle de la sérotonine sur le remodelage osseux a été la définition d’une nouvelle classe d’agents anaboliques osseux.

Un second aspect des interactions entre la régulation du métabolisme osseux et du métabolisme énergétique a été l’identification des mécanismes génétiques moléculaires coordonnant l’acquisition de la masse osseuse et le métabolisme énergétique. Bien que ce sujet ne soit pas spécifiquement abordé dans cette monographie, ce nouveau domaine de la physiologie osseuse est évoqué dans trois articles. Comme l’indiquent Vijay Yadav et coll., il y a maintenant 10 ans, nous avons montré que la leptine, une hormone dérivée des adipo-cytes, dont il faut souligner qu’elle apparaît au cours de l’évolution parallèlement à l’évolution du système osseux, inhibe l’acquisition de la masse osseuse. Cette observation, qui démontre que l’acquisition de la masse osseuse est régulée à un échelon central, constitue un aspect de la biologie osseuse désormais étudié dans de nombreux laboratoires à travers le monde, et abordé dans cette monographie dans l’article de Maria Luisa Brandi. Cet aspect est également
abordé par l’article de Bernard Cortet qui fait le point sur notre compréhension des manifestations squelettiques de l’anorexie mentale et de l’obésité.

Laurence Vico, qui examine les os et les organes qui leur sont le plus étroitement associés, les muscles, montre que l’exercice physique – et par conséquent la masse musculaire – influe directement sur la masse osseuse, certains sports favorisant la formation osseuse (par exemple, le jogging et la gymnastique), tandis que d’autres sont nettement moins ostéogènes (cycling et natation). L’auteur discute ensuite des bénéfices ostéogènes potentiels des vibrations du corps entier comme moyen thérapeutique pour entrainer une augmentation de la masse osseuse.

Heike Bischoff-Ferrari évoque sur une autre connexion entre les os et l’organisme : la peau, et une vieille connaissance, la vitamine D, qui est produite dans cet organe après l’exposition aux rayons ultraviolets B du soleil. L’auteur discute des bénéfices de la vitamine D au plan de la réduction des fractures, en relation avec son double rôle dans la diminution des chutes et l’augmentation de la densité osseuse.

Enfin, dans le dernier article-thème, Daniel Lajeunesse et Johanne et Jean-Pierre Pelletier soulignent les récentes avancées dans deux maladies considérées jusqu’ici comme mutuellement exclusives, l’ostéoporose et l’arthrose. Il semble désormais de plus en plus probable que les mécanismes conduisant à ces deux affections majeures partagent les mêmes mécanismes et soient imputables aux changements affectant le tissu osseux et le tissu osseux sous-chondral. Ces phénomènes ont bien entendu des conséquences thérapeutiques déterminantes, dans la mesure où l’arthrose serait de ce fait susceptible de bénéficier de l’action d’agents inhibant la résorption osseuse sous-chondrale et/ou favorisant la formation osseuse.

Ces nouveaux axes de recherche sont particulièrement intéressants en eux-mêmes, et par les éclairages qu’ils apportent sur les mécanismes de régulation de la masse osseuse. Ils constituent également un rappel que nous sommes loin d’avoir découvert toutes les fonctions exercées par le squelette. Dans la mesure où il est désormais établi que l’acquisition de la masse osseuse est régulée par un grand nombre d’hormones, le squelette apparaît désormais comme étant loin d’être un organe passif, mais qu’il réagit au contraire aux diverses influences agissant sur lui en déterminant la synthèse de ces hormones. Si tel est le cas, le squelette n’est-il pas un organe endocrine régulant le métabolisme énergétique, voire d’autres fonctions endocrines au-delà de celles liées au métabolisme énergétique ? Cette question ouvre des perspectives passionnantes, qui constituent à l’évidence la voie que la biologie moderne du tissu osseux est en train d’emprunter.
Osteoclasts and immune cells share a common origin: the hematopoietic stem cell (HSC). Recognition of this fact has led to the birth of a new discipline, osteoimmunology, which has clarified the involvement of bone cells in diseases initially considered as immunological, and identified the central role of some cytokines, produced by immune cells, in the regulation of bone cells. Recent advances point to the potential involvement of osteoclasts and osteoblasts in the regulation of HSCs directed to an immunological commitment.”

Bone is a tissue of central importance, maintaining several relationships with other organs. Among these, the immune system, with which it shares molecular pathways, transcription factors, and several cytokines responsible for bone and immune cell regulation. A paradigm of this crosstalk comes from the studies of Hiroshi Takayanagi on the mechanisms underlying the development of rheumatoid arthritis, demonstrating the central role of a subset of T lymphocytes in the induction of exaggerated osteoclast activity, thus leading to erosion in the affected joints. RANKL/RANK (receptor activator of nuclear factor–kappaB ligand) is an important pathway shared by bone and the immune system. This pathway is essential for both osteoclastogenesis and lymphocyte differentiation, so that diseases due to inactivating mutations of RANKL or RANK, such as osteopetrosis, result in immunological defects in addition to altered bone phenotype. This review focuses on the description of the principal molecules/pathways shared with the immune system, which under both physiological and pathological conditions, regulate bone remodeling by acting on osteoclast formation and activity. We propose that the evidence available today strongly points to the osteoclast as a cell with immunological properties, in addition to its role in bone resorption.

The perception of bone as a static organ has changed dramatically over the past several years. The literature has clearly shown that bone is a tissue of central importance and that, in addition to its role in locomotion and in the regulation of calcium and phosphate homeostasis, bone actively maintains multiple relationships with other organs.

Recent observations have evidenced crucial crosstalk between bone and the immune system, thus leading to the launch of a new interdisciplinary field, osteoimmunology. Indeed, several cytokines, molecular pathways, and transcription factors are shared by the immune and skeletal systems. Moreover, immune cells, like bone cells, arise from hematopoietic stem cells (HSCs) found in the bone marrow, which is physically as well as functionally associated with bone tissue. Interestingly, cell differentiation from HSCs has been shown to be subject to a fine regulation by the osteoblasts, which form the HSC niche. Kollet et al have consistently found that, once subjected to specific stressful stimuli, activated osteoclasts degrade endosteal components, thus promoting the mobilization of hematopoietic progenitors.

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The unexpected links between bone and the immune system – Teti and Rucci
Studies on autoimmune diseases, such as rheumatoid arthritis, performed by Hiroshi Takayanagi, have provided a pivotal contribution in development of the field of osteoimmunology, with identification of a subset of T cell population that produces high quantities of interleukin (IL)-17, a pro-osteoclastogenic cytokine that increases osteoclast differentiation by direct and indirect mechanisms, thus leading to bone destruction.1 Conversely, animal models lacking molecules pivotal for the regulation of the immune system frequently show an abnormal osteoclast phenotype.1

Based on this evidence, we believe that a more extensive investigation of the mechanisms underlying the bone-immune interplay could allow the identification of new strategies for the management of immune system and bone disorders. In this review, we summarize the recent findings that have contributed to consolidation of the field of osteoimmunology, with particular focus on the close relationship between the osteoclasts and the immune cells.

**Selected abbreviations and acronyms**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>ARF</td>
<td>activation-resorption-formation (sequence)</td>
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<td>ARO</td>
<td>autosomal recessive osteopetrosis</td>
</tr>
<tr>
<td>Blmp1</td>
<td>B lymphocyte-induced maturation protein–1</td>
</tr>
<tr>
<td>BMP</td>
<td>bone morphogenetic protein</td>
</tr>
<tr>
<td>CAMKIV</td>
<td>calcium/calmodulin-dependent protein kinase IV</td>
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<tr>
<td>DC-STAMP</td>
<td>dentritic cell-specific transmembrane protein</td>
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<td>FcRy</td>
<td>Fc-receptor common gamma subunit</td>
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<tr>
<td>FGFR</td>
<td>fibroblast growth factor</td>
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<tr>
<td>HSCs</td>
<td>hematopoietic stem cells</td>
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<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IRF</td>
<td>interferon regulatory factor</td>
</tr>
<tr>
<td>ITAM</td>
<td>immunoreceptor tyrosine-based activation motif</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>M-CSF</td>
<td>macrophage-colony stimulating factor</td>
</tr>
<tr>
<td>MMP</td>
<td>metalloproteinase</td>
</tr>
<tr>
<td>NFATc1</td>
<td>nuclear factor of activated T cells, cytoplasmic 1 (cytoplasmic 2)</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor–kappaB</td>
</tr>
<tr>
<td>ODF</td>
<td>osteoclast differentiation factor</td>
</tr>
<tr>
<td>OPG</td>
<td>osteoprotegerin</td>
</tr>
<tr>
<td>OPGL</td>
<td>osteoprotegerin ligand</td>
</tr>
<tr>
<td>PGE2</td>
<td>prostaglandin E2</td>
</tr>
<tr>
<td>PTH</td>
<td>parathyroid hormone</td>
</tr>
<tr>
<td>RANK</td>
<td>receptor activator of nuclear factor–kappaB</td>
</tr>
<tr>
<td>RANKL</td>
<td>receptor activator of nuclear factor–kappaB ligand</td>
</tr>
<tr>
<td>SOFAT</td>
<td>secreted osteoclastogenic factor of activated T cells</td>
</tr>
<tr>
<td>TGFβ</td>
<td>transforming growth factor-beta</td>
</tr>
<tr>
<td>TRAF</td>
<td>TNF-receptor associated factor</td>
</tr>
<tr>
<td>TRANCE</td>
<td>TNF-related activation induced cytokine</td>
</tr>
</tbody>
</table>

**The bone remodeling process**

It is well known that bone tissue is in dynamic flux, continually renewed lifelong by a physiological process termed bone remodeling.4,5 This process is mandatory for the replacement of immature bone with mechanocompetent bone, as well as for repair of fractures and for proper calcium balance. Indeed, it has been estimated that at least 10% of bone is renewed per year.

Bone remodeling follows the activation-resorption-formation (ARF) sequence (Figure 1). The first step, called the activation phase, starts with stimulation of the lining cells, quiescent osteoblasts, which, in response to appropriate stimuli, increase their own surface expression of receptor activator of the nuclear factor-kappaB (NF-κB) ligand (RANKL), which in turn interacts with its receptor RANK (receptor activator of NF-κB), expressed by preosteoclasts. RANKL/RANK interaction triggers preosteoclast fusion and differentiation to multinucleated osteoclasts. Once differentiated, osteoclasts polarize, adhere to the bone surface, and dissolve bone (resorption phase), then they undergo apoptosis, which is a physiological process, required to prevent excessive bone resorption.

After this resorptive process, there is an intermediate phase preceding bone formation, called a reversal phase, during which some macrophage-like uncharacterized mononuclear cells are observed at the site of remodeling, whose function consists of removal of debris produced during matrix degradation.

The final step, bone formation, is triggered by several growth factors stored in the bone matrix and released after its degradation, including bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and transforming growth factor–β (TGFβ), which are likely to be responsible for recruitment of osteoblasts in the resorbed area. Once recruited, osteoblasts produce new bone matrix, initially not mineralized (osteoid), and then they promote its mineralization, thus completing the bone remodeling process.

Under physiological conditions, the coupling of bone formation with previous resorption occurs faithfully. In contrast, an imbalance between the resorption and formation reflects improper bone remodeling, which in turn affects the bone mass, eventually leading to a pathological condition.

**Osteoblast regulation of osteoclastogenesis**

Although the principal function of the osteoblasts is to synthesize bone matrix proteins and to promote the process of mineralization, a crucial role of osteoblasts in osteoclast biology has been clearly demonstrated by the release of key molecules that regulate osteoclastogenesis and bone resorption. Osteoclasts are multinucleated cells that arise from the monocyte/macrophage cell line.6 Starting from multipotent HSCs, transcription factor PU.1, along with the macrophage-colony...
stimulating factor (M-CSF), allows the commitment toward a common progenitor for macrophages and osteoclasts (Figure 2). In particular, PU.1 positively regulates the M-CSF receptor, c-Fms, while M-CSF stimulates proliferation of osteoclast precursors and upregulates RANK expression. With the expression of c-Fms and RANK receptors, the precursors become fully committed to osteoclast lineage. The main source of RANKL in bone is the osteoblast, which expresses RANKL on its membrane surface, thus inducing osteoclast differentiation by interacting with the RANK receptor expressed by the osteoclast precursors. Therefore, triggering of the RANKL/RANK pathway requires a cell-cell contact (Figure 3, page 344). However, lower quantities of soluble RANKL are also released after enzymatic cleavage of the surface molecule by metalloproteinase (MMP)-14. Another key molecule produced by osteoblasts that interfere with the RANKL/RANK pathway is osteoprotegerin (OPG), a decoy receptor for RANKL with an osteoprotective role. Indeed, OPG is a secreted protein sharing the same structure of the extracellular domain of RANK so that it binds RANKL, preventing its interaction with RANK and subsequent inhibition of osteoclastogenesis.

RANKL/RANK signaling
RANKL is a type II membrane protein belonging to the TNF superfamily, while its receptor RANK is a type I membrane protein. Osteotropic hormones and factors such as 1,25-di-hydroxyvitamin D₃ (1,25(OH)₂D₃), parathyroid hormone (PTH), prostaglandin E₂ (PGE₂), and IL-11 upregulate the expression of RANKL in osteoblast/stromal cell plasma membrane. As previously mentioned, RANKL interacts with its receptor RANK, located on the preosteoclast surface, which in turn activates signaling by recruiting adaptor molecules belonging to the TNF-receptor–associated factors (TRAF) family (Figure 3). Indeed, the RANK cytoplasmic tail contains three binding sites for TRAF6 and this interaction is mandatory for osteoclast differentiation, since TRAF6 knockout mice develop osteoporosis. Binding of TRAF6 to RANK induces trimerization of TRAF6, leading to activation of nuclear factor–κB (NF-κB) and of mitogen-activated protein kinases (MAPKs).
NF-κB includes a family of dimeric transcription factors, which reside in the cytoplasm under nonstimulated conditions. However, they enter the nucleus upon cell stimulation by various factors, including RANKL. NF-κB is central to the osteoclastogenic process since the double knockout of the p52 and p50 subunits leads to blockade of osteoclast formation.12

Another transcription factor crucial for osteoclast differentiation is activator protein 1 (AP-1) complex, which consists of c-Fos, c-Jun, and ATF proteins. In particular, c-Fos is specifically induced by RANK and is critical for osteoclastogenesis, since c-Fos knockout mice develop osteopetrosis due to the lack of osteoclasts.13

NF-κB upregulates the expression of another key molecule for osteoclast differentiation, nuclear factor of activated T cells, cytoplasmic 1 (NFATc1) transcription factor.14,15 This initial induction requires the interaction of NF-κB with NFATc2, which is recruited to the NFATc1 promoter independently of RANKL stimulation. The essential role of NFATc1 in osteoclastogenesis was demonstrated by evidence that NFATc1-deficient embryonic stem cells did not differentiate into osteoclasts, while the ectopic expression of NFATc1 induced osteoclast differentiation also in the absence of RANKL.17

Chromatin immunoprecipitation experiments revealed that NFATc1 is recruited to the NFATc1 promoter region 24 hours after RANKL stimulation, and this occupancy persists during the terminal differentiation of osteoclasts, thus indicating a mechanism of autoamplification.18

In cooperation with AP-1, PU.1, NF-κB, and microphthalmia-associated transcription factor (MITF), NFATc1 regulates the transcription of several target genes involved in osteoclast differentiation and function (Figure 3). These include cathepsin K, calcitonin receptor, tartrate-resistant acid phosphatase (TRACP), β3 integrin, osteoclast-associated receptor (OSCAR),7 and dendritic cell–specific transmembrane protein (DC-STAMP), the latter involved in osteoclast fusion.

RANKL/RANK signaling is shared by bone and the immune system

When we talk about the role of RANKL/RANK in the immune system, we need to point out that RANKL, also known as TNF-related activation-induced cytokine (TRANCE) according to the nomenclature of the immune system, and its receptor RANK, were first identified as molecules expressed by T cells and dendritic cells, respectively, and their physical interaction increased the ability of dendritic cells to stimulate naive T cell proliferation as well as dendritic cell survival.

Therefore, the RANKL/RANK pathway was “born” in an immunologic context. At the same time, bone researchers identified the so called osteoclast differentiation factor (ODF), expressed by the osteoblasts, which increased osteoclast formation,20 and OPG, an osteoblast-derived secreted member of the TNF receptor family, which, in contrast, inhibited osteoclast development and bone resorption acting as a decoy receptor. The molecule able to interact with OPG, named OPG-ligand (OPGL),21 was then identified. Finally, bone researchers and immunologists joined in the conclusion that RANKL/TRANCE, ODF, and OPGL are the same molecule, and that RANKL-expressing T cells can also activate osteoclasts, thus mimicking the effect of osteoblasts. Based on this evidence it is not surprising to find bone loss in patients with disorders characterized by abnormal activation of the immune system, such as rheumatoid arthritis or other chronic inflammatory diseases.

Immunological role of the RANKL/RANK pathway

As far as the role of RANKL in the immune system is concerned, it has been demonstrated that, in addition to bone phenotype, due to the lack of osteoclasts, RANKL-deficient
mice show a defect in the development of secondary lymphoid tissue. However, these mice do not present a severe immunodeficiency, likely due to the fact that lack of RANKL in T cells is compensated by CD40. RANKL also seems to be important for mammary development and has been found to be involved in inflammatory bowel diseases by stimulating dendritic cells.

Recent evidence identified a role for RANKL as a chemokine that can attract RANK-expressing tumor cells and osteoclasts, thus pointing to a role of this factor in tumor-induced bony metastases.

Finally, a recent study (2009) identified an unexpected role of RANKL/RANK in the central nervous system, showing that this pathway was involved in thermoregulation and central fever response in inflammation.

**RANKL/RANK–linked diseases**

The versatility of the RANKL/RANK axis mirrors the complexity of the diseases in which this pathway is lacking. Among them, osteopetrosis is a rare genetic disorder characterized by sclerosis of the skeleton due to reduced or complete lack of osteoclast function and, as a consequence, impairment of bone resorption. This disease is clinically very heterogeneous, ranging from severe to asymptomatic. Autosomal recessive osteopetrosis (ARO) is the most severe form of osteopetrosis, usually diagnosed within the first year of life and in patients with a resultant life expectancy of 3 to 4 years. Similar clusters of patients with ARO harbor mutations in the genes encoding for RANKL and RANK. In contrast with all the other forms characterized by a normal or increased number of osteoclasts that are unable to resorb bone, obviously this is an osteoclast-poor ARO form.

Importantly, beside bone phenotype, there are also immunological defects, such as hypogammaglobulinemia due to impairment in immunoglobulin-secreting B cells. This is in line with evidence showing the importance of RANKL/RANK in the immune system, which should be taken into account in the management of this form of ARO. Indeed, it has been demonstrated that two ARO patients harboring RANK mutations exhibited impaired fever during pneumonia.

**Ig-like receptors and osteoclast regulation**

Beside the well-known RANKL/RANK pathway, osteoblasts can regulate osteoclast differentiation by interacting with immunoglobulin (Ig)-like receptors, such as OSCAR, whose ligand has not yet been clearly identified. These receptors are associated with immunoreceptor tyrosine-based activation motif (ITAM)-harboring adaptor molecules DAP12 and Fc-receptor common gamma subunit (FcRγ). The role of the latter molecules in osteoclast regulation has been underlined by evidence that mice deficient in both DAP12 and FcRγ have an osteopetrotic phenotype. Phosphorylation of the ITAM sequence in DAP12 or FcRγ, resulting after RANK activation, allows the recruitment of splenocyte tyrosine kinase (SYK) and resultant activation of phospholipase C gamma (PLCγ), which in turn triggers calcium signaling. Calcium signaling promotes osteoclastogenesis by activating calcium/calmodulin-dependent protein kinase type IV (CAMKIV), which concurs to c-Fos and calcineurin activation, both cooperating to potentiate NFATc1 autoamplification (Figure 4).

Among the molecules that have a dual role in the regulation of immune cells and osteoclasts, a recent study identified the transcription factor B lymphocyte-induced maturation protein–1 (Blimp1). This is a transcriptional repressor involved in the differentiation of B lymphocytes toward plasma cells by direct repression of the transcription factors Pax5, Bcl, and Myc. Nishikawa and colleagues demonstrated that Blimp1 stimulates osteoclastogenesis by repressing the transcription factors IFN regulatory factor-8 (IRF-8) and v-Maf musculo-aponeurotic fibrosarcoma oncogene family, protein B (MafB), both negatively affecting osteoclastogenesis.
Inflammatory cytokines and osteoclastogenesis

Among the cells of the immune system, T lymphocytes play a crucial role in the regulation of osteoclastogenesis, which is indeed the result of a balance between positive and negative factors produced by T cells. As far as the RANKL/RANK pathway is concerned, it has been demonstrated that activated T cells express RANKL on their surface, thus activating osteoclastogenesis by cell–cell contact. Activated T cells also produce IL-10, IL-12, and IL-18, which, in contrast, negatively affect osteoclastogenesis. As described below, the CD4+ T helper (T₄₁)-cell subset T₄₁ and T₄₂ produce interferon gamma (IFN-γ), which suppresses RANKL signaling by degrading TRAF6, and IL-4, another cytokine with an anti-osteoclastogenic role.

Other cells of the immune system, such as the macrophages, contribute to osteoclast differentiation and function by producing IL-1, IL-6, and TNF-α. Moreover, a recent study showed that lipopolysaccharides (LPS) upregulated the expression of membrane RANKL in human blood neutrophils. LPS-activated neutrophils were then able to stimulate osteoclastogenesis and bone resorption in co-cultures with osteoclast precursors in co-cultures with osteoclast precursors.

Finally, a recent report from Rifas and Weitzmann described the identification of a new T cell cytokine, called secreted osteoclastogenic factor of activated T cells (SOFAT), which induces both osteoblastic IL-6 production and osteoclast formation in the absence of osteoblasts or RANKL, and was insensitive to the effects of the RANKL inhibitor OPG.

Immune diseases and osteoclast activation

- **Rheumatoid arthritis**

One of the milestones that was pivotal in defining the new field of osteoimmunology came from research by Takayanagi et al on rheumatoid arthritis. This is an autoimmune disease characterized by inflammation of synovial joints, which, at variance with CD4+ TH 1 cells, does not produce the anticlastogenic cytokines IFN-γ and IL-4, but secretes high quantities of IL-17. This cytokine, in turn, triggers RANKL expression by synovial fibroblasts. IL-17 also stimulates local inflammation, thus inducing macrophages to secrete proinflammatory cytokines such as TNF-α, IL-1, and IL-6. These cytokines in turn activate osteoclastogenesis, directly as well as by stimulating RANKL expression by synovial fibroblasts. Finally, it has been shown that T₄₀ cells themselves express RANKL, thus activating osteoclastogenesis by direct induction of precursor differentiation.

- **Psoriatic arthritis**

This is a disease characterized by musculoskeletal inflammation, and several studies have reported the crucial role of TNF in its pathogenesis. Elevated levels of TNF have been found in the sera, synovial fluid, and synovial membranes of psoriatic patients. A marked reduction in inflammation and progressive joint damage was consistently observed in patients treated with anti-TNF drugs, which is not only due to their ability to reduce inflammation, but also to reduce osteoclast activation, since it is well known that TNF promotes osteoclast formation. On the other hand, recent reports showed that TNF can affect bone formation by inducing Dickkopf-related protein 1 (DKK-1) to impair bone-forming osteoblast development via inhibition of Wnt signaling.

Is the osteoclast an immune cell?

Based on the aforementioned evidence, it has been hypothesized that osteoclasts are cells that belong to the immune system. This raises the question as to why there is a need for an immune cell to resorb bone. Chambers had previously proposed that the bone matrix is recognized by osteoclasts as a peculiar “foreign body.” In fact, as described in the above (see the bone remodeling process), during the resting condition the bone matrix is covered by a layer of osteoblasts, or lining cells (Figure 1), which segregates the bone matrix from the interstitial fluid, thus probably preventing recognition by the immune system. An external stimulus, such as an inflammatory response, or exposure to PTH/parathyroid hormone-related protein (PTHrP), could trigger osteoclast retraction, so that the mineralized bone matrix can be exposed and recognized as a “foreign body” by immune cells, which have all the requirements to induce osteoclast formation and bone resorption.

Under physiological conditions, this process, once activated, must be switched off and, in fact, there are several paracrine and autocrine mechanisms that negatively regulate osteoclast activity. Consequently, osteoclasts are recalled in the previously resorbed site where they refill the lacunae with new-formed bone matrix and again segregate the bone surface from the interstice so that “foreign bone” is no longer exposed.
to the immune system. If the negative regulation of osteoclast activity fails, this process proceeds longer than necessary, thus resulting in excess bone resorption, with pathological consequences.

Conclusions

It is now clear that bone is a tissue of central importance, therefore, when we study the molecular mechanisms underlying bone remodeling and bone pathological events, we cannot ignore its multiple interactions with other tissues. The discipline of osteoimmunology has shown that osteoclasts and immune cells share a common origin. These two types of cells arise from the HSCs in the bone marrow, another organ closely related to bone. Immunology has also clarified the involvement of bone cells in the development of diseases initially classified in an immunological context, and has identified the central role of some cytokines, known to be produced by immune cells, in the regulation of bone cells. Furthermore, recent advances suggest the potential involvement of osteoclasts and osteoblasts in the regulation of HSCs directed to an immunological commitment. We believe that these findings should encourage immunologists and bone researchers to continue investigating this field, all the more so as better understanding of the relationships between bone and immune cells could help identify new strategies for the management of patients suffering from bone diseases.

References

Keywords: osteoimmunology; bone tissue; immune system; hematopoietic stem cell; osteoclast; cytokine; rheumatoid arthritis; bone remodeling
Impact of psychiatric disease on bone health

by B. Cortet and I. Legroux-Gérot, France

Psychiatric diseases may, via direct and indirect mechanisms, induce bone fragility. This is particularly the case with depression and anorexia nervosa. Studies show a moderate decrease in bone mineral density (of the order of 6%) in the spine and hip of depressed patients vs controls. Similarly, a significant increase in fracture risk is observed, with an up to 2-fold increase in hip fracture risk. The mechanisms of bone fragility in depressed subjects are complex, multifactorial, and have yet to be fully elucidated. One of the major direct mechanisms involves endogenous hyperadrenocorticism—which is less pronounced than in Cushing’s syndrome, and may be due in part to a rise in proinflammatory cytokines (notably interleukin 6), which is reported in depressed patients. Also, antidepressant treatment—in particular serotonin reuptake inhibitors—may have a negative impact on bone. Indirect factors, whose role is disputed, include weight loss and cigarette and alcohol abuse, often reported in depressed subjects. Anorexia nervosa (AN) has become a major problem in recent years. AN gives rise to multiple complications and is frequently associated with bone loss, with osteoporosis occurring in 38% to 50% of cases. Estrogen deficiency has long been known to play a major role, but cannot alone explain bone loss. Recent publications have highlighted the essential role of undernourishment and factors influenced by nutritional status, in particular the growth hormone–insulin-like growth factor I (GH-IGF-I) axis. The management of anorexia nervosa–related bone loss is debated. While restoring menstruation and body weight is mandatory, it does not always ensure correction of bone loss. Studies have failed to show any effectiveness of estrogen treatment.

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Certain psychiatric disorders may have a deleterious impact on bone. This occurs via direct and indirect mechanisms, not all of which have been elucidated. Essentially two psychiatric disorders have undergone extensive research to evaluate this relationship: depression and anorexia nervosa (AN). These two diseases will therefore be addressed in this paper.

IMPACT OF DEPRESSION ON BONE

Depression is a frequent disease, affecting about 16% of the North-American population. One of the first publications to link depression and impact on bone was published by Schweiger et al in 1994. These authors determined...
bone mineral density (BMD) by quantitative computerized tomo-
graphy (QCT) in 70 depressed subjects and 88 controls. The female/male ratio was the same in both groups. The au-
thors found an approximately 15% reduction in BMD in the
depressed subjects, after adjustment for age. Subsequently,
several articles on the same topic were published in which
BMD was measured by dual-energy x-ray absorptiometry
(DXA), the current consensus method; but the findings dis-
agreed: certain authors reported a link between depression
and low BMD,3-14 while others found no such link.15-21 This dis-
crepancy is undoubtedly related to the heterogeneity of the
disease itself. In addition, it is to be noted that most of the sub-
jects enrolled in the studies were, quite logically, on antide-
pressant treatment, often serotonin reuptake inhibitors (SSRIs),
known to have an impact on bone.

Epidemiology of bone impact in depression

Densitometric data
A meta-analysis, avoiding the aforementioned pitfalls, was re-
cently published,22 which included 8 cross-sectional and 6
case-control studies. Cohort studies were also evaluated when
densitometric data were available. In all the studies, BMD was
determined by DXA. In the cross-sectional studies, confounding
factors such as age, gender, menopausal status, weight, and body mass index (BMI) were taken into account in the
analysis of the results. The studies are summarized in Fig-
ure 1. Of the 14 studies, BMD data for all sites (lumbar spine and hip) were only reported in 12 studies, which
were thus finally selected for the meta-analysis. The decrease
in BMD was only significant in 6 cases. Mean between-group
BMD difference was only slight: 53 mg/cm² (95% confidence
interval [CI], 18-87) for the lumbar spine. The difference was
very similar for the hip: 52 mg/cm² (95% CI, 22-83). In the
depressed subjects, the percentage decrease in BMD was
5.9% for the lumbar spine and 6% for the hip.

When results were expressed as T-scores and Z-scores, the
trend was similar, as expected. The decrease, while real, was
only modest. Thus, mean T-scores in depressed subjects were

Figure 1. Mean differences in bone mineral density (BMD) between depressed and nondepressed groups and corresponding 95% confidence intervals (CI) for the spine (A) and hip (B) in 12 studies.

SELECTED ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AN</td>
<td>anorexia nervosa</td>
</tr>
<tr>
<td>BAP</td>
<td>bone alkaline phosphatase</td>
</tr>
<tr>
<td>BMD</td>
<td>bone mineral density</td>
</tr>
<tr>
<td>CRH</td>
<td>corticotropin-releasing hormone</td>
</tr>
<tr>
<td>CTX</td>
<td>C-terminal crosslaps</td>
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<tr>
<td>DXA</td>
<td>dual-energy x-ray absorptiometry</td>
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<tr>
<td>GH</td>
<td>growth hormone</td>
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<tr>
<td>IGF</td>
<td>insulin-like growth factor</td>
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<td>IL</td>
<td>interleukin</td>
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<tr>
<td>NTX</td>
<td>N-terminal crosslaps</td>
</tr>
<tr>
<td>QCT</td>
<td>quantitative computed tomography</td>
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<tr>
<td>SSRIs</td>
<td>selective serotonin reuptake inhibitors</td>
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</table>
Depression and fracture risk

Bone densitometry is only a surrogate marker and the most important issue is whether depressed subjects are at greater fracture risk. As is the case for densitometric assessment, this requires taking into account numerous factors well known to influence fracture risk. It should be pointed out that studies aimed at determining fracture risk are few. Moreover, some of them are open to methodological criticism, particularly those of Kessler et al,^1^ due to the fact that they are retrospective studies.

Four of the 5 prospective studies on fracture risk available to date concluded that depression was associated with an increase in fracture risk. In the remaining study, by Greendale et al,^25^ which evidenced no such increase, the authors nonetheless showed that those patients with the highest urinary cortisol level were at increased risk of fracture. The main findings from these studies are summarized in Table I.\(^{15,20,25-27}\)

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Sample size</th>
<th>Follow-up duration</th>
<th>Fracture type</th>
<th>Adjustment</th>
<th>Principal results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greendale et al,^25^ 1999</td>
<td>684</td>
<td>7 years</td>
<td>Hip, arm, spine, wrist and other</td>
<td>Age, race, gender, concomitant diseases, physical exercise, BMI, smoking, alcohol</td>
<td>No association with fracture risk</td>
</tr>
<tr>
<td>Forsen et al,^26^ 1999</td>
<td>18,612</td>
<td>3 years</td>
<td>Hip</td>
<td>Medication, BMI, smoking, physical exercise, handicap</td>
<td>Risk gradient as a function of depression severity</td>
</tr>
<tr>
<td>Whooley et al,^20^ 1999</td>
<td>7,414</td>
<td>3.7 years</td>
<td>Vertebral and nonvertebral</td>
<td>Age, marital status, educational level, fracture history, fall risk, diabetes, rheumatologic disease history, steroids intake, corticosteroids, estrogen treatment, calcium intake, cognitive function, hip BMD</td>
<td>Increase in fracture risk (vertebral and nonvertebral fractures)</td>
</tr>
<tr>
<td>Sogaard et al,^15^ 2005</td>
<td>12,270</td>
<td>7 years</td>
<td>Nonvertebral, hip, pelvis, humerus, forearm</td>
<td>Age, marital status, smoking, alcohol</td>
<td>Increase in fracture risk in women only</td>
</tr>
<tr>
<td>Mussolino,^27^ 2005</td>
<td>6,195</td>
<td>18.5 years</td>
<td>Hip</td>
<td>Age, gender, race, BMI, smoking, alcohol, physical exercise</td>
<td>Increase in fracture risk</td>
</tr>
</tbody>
</table>

Table I. Depression and fracture risk.

Abbreviations: BMD, bone mineral density; BMI, body mass index.

Pathophysiology of bone impact in depression

- **Hypothalamic-pituitary axis**
  The various hypotheses are summarized in Figure 2 (page 352). There is substantial evidence to confirm the presence of hyperadrenocorticism in the context of depression. The latter results from chronic exposure to stress, which triggers the release of corticotropin-releasing hormone (CRH) by the paraventricular nucleus of the hypothalamus. The process involves frontal cortex, hippocampus, amygdala, and hypothalamus pathways. There is no autonomous hypersecretion of cortisol in depression, so that cortisol levels are markedly lower than those observed in Cushing’s syndrome. This hypothesis is corroborated by clinical data,\(^{15,20}\) which show an increase in plasma cortisol. An increase in urinary cortisol has also been observed, though some authors failed to evidence any such increase.\(^{29}\)

- **Autonomic nervous system**
  Animal data suggest that there is a relationship between hyperactivity of the efferent autonomic nervous system and risk of bone demineralization. However, the implications of this finding in depression are still the subject of considerable debate.
Leptin
The relationship between leptin metabolism and bone metabolism is complex. Findings relating to circulating leptin levels in depressed subjects are contradictory. Thus, understanding of the pathophysiological role of leptin as a factor liable to explain the bone impact in depression requires further investigation.

Impairment of the immune system
Impairment of the immune system in depression has been fairly well established, with an increase in proinflammatory cytokines such as interleukins (IL) 1 and 6 and tumor necrosis factor. Cytokines are stimulants of the hypothalamic-pituitary-adrenal axis, which may account for the hyperadrenocorticism observed in depression. This has been confirmed by a recent study by Eskandari et al, which also reported a reduction in anti-inflammatory cytokines (IL-10 and IL-13) in depressed subjects. This reduction was only significant for IL-13. However, the study population was small, limiting the scope of the study.

Impact of depression on bone and confounding factors
Confounding factors include the classic risk factors for osteoporosis, which can be present in depressed subjects just as in the general population, and a risk factor specific to depression: antidepressant treatment.

Osteoporosis risk factors in depressed subjects
Certain risk factors for bone fragility are more frequently encountered in depressed subjects, eg, tobacco and alcohol abuse. Similarly, one of the symptoms of depression, namely, weight loss, is associated with an increase in fracture risk. These confounding factors are sometimes taken into account in the studies previously cited, but not always, which makes it difficult to interpret findings.

Antidepressants and bone metabolism
Antidepressants, in particular SSRIs, undoubtedly are the most important confounding factor. The presence of serotonin receptors on the osteoblasts and osteocytes lends support to the involvement of these agents. Some studies are adjusted to take into account antidepressant intake, but this is not always the case. The adverse effect on bone of SSRIs is supported by in vitro and animals studies. Severe osteoporosis has been evidenced in serotonin-deficient mice. A clinical study by Cauley et al showed that only SSRIs (and not tricyclic antidepressants) have an adverse impact on bone. More recently, Diem et al were able to show that the effect of SSRIs persisted even after adjustment for the symptoms of depression. They also reported accelerated bone loss in subjects on SSRIs vs nonusers and vs tricyclic antidepressant users.

Obviously, the most important issue is to determine whether antidepressants are associated with an increase in fracture risk. Quite logically, and in line with previous studies, a recent large-scale study in 6763 subjects on tricyclic antidepressants or SSRIs vs 26341 controls matched for age, gender, and geographic origin, reported an increase in hip and femur fracture risk in patients receiving SSRIs: relative risk (RR), 2.35 (95% CI, 1.94-2.84). An increase in fracture risk, albeit of lesser amplitude, was also found in patients on tricyclic antidepressants: RR, 1.76 (95% CI, 1.45-2.15). The
The most frequently used bone-formation markers are osseous to BMD determination, but are not diagnostic tools. Bone markers, used to assess bone remodeling, are complementary to BMD determination, but are not diagnostic tools. The most frequently used bone-formation markers are osteocalcin and bone alkaline phosphatase (BAP); bone-resorption markers include deoxypyridinoline (DPD), C-terminal (crosslaps or CTX), and N-terminal (NTX) extension peptides and telopeptides (carboxyl terminal telopeptide of collagen I [ICTP]). These markers are mainly used in postmenopausal women and their interpretation is more difficult in young women and adolescents. The literature shows wide divergence in findings; study populations are frequently small and it is necessary to distinguish between the studies conducted on female adolescents and those conducted on adult anorexic patients. Like postmenopausal women, AN women show an increase in bone resorption, but studies have also shown that there is a marked decrease in bone formation.

Impact of anorexia nervosa on bone

Anorexia nervosa (NA) has become a major public health concern in industrial countries in recent years. Its prevalence is 0.5%, vs 2% for bulimia. AN is a syndrome combining an exaggerated fear of excessive weight, a disorder of body image, significant weight loss, refusal to maintain a minimum normal weight, and amenorrhea.

The course of the disease is accompanied by a variety of disorders and complications. Bone health is much affected, with a decrease of more than 1 standard deviation (SD) in spine and femur neck bone mass in 92% of female patients, which exceeds 2.5 SD in 38% of cases. The mechanisms of bone loss in AN patients are multiple: hormonal, endocrine, and nutritional. The disease is more severe when it develops during adolescence, a critical period for acquisition of peak bone mass. Bone mass increases gradually through childhood and accelerates during adolescence to reach a peak during Tanner stages 4 and 5. The greater part of bone mass peak determination seems to be genetic (60% to 80%); the remaining 20% to 40% of determination is influenced by nutritional and hormonal factors. For a given age, bone loss is more marked in anorexic women than in women with normal BMI and amenorrhea of hypothalamic origin. Forty percent of anorexic women are osteoporotic vs 16% in the second group. BMI in women in whom AN develops before age 18 years is significantly lower than those in whom it develops later, reflecting the impact of the disease on bone formation.

Assessment of the bone impact of anorexia nervosa

- **Bone mineral density**
  BMD is determined in the spine and femur neck by means of bone densitometry measurements using low-dose radiation. The World Health Organization defines osteoporosis as a BMD that is at least 2.5 SD lower than the mean for young women (T-score < -2.5 SD). However, this definition only applies to postmenopausal women, a fact that must be taken into account when dealing with adolescents who have not always achieved peak bone mass. Lower BMD is consistently reported in anorexic female patients, and osteoporosis is present in about 30% of them.

- **Bone remodeling markers**
  Bone markers, used to assess bone remodeling, are complementary to BMD determination, but are not diagnostic tools. The most frequently used bone-formation markers are osteocalcin and bone alkaline phosphatase (BAP); bone-resorption markers include deoxypyridinoline (DPD), C-terminal (crosslaps or CTX), and N-terminal (NTX) extension peptides and telopeptides (carboxyl terminal telopeptide of collagen I [ICTP]). These markers are mainly used in postmenopausal women and their interpretation is more difficult in young women and adolescents. The literature shows wide divergence in findings; study populations are frequently small and it is necessary to distinguish between the studies conducted on female adolescents and those conducted on adult anorexic patients. Like postmenopausal women, AN women show an increase in bone resorption, but studies have also shown that there is a marked decrease in bone formation.

This shows that the bone loss in AN patients is also related to other mechanisms, such as estrogen deficiency, and that nutritional or nutrition-dependent factors are also involved. This is confirmed in the literature by the fact that bone loss in AN patients is more marked than that in women of the same age suffering from hypogonadism.

Few studies have addressed fracture risk in AN populations. Lucas et al reported a retrospective study in 208 AN patients over 13 years with 58 fractures. Compared with the expected number of fractures, the risk in AN patients was 3-fold greater. Fractures occurred more frequently in inpatients than in outpatients, and bone insufficiency–related cracks were also more frequent in inpatients. A study in female patients with a mean AN duration of 5.8 years reported a 7-fold greater fracture risk than in healthy women of the same age. Fractures occurred more frequently at the usual sites (vertebrae, followed by the radius and the distal extremity of the femur).

- **Hormonal factors**
  Studies of the time course of BMD in female AN patient populations show that when AN is diagnosed before age 18, BMD is significantly lower than when diagnosed at a later age, reflecting the impact of the disease on acquisition of peak bone mass.

Amenorrhea is a diagnostic criterion for AN. Estrogen deficiency is known to play a major role in bone mass loss in the AN population. The mechanisms underlying estrogen deficiency in AN have yet to be fully elucidated. They are probably multifactorial, and include hypothalamic dysfunction, drug effects, and food restriction.
weight loss, and dysregulation of neurotransmitters such as GnRH. The literature shows a correlation between BMD and the duration and age of onset of amenorrhea.\textsuperscript{35,39,44-46}

Estrogen deficiency alone cannot explain bone loss in anorexic female patients. Bone mass gain precedes resumption of menstrual cycles in recovering anorexic patients, while estrogen therapy does not prevent bone loss in adolescents.\textsuperscript{42} Other factors are involved in bone loss in AN. Bone remodeling is differently affected in AN female patients compared with postmenopausal osteoporotic women. Bone resorption and formation are increased, with a balance in favor of resorption, in postmenopausal women, whereas in AN, although bone resorption is slightly greater, the predominant disorder is decreased bone formation.\textsuperscript{37,38} though some authors report that it is normal.\textsuperscript{39} In any event, it is never increased. Reduced bone formation in AN explains the relative failure of antiresorptive treatments and, particularly, estrogens.\textsuperscript{42} In all, this suggests an essential role for undernourishment and factors influenced by nutritional status in the bone loss of AN.

- **Nutritional and endocrine factors**

The role of nutritional and endocrine factors is supported by the literature, which shows a strong correlation between female patient BMD and nutritional indices such as BMI, lean mass, fat mass, insulin-like growth factor--I (IGF-I), and leptin.\textsuperscript{37,38} In a previous study, the author and his colleagues showed a correlation between hip BMD and IGF-I in 113 female patients with AN.\textsuperscript{46} Hotta et al\textsuperscript{47} showed that the osteoporotic risk is higher when BMI is less than 15 kg/m\textsuperscript{2}. Other authors\textsuperscript{50,51} have also reported a correlation between bone formation markers (ostecalcin and BAP) and nutritional markers such as BMI, fat mass percentage, IGF-I, and a negative correlation between estradiol and bone resorption markers.

At puberty, the levels of GH-IGF axis hormones increase to stimulate the proliferation and differentiation of osteoblastic precursors. IGF-I is a bone tropism hormone that stimulates bone formation and growth by acting on osteoblasts and stimulating collagen synthesis. Studies have shown an impairment of the GH-IGF-I axis in AN patients.\textsuperscript{46,49} Female AN patients display resistance to GH, with high GH levels, but low IGF-I levels. Stoving et al\textsuperscript{48} monitored 24-hour GH secretion in 8 anorexic female patients and showed an increase in the number, duration, and intensity of GH peaks. The authors also showed an increase in basal secretion (20-fold vs 4 fold for pulsatile secretion). The increase in the intensity of GH peaks is ascribed to weight loss, while the number of peaks is related to hypoestrogenism. There was no difference in GH half-life in anorexic patients compared with healthy controls. Sacchi et al\textsuperscript{49} published similar results. Several authors have reported a decrease in IGF-I levels, but also in binding proteins (IGFBP), in particular IGFBP3 and 2, in anorexic female patients.\textsuperscript{50,51} sometimes with an increase in IGFBP1. The decrease in circulating binding-protein levels may in part explain the resistance to GH, preventing the transfer of IGF-I toward the target organs. In addition, IGFBP3 is reported to be a good predictive factor for bone loss in anorexic patients, independently of BMI and IGF-I.

An important role is played by the hormone leptin, an anti-oxygenic adipokine secreted by adipose tissue. Leptin’s physiological effects on bone are debated, particularly as they differ depending on whether its peripheral or central action is considered. Measuring BMD and several hormonal factors in a recent cohort study of 103 young women with AN,\textsuperscript{52} the authors found a mean Z-score of −1.17 for the spine, −1.33 for the hip, and −1.11 for the femur neck, and a modest, but significant, positive correlation between leptin levels and spinal BMD ($r = 0.30$). The correlations were significant, but of lesser amplitude, for the femur neck and whole hip ($r = 0.23$ and $r = 0.21$, respectively). Multiple regression analysis showed that 27% of spinal BMD variability was explained by differences in duration of amenorrhea and leptin levels. Figure 3, which plots BMD values as a function of leptin level divided into 3 tertiles, shows a marked and significant difference between the patients in the lowest tertile (mean Z-score: −1.25) and higher tertile (mean Z-score: +0.75).\textsuperscript{52}

Hyperadrenocorticism and calcium and vitamin D deficiency are reported in AN, in some cases compounded by excessive exercise. Thus, high cortisol levels can be found,\textsuperscript{38,46} although the circadian rhythm is spared. Similarly, the dexamethasone suppression test frequently evidences an increase in urinary free cortisol. Hyperadrenocorticism may be related to impairment of hypothalamic function or CRH hypersecretion. Grinspoon et al\textsuperscript{50} reported hyperadrenocorticism in only 22% of anorexic patients with severe bone loss. We found similar results in our study.\textsuperscript{46} Audi et al\textsuperscript{51} did not find any signif-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Relationship between circulating leptin level (abscissa: tertiles 1, 2 and 3) and bone mineral density expressed as lumbar spine Z-score (as ordinates).}
\end{figure}
icant difference in urinary free cortisol levels between AN patients and controls. This suggests that while hyperadrenocorticism is a potential cause of bone loss, it is not the only mechanism involved. The role of vitamin and calcium deficiency in bone loss remains uncertain. In the study by Audi et al., vitamin D deficiency (25-OH-D3) was observed in 24.6% of AN patients. Urinary calcium was somewhat higher in the group of AN patients in the active phase, and lower in those having regained weight, but still with amenorrhea, and those who had recovered. Soyka et al. reported dietary calcium deficiency (<1300 mg/day) in 42% of the AN patients in their study population, but also in 50% of the controls. Similarly, vitamin D deficiency was present in 42% of AN patients and 44% of controls.

**Course of bone loss after weight recovery**

A few studies have addressed the time course of BMD in recovered anorexic patients. Despite the improvement in bone mass with body weight normalization, certain studies report persistent osteopenia in a high proportion of postanorexic patients. Hartman et al. in a study of 19 female patients with a history of AN, determined bone mass at age 21 years and severe and is associated with a substantial fracture risk, the mechanism of which has yet to be fully elucidated and is probably multifactorial. Early screening is necessary and BMD must be determined as soon as AN is diagnosed.

**References**


**Impact of psychiatric disease on bone health**
Les affections psychiatriques peuvent, par le biais de mécanismes directs et indirects, engendrer une fragilité osseuse. Les affections psychiatriques peuvent, par le biais de mécanismes directs et indirects, engendrer une fragilité osseuse. Les affections psychiatriques peuvent, par le biais de mécanismes directs et indirects, engendrer une fragilité osseuse. Les affections psychiatriques peuvent, par le biais de mécanismes directs et indirects, engendrer une fragilité osseuse. Les affections psychiatriques peuvent, par le biais de mécanismes directs et indirects, engendrer une fragilité osseuse. Les affections psychiatriques peuvent, par le biais de mécanismes directs et indirects, engendrer une fragilité osseuse. Les affections psychiatriques peuvent, par le biais de mécanismes directs et indirects, engendrer une fragilité osseuse. Les affections psychiatriques peuvent, par le biais de mécanismes directs et indirects, engendrer une fragilité osseuse. 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Serotonin: a new player in the regulation of bone remodeling

by V. K. Yadav, P. Ducy, and G. Karsenty, USA

Serotonin is a bioamine synthesized in the brain and gut that regulates diverse functions from mood to gastrointestinal tract motility. This diversity in serotonin function(s) is achieved through one or several of its 14 distinct receptor(s) expressed on the target cells. The emerging concept that brain- and gut-derived serotonin regulate bone remodeling in opposite manner has revealed novel mechanism(s) by which bone mass is regulated and maintained. Advances in our understanding of serotonin synthesis, receptor activation, and participation in distinct regulatory networks demonstrate a role for serotonin in osteoblast and osteoclast functions. This review focuses on this new “expanded serotonin biology” and discusses how drugs targeting serotonin synthesis or signaling can be harnessed for treating low-bone-mass diseases.

Skeleton in vertebrates serves multiple mechanical, hematopoietic, and endocrine functions. In order to perform its functions properly, skeleton continuously renews itself through a homeostatic process known as bone remodeling—a process carried out by osteoblasts and osteoclasts to maintain a fine balance between bone formation and resorption. Bone remodeling occurs constantly and simultaneously in numerous parts of skeleton and the maintenance of a normal, healthy skeletal mass depends on continuous exchange of information taking place among osteoblasts, osteoclasts, osteocytes, constituents of the bone matrix, and other organs. Therefore, understanding what factors are influencing bone mass in the context of other signals is important. The fact that osteoporosis is a heritable trait provides an opportunity to use modern molecular genetics to obtain mechanistic insights that were previously unobtainable. If we could find genetic variants with known or at least tractable functions that are unequivocally associated with osteoporosis, we might be able to build up a picture of what sorts of biological factors determine why some people are more susceptible to osteoporosis than others.

Lrp5: a multifaceted molecule

The low-density lipoprotein receptor (LDLR)-related protein (Lrp)-5 is part of a subset of the LDLR family of cell surface proteins. Since its cloning in 1998, Lrp5 has taken biologists to voyages of discoveries from lipoprotein clearance to glucose homeostasis to bone remodeling. Not surprisingly, it has been shown to bind to multiple ligands and activate a multitude of downstream cascades in distinct cell types.
to regulate different processes (Figure 1). In hepatocytes, Lrp5 binds apolipoprotein E (ApoE) and plays a role in the hepatic clearance of ApoE-containing chylomicron remnants, a major plasma lipoprotein carrying diet-derived cholesterol.\(^4,5\) In pancreatic islets, Lrp5 regulates insulin secretion and consequently Lrp5-deficient animals are glucose intolerant.\(^6\) Consistent with its role in glucose homeostasis, the LRP5 gene is mapped within the region (IDDM4) linked to type 1 diabetes on chromosome 11q13.\(^7\) LRP5 is also the gene responsible for osteoporosis-pseudoglioma (OPPG) syndrome and high-bone-mass (HBM) syndrome in humans due to an isolated change in bone formation.\(^8-10\)

The main question surrounding Lrp5 biology, since its identification as the cause of OPPG, has been to define how its absence can cause the developmental onset of blindness and postnatal onset of osteoporosis characterizing this disease.\(^8-10\) Several recent studies have now shed\(^11\) new light on the mechanisms associated with these two functions of Lrp5. Indeed, ample studies have conclusively demonstrated that Lrp5 uses the Norrin and Wnt signaling pathways during embryogenesis to regulate vascularization in the eyes.\(^12-14\) That dysregulation of Wnt signaling plays a role in the development of blindness in a Lrp5-dependent manner fuelled interest in this signaling pathway, leading to the identification of critical Wnt-dependent mechanisms involved in controlling early differentiation of osteochondroprogenitor cells during embryogenesis as well as osteoblast and osteoclast functions.\(^11,15-17\)

Some of these targets have already made it to preclinical trials, viz, sclerostin.\(^18\) However, and to our dismay, using an unbiased microarray approach, we serendipitously identified that the mechanism through which Lrp5 loss- and gain-of-function mutations regulate bone formation is by regulating serotonin production in the gut.\(^19\) This dual role of Lrp5—one developmental (directly dependent on Wnt signaling in the eye) and the other postnatal (relying on the indirect effect of gut-derived serotonin on bone cells)—is consistent with the multifunctionality of Lrp5, which participates in a wide variety of signaling cascade(s).

We should emphasize that the fact that the deletion of Lrp5 in osteoblasts progenitors or mature osteoblasts did not result in a discernible effect on bone mass in our studies does not exclude, however, that Lrp5 could play a role, in a Wnt-dependent manner, or not, in regulating the response of osteocytes to mechanical loading.\(^20-21\) Further studies analyzing, in parallel, mice deficient in Lrp5 globally as well as conditionally in osteocytes will be pivotal to address this specific point.

### Ever-expanding tenets of serotonin biology

Serotonin (5-hydroxytryptamine) was discovered in 1948 as a factor causing vascular contractions, hence the name of the molecule serotonin (L, \textit{serum} + Gk, \textit{tonos}, tone).\(^22\) Since then serotonin biology has expanded exponentially and it is now recognized as a pivotal regulator in many central and peripheral functions.\(^23\) Serotonin is generated through an enzymatic pathway in which L-tryptophan is converted into L-5-OH-tryptophan by an enzyme called tryptophan hydroxylase (Tph); this intermediate product is then converted to serotonin by an aromatic L-aminoacid decarboxylase.\(^24,25\) There are two Tph
genes that catalyze the rate-limiting step in serotonin biosynthesis: Tph1 and Tph2. Tph1 is expressed mostly, but not only, in enterochromaffin cells of the gut and is responsible for the production of peripheral serotonin. Tph2 is expressed exclusively in raphe neurons of the brainstem and is responsible for the production of serotonin in the brain. Remarkably, serotonin does not cross the blood–brain barrier; therefore it should be viewed from a functional point of view as two distinct molecules depending on their site of synthesis. Brain-derived serotonin (BDS) acts as a neurotransmitter, while gut-derived serotonin (GDS), till now, has only been appreciated as an autocrine/paracrine signal that regulates mamma-
mary gland biogenesis, liver regeneration, and gastrointestinal tract motility.

Regulation of bone formation through gut-derived serotonin

Our work on the mechanism(s) underlying OPPG and HBM led us to identify Lrp5 as one of the regulators of GDS (Figure 2). Conditional inactivation of Lrp5 and Tph1 in the gut cells identified that GDS functions as a hormone that directly inhibits osteoblast proliferation and bone formation through the Htr1b-CREB signaling pathway. Nodes in the pathway that are amenable to therapeutic interventions are highlighted. Structures not to scale.

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); CREB, cAMP response element binding (protein); Htr1b, 5-hydroxytryptamine receptor 1B; HTT, serotonin transporter; Lrp5, low-density lipoprotein receptor (LDLR)-related protein (Lrp)-5; Tph1, tryptophan hydroxylase–1; Trp, tryptophan.
**Negative association of peripheral serotonin levels with bone mass in humans**

The identification of a gut-derived serotonin-bone endocrine axis (Figure 2) begged the question of its biomedical importance in humans. Modder et al.\(^{28}\) analyzed serum serotonin levels in a population-based sample of 275 women and related these to bone mineral densities (BMD) at distinct skeletal sites and bone microstructural parameters. They found that serum serotonin levels were inversely associated in these women with body and spine areal bone mineral density (aBMD) as well as with femur neck total and trabecular volumetric bone mineral density (vBMD).\(^{28}\) Moreover, multiple LRP5 mutations associated with decreased BMDs have been analyzed and all published studies thus far show that these mutations are associated with a 2-to-4 increase in serum serotonin levels.\(^{19,29}\)

Conversely, analysis of two HBM patients in the US as well as a recent study of 9 HBM European patients, who harbor the T2531 gain-of-function mutation of LRP5, showed that their serotonin concentrations in platelet-poor plasma were significantly lower compared to sex- and age-matched controls.\(^{19,30}\) Collectively, these studies performed in different continents by different investigators, provide convincing evidence to support a physiological role for circulating serotonin in negatively regulating bone formation in humans related to one it plays in mice.

**Brain-derived serotonin: an expected player in the regulation of bone mass**

In our quest to understand the serotonin regulation of bone mass in vertebrates, we then inactivated Tph2, the gene that catalyzes the rate-limiting step in the biosynthesis of BDS. The absence of serotonin in the brain resulted in a severe low-bone-mass phenotype affecting the axial (vertebrae) and appendicular (long bones) skeleton.\(^{31}\) This phenotype was secondary to a decrease in bone formation parameters (osteoblast numbers and bone formation rate) and to an increase in bone resorption parameters (osteoclast surface and circulating Dpd levels).\(^{31}\) Hence, BDS is a positive and powerful regulator of bone mass accrual acting on both arms of bone remodeling.\(^{31}\)

While we were doing these studies we noticed, upon opening the abdominal cavities, that Tph2-deficient animals had a dramatic decrease in their adipose mass.\(^{31}\) This prompted us to analyze in great detail their energy metabolism phenotype. The decrease in their fat mass was due, in part, to the fact that these mice ate less and spent much more energy compared to their wild-type littermates.\(^{31}\) This observation was not entirely surprising since serotonin is known to play important roles in many other physiological processes. However, what caught our attention was the fact that the three most notable phenotypes of adult Tph2-deficient animals ie, decrease in bone mass, decrease in appetite, and increase in energy expenditure are a mirror image of what is observed in mice that lack leptin.\(^{32,33}\)

Leptin is an adipocyte-derived hormone that regulates many functions, viz, appetite, energy expenditure, bone mass, etc.\(^{34-39}\) Studies in the last 16 years have highlighted a more complete neural and neurochemical circuit diagram for the leptin regulation of these functions.\(^{34-36}\) These neural circuits involve many distinct neuronal populations in the brain, including neurons of arcuate, ventromedial, and lateral hypothalamus, and neurons of the nucleus tractus solitarius (NTS) etc.\(^{34-36}\) Three correlative experiments suggested that leptin might signal in the serotonin neurons, among others, to regulate some of its downstream functions. First, the leptin receptor (ObRb) is expressed on serotonin neurons located in the raphe nuclei of brainstem, where BDS is produced, and is functional.\(^{31,41}\) Second, serotonin neurons project to the key hypothalamic nuclei responsible for the regulation of appetite, energy expenditure, and bone mass.\(^{32}\) Third, patients with selective serotonin reuptake inhibitors (SSRIs) have been reported to have changes in their appetite and bone mass.\(^{33,44}\)

To explore that leptin might utilize serotonin as one of its downstream mediators to regulate these three functions, we inactivated the leptin receptor in different nuclei of the hypothalamus or in the serotoninergic neurons of the brainstem.\(^{31}\) Mice lacking ObRb either in Sf1-expressing neurons of the ventromedial hypothalamus (VMH) nuclei or in Pomc-expressing neurons of the arcuate (ARC) nuclei had normal sympathetic activity, bone remodeling parameters, and bone mass; they also had normal appetite and energy expenditure, and when fed a normal diet, did not develop an obesity phenotype.\(^{35,46}\) In contrast, mice that lack ObRb in Sert-Cre positive serotonin neurons (ObRb\(_{\text{SERT-}}\)\(^{-}\)) developed a high bone mass phenotype; they also had an increase in appetite and displayed low-energy expenditure. As a result, ObRb\(_{\text{SERT-}}\)\(^{-}\) mice, when fed a normal diet, developed an obesity phenotype. These genetic studies demonstrated that leptin signals, in part, in the serotonin neurons of the brainstem to regulate, bone mass, appetite, and energy expenditure (Figure 3). The identification of serotonin as one of its mediators adds to the list of the multitude of messengers (viz, dopamine, melanocortins, etc.) utilized by leptin in the brain to affect peripheral functions.\(^{31,34-36}\)

The demonstration that a leptin-dependent central control of bone mass, appetite, and energy expenditure occurs, among other neural relays, through its ability to inhibit serotonin production, raised questions about the location and identity of serotonin receptors on hypothalamic neurons mediating these functions. Double fluorescence in situ hybridization and nuclei-specific gene inactivation experiments revealed that serotonin promotes bone mass accrual through Htr2c receptors expressed on the VMH nuclei, while appetite was promoted through Htr2b and Htr1a receptors expressed on ARC nuclei of the hypothalamus. Further analysis revealed that Htr2c receptor expression on VMH nuclei is on route to the sympathetic center of the brain, while Htr1a and Htr2b achieve their functions on appetite most likely through modulation of
melanocortin signaling (Figure 3). These studies emphasized that with respect to the bone mass and energy metabolism effects of leptin signaling in the brain, a systems approach involving anatomically distinct neural elements will provide a more complete explanation of leptin actions in the brain.

**Gain of function in serotonin signaling and bone mass**

Our loss of function studies with GDS and BDS dissociated the role played by peripheral and central serotonin signaling in the regulation of bone mass. As with any other study, these studies raised many more questions than they answered. For instance, what effect would an increase in serotonin injections on the trabecular bone mass in their study be consistent with our and earlier mouse genetic studies. We reported that mice harboring a loss of function mutation for Lrp5 gene have increased levels of GDS and a low bone mass at vertebral sites. Battaglino et al tested the direct effects of SSRIs on bone mass and they consistently observed an increase in trabecular bone mass in these animals. These latter results, given the effects of SSRIs in humans, were surprising at the time they were reported, but with the advancement of knowledge related to serotonin signaling in the brain and periphery we can today explain these results. Likely the observed effects were due to the fact that, under the conditions tested in their study, SSRIs were having more pro-

![Figure 3. Neuronal relays underlying leptin regulation of bone mass, appetite, and energy expenditure.](image)

Leptin inhibits release of brainstem-derived serotonin, among other neuronal relays, which favors bone mass accrual and appetite through its action on hypothalamic neurons. Serotonergic neurons are in blue; ARC is in green; NTS is in orange; and VMH is in purple. Structures not to scale. Abbreviations: ARC, arcuate; NTS, nucleus tractus solitarius; PVH, paraventricular hypothalamus; VMH, ventromedial hypothalamus.

Several approaches have been used in the past to understand this deleterious effect of SSRIs on bone mass. Gustafsson et al, using naïve rats as a model of serotonin effect on bone mass, analyzed site-specific alterations in the long bone when rats were injected daily with serotonin. These authors reported a decrease in trabecular bone mass and an increase in cortical thickness in long bones. The negative influence of found influences on BDS, a positive regulator of bone mass. Warden et al, taking another approach for a model of chronic use of SSRIs, reported that mice that lack serotonin transporter (Htt-/- mice) have decreased bone mass at both cortical and trabecular sites.

Their study is consistent with Richards et al and other clinical reports that show that patients taking SSRIs often have a decrease in bone mass. Surprisingly, Htt-/- mice have undetectable levels of serotonin in their blood and a twofold reduction in brain serotonin content (VKY, unpublished observations). The low bone mass observed in Htt-/- mice would suggest that BDS compared to GDS has a dominant role in the overall regulation of bone mass through serotonin. Indeed, analyses of mice lacking both the Tph1 and Tph2 genes display a low bone mass phenotype demonstrating that despite accounting for >5% of total serotonin pool in the body, BDS dominates in the overall regulation of bone mass. Since SSRIs cross the blood–brain barrier, and osteoporosis is only observed when they are taken in the long term, development of SSRIs with selective central actions would be worth exploring in the future for curing depression while minimizing their side effects on bone.
Therapeutic implications of serotonin regulation of bone mass

The richness and complexity of the serotonin modulation of bone mass discussed in this review provide both a pharmacological opportunity and a challenge. On the one hand, the involvement of specific serotonin receptors on osteoblasts and hypothalamic neurons provides an opportunity to pharmacologically target these specific receptors for the treatment of osteoporosis. On the other hand, the fact that each of these serotonin receptors participates in multiple physiologic processes presents a challenge, since even a drug targeting a single serotonin receptor is likely to have effects on multiple body systems. For example, although Htr2c agonists may be used to increase bone mass through its effect in the brain, their clinical use would be limited by their effects on other organ systems, such as sympathetic tone or melanocortin signaling. Fortunately, the system is less complex and more amenable to therapeutic interventions in the periphery. Since the effect of GDS, a negative regulator of bone formation, is dominant there, one would be able to suppress its levels mildly in order to avoid side effects of drugs targeting the receptors directly. This way one would be able to maintain basal level of signaling in other systems dependent on serotonin while at the same time getting the therapeutic outcome in sensitive systems such as bone, which responds robustly to >50% modulation in peripheral serotonin levels.

As GDS is a potent inhibitor of osteoblast proliferation and bone formation, we tested the contention that pharmacologically suppressing GDS would be able to prevent, or cure, gonadal-ectomy-induced bone loss. Serendipitously, we came across an inhibitor that was inhibiting peripheral serotonin production without having any detectable effect on brain serotonin content. This is, and will be, a prerequisite for any drug that is going to target serotonin synthesis or signaling, as brain serotonin has opposite influence on bone mass accrual and in fact is beneficial to bone. The drug, LP533401, a Tph1 inhibitor, was effective in preventing and even curing osteoporosis in mice and rats at an oral close of less than 25 mg/kg/day through an isolated increase in bone formation. The effect of Tph1 inhibitors on bone mass establishes that inhibition of GDS biosynthesis can rescue ovariectomy-induced osteoporosis in the mouse through an anabolic mechanism. These further validate the role of GDS as a regulator of bone formation and provide foundation for the development of other molecules that target the Tph1/Htr1b/osteoblast pathway for the treatment of low bone mass diseases, either alone or in combination with other existing therapies (Figure 2).

Future studies would be necessary to investigate four specific issues: First, the absolute threshold levels at which suppression in peripheral serotonin signaling is anabolic to the bone. Second, to analyze in more detail plasma- vs serum-vs platelet-derived serotonin in the regulation of bone mass. Third, to thoroughly characterize any toxicity or side effects the drugs that target this pathway might have on any of the functions of other peripheral organs. Fourth, and most importantly, if these types of drugs can be used to treat low bone mass conditions associated with specific genetic mutations in mouse models of human diseases such as osteoporosis pseudoglioma.

As research on the role of serotonin and its receptors in bone physiology progresses, the difficulty of these challenges will become clearer. In the process we will likely discover new therapeutic targets for osteoporosis treatments as well as gain a better understanding of the beauty and complexity of bone biology.

This work was supported by a NIH grant (DK85328) and a Rodan fellowship from IBMS to VKY. I apologize to numerous researchers whose work I was unable to discuss due to space constraints.

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LA SÉROTONINE : UN NOUVEL ACTEUR DANS LA RÉGULATION DU REMODELAGE OSSEUX

La sérotonine est une bioamine synthétisée dans le cerveau et l’intestin, régulant différentes fonctions allant de l’humeur à la motilité du tractus gastro-intestinal. Cette diversité dans les fonctions de la sérotonine s’exerce par l’intermédiaire d’un ou de plusieurs de ses 14 récepteurs distincts exprimés sur les cellules cibles. La constatation que la sérotonine cérébrale et la sérotonine intestinale agissent en sens contraires sur le remodelage osseux attesté de l’existence de mécanismes nouveaux impliqués dans la régulation et le maintien de la masse osseuse. Les avancées dans la compréhension de la synthèse de la sérotonine, de l’activation de ses récepteurs et de sa participation à des réseaux de régulation mettent ainsi en évidence le rôle de la sérotonine dans les fonctions des ostéoblastes et des ostéoclastes. Cet article fait le point sur cette nouvelle « biologie étendue de la sérotonine » et examine comment exploiter les médicaments ciblant la synthèse ou la signalisation de la sérotonine dans le traitement des maladies se traduisant par une diminution de la masse osseuse.

Keywords: serotonin; gut; bone; osteoblast; osteoclast

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Serotonin: a new player in the regulation of bone remodeling – Yadav and others

MÉDICOGRAPHIA, Vol 32, No. 4, 2010 363
The association between diabetes and bone health has long been a matter of debate. Both type 1 diabetes and type 2 diabetes have been linked to increased risk of fractures, with bone mineral density being decreased in type 1 diabetes and increased in type 2 diabetes. Insulin has an anabolic effect on bone, and the qualitatively different effects of type 1 and type 2 diabetes on bone mass are consistent with the opposite insulin-secretory states (hypoinsulinemia vs hyperinsulinemia). The existence of an elevated fracture risk in type 2 diabetes, despite the underlying hyperinsulinemia, has led to speculation about differences in bone quality between type 1 diabetes and type 2 diabetes. This could be explained by the fact that increased blood glucose levels are associated with increased urinary calcium loss, resulting in a negative calcium balance. There is also speculation about the role of the resistance to parathyroid hormone observed in diabetes, and its effect on calcium and bone turnover. Also, collagen glycosylation may alter bone biomechanical competence. Falls associated with diabetes-related comorbidities are another possible cause of low-trauma fractures. Adequate glycemic control and prevention of diabetic complications are the mainstay of therapy to lower fracture risk, with the caveat that thiazolidinediones increase fracture risk in postmenopausal women with type 2 diabetes.

Bone health and diabetes

by M. L. Brandi, Italy

Type 1 diabetes is clearly associated with bone loss and suppressed bone formation, as more than 50% of type 1 diabetic patients are thought to have bone loss vs healthy age-matched subjects and almost 20% of diabetic patients aged 20 to 56 meet the criteria for osteoporosis. Adequate glycemic control and prevention of diabetic complications are the mainstay of therapy to lower fracture risk, with the caveat that thiazolidinediones increase fracture risk in postmenopausal women with type 2 diabetes.

More than 180 million people worldwide suffer from type 2 diabetes, a disease that more than doubles the risk of death, mainly from cardiovascular disease. Interestingly, the medical literature provides evidence of a convergence between diabetes, a metabolic disease, and potential mechanisms accounting for osteoporosis. Skeletal involvement in diabetes was first suggested more than 80 years ago, prompted by radiological findings of retarded bone development and bone atrophy in children with type 1 diabetes. In 2007, a systematic meta-analysis in women with type 2 diabetes reported that, although there was no significant increase in vertebral or distal forearm fractures, hip fracture risk was elevated 1.7-fold. Furthermore, it is now recognized that diabetes and hip fractures share common risk factors. Nevertheless, despite a large body of accumulated data on the skeletal effects of diabetes, many questions remain unresolved, with biochemical...
Bone health and diabetes – Brandi

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and imaging studies producing conflicting findings. This is likely to be due in large part to the complex pathophysiology of diabetes, the diversity of skeletal sites examined, the multitude of techniques used for measuring bone mass, and variations in the duration, severity, and treatment of diabetes in the different studies. This paper reviews our current understanding of the pathogenetic bases of bone disease in diabetes.

Pathophysiology

The biological relevance of bone remodeling

There is a constant turnover of bone through bone remodeling, via a biphasic process occurring throughout the skeleton over a period of approximately 3 months. It includes destruction (resorption) of preexisting bone, a function exerted by a specialized bone-specific cell, the osteoclast, followed by de novo bone formation, a function exerted by another bone-specific cell, the osteoblast. Normally, resorption and formation of bone occur not only sequentially, but in a balanced manner in order to maintain bone mass nearly constant during most of adulthood. This qualifies bone remodeling as a true homeostatic function controlled by cytokines acting locally and hormones acting systemically.

Maintenance of constant bone mass is the aspect of bone remodeling we are most familiar with, because osteoporosis, the most frequent bone disorder, is a bone-remodeling disease. Osteoporosis results from an increase in bone resorption exceeding bone formation. Bone remodeling can be studied by means of biological markers in serum and urine, or bone mineral density (BMD). BMD is a strong predictor of fracture risk, but bone mineral quantity is only one component of bone strength, and various disorders, including diabetes, can be associated with poor bone quality.

The relatively recent observation of a convergence between bone and energy homeostasis suggests that energy metabolism and bone mass are regulated by the same hormones, such as leptin, adiponectin, neuropeptide Y, and substance P. A remarkable feature of most types of hormonal regulation is that they are controlled by feedback loops, such that the cells targeted by a hormone send signals influencing the hormone-producing cells. When applied to skeletal biology, the concept of feedback regulation suggests that bone cells exert an endocrine function.

This was recently demonstrated by the finding that the skeleton exerts an endocrine regulation of glucose homeostasis through the “secretion” of osteocalcin, one of the very few osteoblast-specific proteins, which improves glucose homeostasis by favoring β-cell proliferation and insulin secretion (Figure 2, page 366). Teleologically, the proliferation function of osteocalcin may have arisen during evolution to maintain the size of the pancreatic islets constant in periods of food deprivation.

Bone phenotypes in type 1 and type 2 diabetes

Type 1 diabetes, also called insulin-dependent diabetes mellitus, is characterized by little or no insulin production and hyperglycemia. Improved glucose monitoring, insulin delivery methods, and pharmacologic treatments are increasing patient lifespan. However, as a result, there is a parallel increase in the risk of complications due to extended exposure to diabetes. Attention has been recently focused on diabetic bone pathology, as type 1 diabetes was found to be clearly associated with bone loss and suppressed bone formation. As reported by McCabe comparing type 1 diabetic patients and healthy age-matched subjects, it is estimated that more than 50% of type 1 diabetic patients have osteopenia or osteoporosis.
of type 1 diabetic patients have bone loss and almost 20% of patients aged 20 to 56 meet the criteria for osteoporosis. Quite logically in this connection, type 1 diabetes has been shown to be a risk factor for delayed fracture healing. Bone loss can begin as early as at onset of diabetes in children, but there are reports of children with type 1 diabetes who do not exhibit bone loss. Bone loss occurs predominantly in the appendicular skeleton. A concern is that existing bone loss in type 1 diabetic patients could compound the fracture risk associated with conditions such as menopause and aging.

The mechanisms contributing to type 1 diabetic bone loss are unknown, but several theories have been put forward. Analysis of type 1 diabetic bone remodeling serum markers suggests that bone turnover is unaltered or decreased, while bone formation is decreased, as indicated by reduced serum levels of osteocalcin and histomorphometric studies. The potential contributors to type 1 diabetic bone phenotypes are listed in Table I.

Type 2 diabetes, also called non-insulin-dependent diabetes mellitus, develops when cells become resistant to insulin signaling, and accounts for more than 90% of diabetes cases. Diet, obesity, and reduced physical activity are several of the factors that are thought to contribute to the development of type 2 diabetes. Available data concerning an association between reduced BMD and type 2 diabetes are equivocal. Type 2 diabetes mellitus in the literature has been reported to be associated with increased, unchanged, or decreased BMD. However, most large-scale epidemiological studies indicate normal or above-normal BMD. Possible contributing factors to the higher BMD of type 2 diabetes mellitus are listed in Table II.

- Reduced osteoblast differentiation
- Increased marrow adiposity
  - Reduced insulin signaling
  - Hyperglycemia
  - Adipokine and endocrine changes
  - Inflammation and cytokines
  - Hyperlipidemia

Table I. Potential contributors of the bone phenotypes in type 1 diabetes mellitus.

- Obesity
- Hyperinsulinemia
- Increased androgen levels associated with obesity (in women)

Table II. Potential contributors of high BMD in type 2 diabetes mellitus.

Risk of fracture in type 1 and type 2 diabetes mellitus
The most convincing evidence that osteoporosis is a complication of diabetes mellitus comes from epidemiological studies that have shown an increased risk of fragility fractures. Diabetes and hip fracture share common risk factors (eg, physical inactivity, advanced age); in contrast, obesity, a risk factor for diabetes, is associated with a lower risk of fractures, and any apparent modification in fracture risk by diabetes is likely to reflect a confounding effect of these and other extraneous factors.

Investigations into fracture risk in type 1 diabetes have yielded inconsistent results, with increased incidence of hip fracture being reported in some studies, but not in others. A recent meta-analysis in patients with type 1 diabetes mellitus...
reported that this population is at six- to sevenfold higher risk of hip fracture than nondiabetic individuals. Cross-sectional and prospective studies have shown type 1 diabetes to confer an increased risk of fragility fracture at other sites, in both men and women.

Even though a recent meta-analysis involving a total of 836,000 participants concluded that hip fracture risk was elevated 1.7-fold in women with type 2 diabetes mellitus, some studies have reported either no increase in hip fractures or risks restricted to patients with a higher duration of disease. The reports of increased fracture risk are somewhat unexpected because 2-dimensional areal BMD is normal or elevated in persons with type 2 diabetes, and this implies that diabetic individuals are at decreased risk of fracture. Moreover, the meta-analysis found no significant increase in vertebral or distal forearm fractures in these patients. At present, there is no clear explanation for this apparent contradiction. An increased risk of falling in diabetic patients could account for the elevated hip fracture risk in the face of normal or elevated BMD. A possible explanation for increased bone fragility in diabetes mellitus is the accumulation of advanced glycation end products within bone collagen, leading to increased stiffness of the collagen network. Increased blood glucose levels could also have direct deleterious effects on bone cells, with consequences on bone biomechanical competence. Moreover, adipose tissue (usually increased in type 2 diabetes mellitus) produces cytokines, namely, adipokines, such as leptin, resistin, and adiponectin, which may negatively modulate BMD.

Figure 3 depicts the potential mechanisms contributing to fracture susceptibility in diabetes mellitus.

**Effects of antidiabetic agents on bone**

Oral antidiabetic drugs are commonly used to improve glycemic control, but there are concerns that some may increase the risk of cardiovascular events. Moreover, several epidemiological studies have investigated the effects of antihyperglycemic treatment on fracture risk in diabetes. In the largest of these, in which all individuals diagnosed with fracture in Denmark in 2000 were matched with controls, it was reported that metformin and sulfonylurea treatments were associated with reduced incidences of fracture, while insulin was associated with a nonsignificant trend toward reduced risk of hip, forearm, and spine fractures.

Conversely, recent evidence suggests that the thiazolidinediones, first introduced for the treatment of type 2 diabetes mellitus in 1999, may affect the skeleton, with an increase in fracture risk in women randomized to rosiglitazone versus those randomized to metformin or glyburide monotherapy. In this study, fracture events were not increased in men and did not increase with time. These results were also confirmed in preliminary data from another study.

Interestingly, pioglitazone, the other currently available thiazolidinedione, may have similar skeletal effects, with the majority of fractures occurring at nonvertebral sites, including the lower limb and distal upper limb.

As these findings support the hypothesis of a class effect of thiazolidinediones in increasing fracture risk in women with type 2 diabetes mellitus, letters to health care providers have been issued by the manufacturers. However, doubts still

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**Figure 3. Potential mechanisms contributing to low bone mass and increased fracture susceptibility in diabetes mellitus.**

exist about the clinical relevance of this phenomenon, and more studies are needed to address a number of still pending questions,\textsuperscript{31,46} such as the precise mechanism of action of these agents (Figure 4).

Physicians should carefully check for the existence of risk factors for osteoporosis and fractures in their patients before putting them on thiazolidinedione treatment, and an adequate clinical follow-up of treated patients is strongly recommended.

Future prospects

The prevalence of diabetes mellitus is increasing rapidly in the population, with the implication that adverse outcomes of the condition are likely to grow in importance as well. Considerable concern has been expressed about fracture risk in these patients. Although fractures may now be prevented thanks to the availability of effective treatments, no clear rationale exists for treating patients with type 2 diabetes with antifracture agents able to increase BMD, and our knowledge base is not strong enough for a more effectively tailored prophylaxis to be designed for this group. Additional research is needed to better define the determinants of bone strength in diabetic individuals, including the abnormal properties of bone that might respond to treatment of diabetes itself. Conversely, the differences between type 1-diabetic- and age-associated bone loss stress the importance of selecting condition-specific individualized treatments for osteoporosis. Because in type 1 diabetes the bone defect results predominantly from a decrease in bone formation, anabolic therapies appear likely to be the most effective treatment.

Future studies should contribute to a more thorough understanding of the mechanisms of diabetic bone loss, enabling the development of newer and more effective drugs. Optimizing therapies that prevent bone loss or restore bone density will allow diabetic patients to live longer, with strong healthy bones.

This work was supported by FIRMOD Fondazione Raffaella Becagli to MLB.

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References


Les liens entre diabète et santé osseuse font débat depuis longtemps. Diabètes de type 1 et de type 2 sont tous deux associés à une augmentation du risque de fracture, la densité minérale osseuse étant diminuée dans le diabète de type 1 et augmentée dans le diabète de type 2. L’insuline présente un effet anabolique sur l’os. Ses effets sur la masse osseuse s’exercent de façon qualitativement différentes dans le diabète de type 1 et de type 2, en rapport avec les profils sécrétories insuliniques opposés qu’on y observe (hypo-insulinémie vs hyperinsulinémie). L’existence d’un risque de fracture élevé dans le diabète de type 2 malgré l’hyperinsulinémie sous-jacente laisse présumer de différences de qualité osseuse dans les diabètes de type 1 et 2. Celles-ci s’expliqueraient par une augmentation de la perte en calcium urinaire liée à l’élévation de la glycémie, conduisant à un équilibre calcique négatif. Le rôle de la résistance à l’hormone parathyroïdienne observée dans le diabète et ses effets sur le calcium et le renouvellement osseux sont également évoqués. En outre, la glycosylation du collagène peut altérer les caractéristiques biomécaniques osseuses. Les chutes associées aux comorbidités liées au diabète sont une autre cause possible de fractures provoquées par des traumatismes de faible intensité. La diminution du risque de fractures repose essentiellement sur un contrôle glycémique adapté et la prévention des complications diabétiques, en notant que les thiazolidinediones augmentent le risque de fracture chez les femmes ménopausées diabétiques de type 2. Pour conclure, la santé osseuse est une question importante dans le diabète. Les thiazolidinediones doivent être prescrites avec prudence aux femmes ménopausées ayant une faible masse osseuse et aux patientes ayant des antécédents de fracture de fragilité. Cet article passe en revue l’état actuel de nos connaissances sur les liens entre diabète et santé osseuse.

**Keywords:** osteoporosis; fracture risk; bone mineral density; diabetes; postmenopause; parathyroid hormone; leptin; thiazolidinedione
In patients with progressive chronic kidney disease (CKD), the homeostatic mechanisms regulating calcium and phosphate metabolism suffer important changes, resulting in low serum levels of calcitriol and calcium and phosphorous retention. The regulatory mechanisms fail and several chronic kidney disease mineral bone disorders (CKD-MBD) occur, including bone disease, vascular calcifications, cardiovascular disorders, bone fragility fractures, and reduced survival. Vascular calcification, bone loss, and increased fracture risk are severe disorders associated with aging in chronic CKD, but also generally speaking. Several epidemiological studies have shown the relationship between impaired bone metabolism, vascular calcification, and increased mortality. Recent data suggest this association may not just be a consequence of aging. The frequent occurrence of severe cases of vascular calcification together with low bone activity and osteoporosis suggests direct biological links may exist between bone and the vascular system. New challenging experimental data suggest that once severe vascular calcifications set in, vessels may develop a mechanism to diminish vascular mineralization in the arterial wall, and that this defensive mechanism may have a negative impact that favors the reduction of bone mass.

In healthy individuals, the kidneys regulate calcium and phosphorus homeostasis through active tubular mechanisms. Hormones and factors that contribute to kidney regulation of calcium and phosphorus include 1,25-dihydroxyvitamin D (1,25 [OH]2D or calcitriol), parathyroid hormone (PTH), and fibroblast growth factor-23 (FGF-23). In patients with progressive chronic kidney disease (CKD), the normal homeostatic mechanisms are challenged, leading to important compensatory changes in serum levels of calcium, phosphorus, calcitriol, FGF-23, and PTH. All these changes lead in part to several manifestations that for almost 60 years have been known as “renal osteodystrophy.” In addition, clinical, epidemiological, and experimental data have identified a clear association between the aforementioned changes in biochemical markers and some relevant outcomes such as vascular calcification, myocardial dysfunction, and mortality. As a result, a new term—chronic kidney disease–mineral bone disorder (CKD-MBD)—has been recently coined to encompass all these disorders.

Clinical impact and pathogenesis of mineral and bone disorders
The calcium, phosphorus, vitamin D, PTH, and FGF23 axis is closely regulated and interrelated. Several of the compensatory variations in the aforementioned factors...
take place at the same time under the control of complex feedback mechanisms.\textsuperscript{3-5} The progression of CKD leads to a decrease in active renal mass and then to a reduction in 1-alpha hydroxylase in the kidney, which in turn results in low levels of calcitriol, the physiological active form of vitamin D, impairing calcium absorption in the intestine and favoring the reduction in serum calcium. As a result, the decreases in serum calcium stimulate parathyroid hormone (PTH) synthesis and release, increasing bone turnover, bone resorption, and the stimulation of 1-alpha hydroxylase. All these mechanisms lead to compensatory increases in serum calcium.

In addition, the progressive reduction in renal function impairs phosphorus excretion, leading to increases in serum phosphorus, which stimulates the synthesis of both FGF23 and PTH. These two factors work in the same direction, increasing urinary phosphorus excretion. However, it is important to stress that, regarding vitamin D metabolism, the response is more complex, and FGF23 and PTH work in opposite directions: regarding calcitriol synthesis, FGF23 inhibits 1-alpha hydroxylase, reducing calcitriol synthesis, whereas PTH stimulates it.\textsuperscript{6-8} As renal function decreases, all these complex and tightly interrelated mechanisms of parathyroid gland regulation become insufficient and fail to adequately control parathyroid gland function and calcium and phosphorus homeostasis.

As a result, low serum levels of calcitriol and calcium, coupled with a trend toward phosphorus retention, prevail in the more advanced stages of CKD.\textsuperscript{3-5} Furthermore, in CKD stage 5D, severe forms of secondary hyperparathyroidism are frequently found, with diffuse and nodular parathyroid hyperplasia, as well as clinically relevant monoclonal growth with reduction in serum calcium and active vitamin D therapy. Finally, due to the lack of adequate parathyroid gland control, there is a clear trend toward autonomous parathyroid gland behavior (tertiary hyperparathyroidism), which frequently requires surgical removal of the glands.

Many of the aforementioned abnormalities and others beyond the scope of this review end up not only inducing several varieties of bone disease, but also vascular calcifications, cardiovascular disorders, bone fragility fractures, and a higher mortality risk. The recently coined term CKD-MBD encompasses all these mineral and bone metabolism disorders.\textsuperscript{2-12} As CKD is subdivided according to the degree of renal function into five stages, it is important to stress that marked differences exist between the initial and final periods of CKD.

### Clinical impact and pathogenesis of vascular calcification

The predisposition of CKD patients toward the development of vascular calcification was mentioned for the first time in the 19th century; since then, many studies have looked into this issue. Vascular calcification can be classified into three types according to the size and structure of the arteries: elastic or large-caliber arteries, muscular or medium-caliber arteries, and small-caliber arteries.\textsuperscript{13}

Elastic or large-caliber arteries show a relatively thin wall in proportion to their diameter, and the tunica media contains more elastic fibers than smooth muscle fibers. Muscular or medium-caliber arteries contain a greater proportion of smooth muscle fibers than elastic fibers in the tunica media; finally, small-caliber arteries contain only smooth muscle fibers in the tunica media. The classic description of arterial calcification specifies that it may occur in two locations: the intima and the media layers.\textsuperscript{14} Nevertheless, this classic concept is not fully accepted by all authors.\textsuperscript{15,16}

Intimal calcification begins and progresses under the influence of both genetic and lifestyle-related circumstances. It is associated with a sequence of atherosclerotic events such as endothelial dysfunction, intimal edema, lipid cell formation, plaque rupture, and formation of the thrombus.\textsuperscript{17} They have a patchy distribution along the length of the artery and cause local stenoses and occlusions. They are associated with several risk factors such as inflammation, alterations in lipid metabolism, obesity, hypertension, diabetes, smoking, and a family history of heart disease.

Media calcification occurs in the elastic lamina of large-caliber and medium-to-small-sized arteries; it is either independent of atherosclerosis or associated with it. X-ray imaging shows them as railway tracks. They are commonly found in the aorta, but also appear in arteries that are less likely to develop atherosclerosis, such as the visceral, abdominal, limb, and femoral arteries.\textsuperscript{18} Calcification of the media increases linearly with age and is frequently found in CKD, vitamin D metabolism disturbances, and diabetes, among other situations.\textsuperscript{19-22}

Table I (page 372) summarizes the most prevalent traditional, uremia-related, and nontraditional risk factors for vascular calcification in CKD patients. Like in the general population, the

### Selected Abbreviations and Acronyms

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CaSR</td>
<td>calcium sensing receptor</td>
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<tr>
<td>CKD</td>
<td>chronic kidney disease</td>
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<tr>
<td>CKD-MBD</td>
<td>chronic kidney disease–mineral bone disorder</td>
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<tr>
<td>EVOS</td>
<td>European Vertebral Osteoporosis Study</td>
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<tr>
<td>FGF</td>
<td>fibroblast growth factor</td>
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<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
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<tr>
<td>MBD</td>
<td>mineral bone disorder</td>
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<tr>
<td>PTH</td>
<td>parathyroid hormone</td>
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<tr>
<td>SFPR</td>
<td>secreted frizzled-related protein</td>
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<tr>
<td>VDR</td>
<td>vitamin D receptor</td>
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traditional cardiovascular risk factors, present in a large proportion of patients with CKD, are responsible to a great extent for the progression of vascular calcification. Among these, nontraditional cardiovascular risk factors, including uremia-related risk factors, time on dialysis, and hyperphosphatemia, are the risk factors more strongly associated with increased vascular calcification and mortality. Elevated C-reactive protein (CRP) and interleukin (IL)-6, as expressions of chronic inflammation, have been also frequently associated with vascular calcification. In a recent study, the prevalence of aortic calcification was compared with 13% of control subjects (similar age, with normal renal function). However, vascular calcification is not only seen in CKD patients; a subgroup of randomly selected European subjects older than 50 years (European Vertebral Osteoporosis Study [EVOS]) showed aortic calcification in 54.2% of men and 43.1% of women.

In a recent study, the prevalence of aortic calcification was higher in hemodialysis patients (79%) than in a random-based and age-matched general population (37.5%). Time on renal replacement therapy has been also positively associated with vascular calcification, mainly in medium-caliber arteries; in fact, each year on renal replacement therapy increased the risk of vascular calcifications by 15%. In addition, the number and severity of vascular calcifications have been positively associated with mortality, both in the general population and in CKD patients. In CKD, an up to 10 to 30 times higher mortality than in the general population has been reported. Women on hemodialysis showed an increased risk of severe aortic calcifications compared with women from the general population, probably due to a combination of atherosclerosis and arteriosclerosis.

Until recently, vascular calcification was considered the result of a simple precipitation of circulating calcium and phosphate. However, the mechanism by which the process of vascular calcification is produced is complex; it does not consist in a simple precipitation of calcium and phosphate; on the contrary, it is an active and regulated process in which, step by step, vascular smooth cells undergo apoptosis and vesicle formation, changing the phenotype of smooth vascular cells into osteoblast-like cells. Vascular calcification can be considered as the result of the lack of the physiological equilibrium between the promoters and inhibitors of the calcification process, in which several uremic factors—phosphorus topping the list—play a key role.

In humans and mammals, serum concentrations of calcium and phosphate exceed the calcium×phosphate solubility product; however, no intravessel precipitation takes place. This fact stresses the important role played by physiological inhibitors of calcification, which counterbalance the well-known effect of calcification promoters. The list of promoters and inhibitors of the calcification process has increased in recent years. The main interest has focused on the “modifiable promoters of calcification” with the aim of developing strategies to minimize them. Some have been associated with the risk of mortality, such as phosphorus, calcium, vitamin D, PTH, dyslipidemia, inflammation, nutrition, CRP, homocysteine, fibrinogen, and albumin. Among these, serum phosphorus needs to be highlighted as one of the more important risk factors, which is strongly associated with increased vascular calcifications and mortality.

Today, the fact that elevated phosphorus is a key factor in the differentiation of smooth vascular cells into osteoblast-like cells, triggering signals that will stop the promotion of mineralization, is well accepted. In vitro experiments have demonstrated that elevated phosphorus levels act directly on the transcription of bone-related genes, such as Cbfa-1 and osteocalcin, resulting in the activation of several osteogenic pathways. In addition, phosphorus is able to act as a secondary intracellular messenger activating several molecular pathways involved in bone formation. Other important factors from this list include the following most studied mineral-

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**Table I. Risk factors associated with vascular calcification in chronic kidney disease patients.**

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**Abbreviations:** CRP, C reactive protein; IL-1, interleukin 1; IL-6, interleukin 6; TNFα, tumoral necrosis factor-α.


**Text:**

CKD is associated with a high prevalence of vascular calcifications, which leads to a high prevalence of cardiovascular disease and reduced life expectancy. A high prevalence of vascular calcifications has also been reported in the early stages of CKD, where it has been shown that 40% of patients (CKD stages 2 to 4, mean glomerular filtration rate [GFR] 33 mL/min) have calcification of the coronary arteries, compared with 13% of control subjects (similar age, with normal renal function). However, vascular calcification is not only seen in CKD patients; a subgroup of randomly selected European subjects older than 50 years (European Vertebral Osteoporosis Study [EVOS]) showed aortic calcification in 54.2% of men and 43.1% of women.

In a recent study, the prevalence of aortic calcification was higher in hemodialysis patients (79%) than in a random-based and age-matched general population (37.5%). Time on renal replacement therapy has been also positively associated with vascular calcification, mainly in medium-caliber arteries; in fact, each year on renal replacement therapy increased the risk of vascular calcifications by 15%. In addition, the number and severity of vascular calcifications have been positively associated with mortality, both in the general population and in CKD patients. In CKD, an up to 10 to 30 times higher mortality than in the general population has been reported. Women on hemodialysis showed an increased risk of severe aortic calcifications compared with women from the general population, probably due to a combination of atherosclerosis and arteriosclerosis.

Until recently, vascular calcification was considered the result of a simple precipitation of circulating calcium and phosphate. However, the mechanism by which the process of vascular calcification is produced is complex; it does not consist in a simple precipitation of calcium and phosphate; on the contrary, it is an active and regulated process in which, step by step, vascular smooth cells undergo apoptosis and vesicle formation, changing the phenotype of smooth vascular cells into osteoblast-like cells. Vascular calcification can be considered as the result of the lack of the physiological equilibrium between the promoters and inhibitors of the calcification process, in which several uremic factors—phosphorus topping the list—play a key role.

In humans and mammals, serum concentrations of calcium and phosphate exceed the calcium×phosphate solubility product; however, no intravessel precipitation takes place. This fact stresses the important role played by physiological inhibitors of calcification, which counterbalance the well-known effect of calcification promoters. The list of promoters and inhibitors of the calcification process has increased in recent years. The main interest has focused on the “modifiable promoters of calcification” with the aim of developing strategies to minimize them. Some have been associated with the risk of mortality, such as phosphorus, calcium, vitamin D, PTH, dyslipidemia, inflammation, nutrition, CRP, homocysteine, fibrinogen, and albumin. Among these, serum phosphorus needs to be highlighted as one of the more important risk factors, which is strongly associated with increased vascular calcifications and mortality.
ization promoters and inhibitors: BMPs (bone morphogenetic proteins), an important family of proteins involved in bone formation and vascular calcifications; Cbfa-1; the MxWnt axis; vitamin D; calcium; phosphorus; tumor necrosis factor-α (TNFα); oxidative stress; matrix GLA protein (MGP); osteoprotegerin (OPG); fetuin A; pyrophosphates; and bisphosphonates.13,29-31,36,37

Links between bone metabolism and vascular calcification

Bone loss, increased fracture risk, and vascular calcification are severe disorders associated with aging in CKD patients and the general population.19,20,22,38 Furthermore, several epidemiological studies suggest a relationship between impaired bone metabolism, vascular calcification, and increased mortality.

The pathogenetic factors linking bone fragility with vascular calcification are not fully understood, but this relationship has been known for almost 20 years, when for the first time a significant inverse correlation between osteoporosis and aortic calcification was reported.39 However, during the following years, this association was probably underestimated because osteoporosis and vascular calcification were considered non-modifiable age-dependent disorders. Nevertheless, recent data suggest this association may not be just a consequence of aging.20,25 The role of aging cannot be completely dismissed, but the clinical coincidence of vascular calcifications with low bone activity and osteoporosis suggests there might be direct biological links between arteriosclerosis and osteoporosis. In fact, osteoporosis and vascular calcifications are influenced by several common risk factors such as inflammation, dyslipidemia, oxidative stress, as well as estrogen, vitamin D, and K deficiencies. Some population-based longitudinal studies have demonstrated an association between osteoporosis and vascular calcification or arterial stiffness.25 A large-cohort study published in 2004 showed that the degree of vascular calcification inversely correlated with bone mineral density. Furthermore, in part of the same cohort followed up for 2 years, the progression of vascular calcification inversely correlated with the rate of bone loss.40

In agreement with previous results, a recent study showed that after 4 years of follow-up, individuals who showed the most severe vascular calcification or the greatest progression of vascular calcification were those who showed not only the lowest bone mass, but also the highest incidence of new osteoporotic fractures.25 In addition, as expected, bone mass decreased and nontraumatic vertebral fractures increased in both sexes, as age increased. Also, serum levels of 25(OH)D3 inversely correlated with vascular calcification and bone mass, and positively correlated with the prevalence of secondary hyperparathyroidism and nontraumatic vertebral fractures. The progression of aortic vascular calcifications (new calcifications or increase in the size of preexisting calcifications) was significantly higher in patients who had a previous aortic calcification regardless of severity (mild, moderate, severe; P<0.001, age-adjusted). Interestingly, after 4 years of follow-up, mortality was also significantly and positively associated with the rate of severe vascular calcifications in men and with the rate of nontraumatic bone fractures in women.20

Similar results have also been published about patients on hemodialysis, which showed that vascular calcification in some areas (eg, the large and medium-caliber arteries [uterus-sperm], femoral, iliac; hands [digital, palm arch, radial]), was associated with an increased risk of vertebral fractures.21 In addition, comparing findings from hemodialysis patients with those of the EVOS study (age- and sex-matched population), the risk of aortic calcification was significantly higher in hemodialysis patients (men: odds ratio [OR], 7.7; women: OR, 9.0). In addition, women on hemodialysis with severe vascular calcifications (any localization), as well as women with vertebral fractures, showed a high mortality risk after all adjustments including age (Figure 1). Similarly, women who died during the
2-year follow-up period had a prevalence of vertebral fractures 3 times higher (58.8% vs 19.3%) than those women who were alive at the end of the observation period (adjusted for the same variables) (Figure 2).

Age and diabetes were strongly associated with vascular calcifications, but other well-know modifiable risk factors such as serum PTH, Ca, and P levels, vitamin D, calcium-based phosphate binders intake, dyslipidemia, hypertension, and smoking were not associated with the prevalence, severity, or progression of vascular calcification. If we combine the clinic and epidemiologic data, the association between serum 25(OH)D3 levels, vascular calcification, bone mass, and non-traumatic bone fractures, we may speculate that all of these could be linked by causes other than aging.\textsuperscript{20,25,26,41}

The relationship between vascular calcification and low bone turnover has also been assessed by histomorphometry in hemodialysis patients.\textsuperscript{25} A negative relationship between low bone turnover and the degree of vascular calcification has been found.\textsuperscript{41-43} An inverse relationship between coronary calcification and vascular stiffness with mineralized bone volume has been recently published.\textsuperscript{42} Nevertheless, despite the weight of the evidence, the relationship between low bone turnover and vascular calcification is still a matter of debate. A recent publication found that vascular calcification was not influenced by bone turnover when a multivariate analysis was performed,\textsuperscript{44} even though a high percentage of patients with high bone turnover were included in this study. It is known that high PTH levels are another important pathogenetic factor positively associated with vascular calcification. In fact, it has been reported that correction of the balance in bone turnover, whether the latter was high or low, protects against the progression of vascular calcification.\textsuperscript{41} In any event, overall, the sum of epidemiological and clinical studies strongly suggests that the prevalence and progression of vascular calcification are related to bone mass, bone turnover and mineralization, bone loss, and osteoporotic fragility fractures.

Likely negative effect of vascular calcification on bone health: a challenging hypothesis for further research

An intriguing question is whether the presence of vascular calcification can have a further negative impact on bone metabolism. In a recent study, rats developing severe vascular calcification after a phosphorus load showed no increase in bone mass at any of the sites studied after 20 weeks.\textsuperscript{34} In contrast, rats with no phosphorus load develop no vascular calcification. Furthermore, bone mass increased during the study period as expected. Microarray analysis of the aortas with severe vascular calcification evidenced overexpression of secreted frizzled-related proteins (SFRPs). It is well-known that SFRPs are inhibitors of the canonical Wnt signaling pathway, which is actively involved in bone formation and vascular calcification.\textsuperscript{34,45,46}

The increase in SFRPs in areas of severe vascular calcification may be indicative of a wall artery–defensive mechanism triggered to block the activation of the Wnt pathway, aimed at attenuating mineralization in the calcified aortic wall. Since SFRPs are secreted proteins, they can act not only locally on the artery wall to reduce the mineralization, but may be able to reach the bone, where they could act as they do in the vessels to decrease mineralization, resulting in reduction of bone mass. This is a challenging feedback hypothesis that could help explain the findings reported in the clinical and epidemiological studies discussed above, in which the most severe cases of progressive vascular calcification were associated with low bone mass and a greater percentage of bone fractures.

In summary, in both the general and CKD populations, vascular calcification and its severity seems to correlate inversely related with bone mass, with a resultant increase in bone fractures. In addition, the increase in vascular calcification and bone fractures is associated with reduced survival. Interestingly, once vascular calcifications appear and progress, arteries may develop a defensive mechanism aimed at attenuating or regressing vascular mineralization of the arterial wall, and this in turn may exert a negative impact on bone health.\textsuperscript{1}

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Keywords: bone; vascular calcification; osteoporosis; bone density; bone fracture; low bone mass; bone disease; chronic kidney disease; mineral bone disorder
Chez les patients ayant une insuffisance rénale chronique progressive (IRC), les mécanismes homéostatiques régulant le métabolisme phosphocalcique subissent des modifications importantes, conduisant à de faibles concentrations sériques de calcitriol et de calcium et à une rétention de phosphore. L’IRC altère les mécanismes de régulation qui deviennent inefficaces, favorisant ainsi l’apparition de divers troubles minéraux à l’origine de pathologies osseuses, de calcifications vasculaires, de troubles cardio-vasculaires, de fractures osseuses de fragilité, avec comme conséquence un effet péjoratif sur la durée de vie. Les calcifications vasculaires, la perte osseuse et l’augmentation du risque de fracture sont des troubles sévères associés au vieillissement, tant dans le contexte de l’IRC que de façon générale. Plusieurs études épidémiologiques ont montré un lien entre l’altération du métabolisme osseux, les calcifications vasculaires et l’augmentation de la mortalité. Des données récentes suggèrent que cette association n’est pas seulement une conséquence du vieillissement. La fréquence de cas sévères de calcifications vasculaires associés à une faible activité osseuse et à l’ostéoporose laisse supposer l’existence de liens biologiques directs entre le tissu osseux et le système vasculaire. Selon des données expérimentales récentes, le développement de calcifications vasculaires graves entraînerait l’apparition de mécanismes de défense vasculaires visant à diminuer la minéralisation vasculaire de la paroi artérielle, et ces mêmes mécanismes auraient un effet négatif favorisant la réduction de la masse osseuse.
Physical activity acts directly on bone through mechanical stress, and indirectly by changes in cardiovascular, ventilatory, metabolic, and hormonal parameters. Studies in athletes show that activities such as running, performing gymnastics, and weight lifting induce bone gain, whereas cycling and swimming are poorly osteogenic. Bone gain is mostly observed in the parts of the body involved in the exercise. Failing to continue exercising during adulthood could be detrimental to bone gain. In the early stages of puberty, exercising increases bone mass, whereas in postmenopausal women and in the elderly, exercise does not always provide bone gain. Nevertheless, it may prevent osteopenia and improve muscle tone, cardiovascular function, and balance, thus limiting the risk of falling. As is the case for some young people, too much training can be harmful, as evidenced by cortical thinning in older cyclists who train more than 6 hours per week. This is evidence of a nonlinear effect of exercise on the skeleton. High-impact exercises are hardly applicable to fragile subjects. Whole-body vibrations (WBV) may have osteogenic potential. In animal models of bone loss, WBV improves bone mass and quality. In humans, certain studies show a potential benefit of WBV with regard to muscle, bone, and posture. The therapeutic use of WBV is not standardized, and the impact and scope of application still needs to be defined in terms of frequency, amplitude, duration, etc. This will require tailoring WBV to the characteristics of the users and assessing its effect on the whole body as well as on individual compartments (cartilage, peripheral circulation, tendons).

As early as 1892, Wolff suggested that the distribution of mechanical stress at the tissue level determines bone architecture. In 1971, Thompson and Frost introduced the concept of adaptation of skeletal tissue to stress, through regulation of bone cell populations. Exposure to stress causes the tissue to deform, resulting in local alterations designated as microstrains (10 000 microstrain (µε) = 1% change in length, or 1 strain (ε) = 100%).

Moreover, it appears that the capacity of bone to adapt to mechanical stress occurs during dynamic stress (cyclic), whereas static stress entails no tissue response. These mechanical signals act on the bone cells themselves, which, through a cascade of reactions starting from the extracellular matrix, transform the mechanical signal into a biological response. This phenomenon is known as mechanotransduction.
Mechanoadaptation of the bone—tissue support

**Mechanotransduction: in vitro studies**

Bone cells, particularly those of osteoblastic lineage, are the most studied cells. This lineage comprises mesenchymal precursor cells to osteocytes, which is the final stage of differentiation, and represents 90% of bone cells. Understanding the mechanotransduction of all these stages is crucial: in precursors, it can guide their commitment to osteoblastogenesis at the expense of adipogenesis; in osteoblasts, it affects the physicochemical properties of the newly synthesized matrix; and finally in osteocytes, it coordinates bone remodeling. One of the major cellular components of mechanotransduction is the cytoskeleton. Indeed, it is an intracellular cable network comprising microtubules that are resistant to contractile strains of actin filaments and intermediate filaments that stabilize microtubules and microfilaments of actin.

This compression-tension network physically links with the extracellular matrix through transmembrane receptors (particularly integrins, mechanical transfer areas). Intracellular tension forces are therefore able, through the connected system, to balance out the forces of the extracellular matrix (and vice versa). This regulatory mechanism influences and integrates the effects of biochemical factors, by using or crossing these same regulatory pathways.

The model that takes into account all these forces, which, separately or jointly, affect the fate and/or activity of the bone cells, is referred to as the tensegrity (= tensional integrity) model (Figure 1).

**At tissue level**

The tensegrity model also applies to a musculoskeletal system in which the bones are compressed under the effect of gravity (weight, load) and under tension caused by the action of muscles, tendons, and ligaments. Such hierarchical structures can explain the mechanical transmission of information and coordinated response of an organ to a stimulus by mechanical coupling.

In bone, osteocytes undergo deformation variations resulting from movements that give rise to compression, tension, and torsion forces. Without functional osteocytes (targeted deletions) bone cannot adapt to changes in mechanical stress. In addition, pressure gradients caused by the tissue as it deforms create a flow of extracellular fluid around the osteocytes. However, mechanical and shear forces are not the only phenomena that occur: the deformation creates piezoelectric effects and the fluid causes the formation of electric fields called “streaming potentials.” Each of these three phenomena plays a role in mechanotransduction.

The resistance of bone to stress, to which it is constantly subjected (posture, physical activity), is determined both by its macroscopic characteristics (shape, size, structure) and a series of microscopic material and structural properties of the tissue. The stiffness (elastic zone of the bone) and the toughness (plastic zone of the bone) are examples of the biomechanical torque, which is the most studied in mineralized tissues. As a result of the nature of the materials, it is difficult to associate a very high stiffness with an extensive range of mechanical resistances. There are different ways of combining the two, but it might make the material extremely anisotropic, in the sense that it becomes rigid and hard in one direction, but weak and fragile in other directions. The balance between the function and structure of mineralized biological materials has led to a compromise between stiffness and toughness. Bone stiffness is mainly related to its mineral fraction, rendering it resistant to compressive forces. In contrast, the organic fraction, consisting mainly of collagen, gives bone its toughness and renders it resistant to tensile forces.

Another aspect of the mechanoadaptation of bone is the formation of microcracks resulting from bone fatigue caused by cyclic loading of critical areas that concentrate the stress and which are known to increase with age. The theory of bone’s adaptation to stress by microcracks is reinforced by findings from in vivo and ex vivo studies, which have analyzed...
the initiation and propagation of microcracks in bone samples. Microcracks were experimentally generated in vivo by physiological deformations, and the relatively significant remodeling activities were found to correlate with the experimentally induced damage. These activities that induce elevated remodeling are responsible for maintaining the structural integrity of bone and repairing fatigue damage produced by normal mechanical use. The osteocytes are responsible for this regulatory process by stimulating bone resorption at the site of a microcrack, either because their apoptosis initiates a cycle of resorption and the remodeling of a unit, or because the rupture of osteocyte dendrites affects signaling networks such as RANKL/OPG (receptor activator of nuclear factor kappaB ligand / osteoprotegerin). During the remodeling process, sclerostin, synthesized by osteocytes, has been shown to be a new player that inhibits bone formation. Its synthesis is stimulated by immobilization, which induces inhibition of the beta-catenin Wnt pathway and is inhibited by stress.

It thus appears that accumulation of fatigue is a stimulus of bone modeling/remodeling, which could explain the osteogenesis triggered by certain types of sports. Moreover, it seems possible that a mechanically overstretched bone or bone in an osteoporotic subject may not be able to "repair" the microdamage, thus creating a situation conducive to fracture. This aspect of bone fatigue is poorly understood, due to a lack of noninvasive tools for the visualization of microcracks.

Effects of different types of sports
Differences exist with respect to the type of sport performed. Running, gymnastics, and weight lifting induce bone growth of increasing amplitude. In contrast, with low-impact sports with limited loads, such as swimming, the effects on the bone mass in the lower limbs, or even the whole body, are negligible. Other findings suggest that a physical activity involving a significant impact, physical contact, and/or rotational forces, not only has beneficial effects on the areas under load, but also on the peripheral and axial bones not subjected to load. The magnitude of the difference between a state under load or not seems to be the decisive parameter. Indeed, weight lifters who are subjected to very high stresses appear to increase their bone mass more compared with any other sport. A distinction should be made between sports that generate mechanical stress based on the mode of loading (weight lifting) and those that generate mechanical stress through repeated impacts (running).

The musculoskeletal system of humans has evolved to adapt to endurance running. We can thus imagine that in load-bearing bones, large forces are needed to generate unusual strains. With regard to swimming, the loads developed by movements against the resistance of water, as well as the muscle contractions generating them are insufficient for inducing stimuli to the inferior limb bones. However, when it comes to non-load-bearing bones, such as the humerus, the deformations caused by muscle contractions are osteogenic. Consequently, the effect of pulling the muscles by their attachments on the bone has an impact on bone that depends on the function of load-bearing bones in the considered area. This has been confirmed in astronauts, another extreme model in which bone mass is lost in load-bearing
 bones, but not in non-load-bearing bones despite the substantial exercise programs they follow, but with which they cannot—or rarely—exceed accelerations above 1 g.

**Thresholds effects**

Under certain circumstances, carrying out a sport that is known to be osteogenic may have a deleterious effect on bone tissue. Intensive running by adults not accustomed to training can cause stress fractures. In marathon runners, both male and female, bone deficiency is frequently observed at the lumbar spine. In highly trained female athletes, a problem known as the “female athlete triad” (eating disorders + amenorrhea/oligomenorrhea + decreased bone mineral density) is more accentuated because of the harmful effects of overtraining on the hormonal cycle and of inadequate nutrition. This stresses the interdependence of the determinants of bone mass. These data suggest that exercise of too great intensity is damaging to the bone tissue. Indeed, a study in women and men over 50 shows that exercising (with loads) more than 5 hours per day (running, dancing or brisk walking), results in a decrease in spinal mineral density. This mineral deficiency can be explained by age, body mass, or estrogen status. We have also shown that increased bone resorption occurs in men over 60 practicing more than 6 hours of sport per week. These studies point to a nonlinear effect of exercise on bone mass. Another study showed that in soccer players training for up to 6 hours per week, femoral mineral density increased in proportion to the duration of training, but plateaued beyond this limit, without additional benefit to the bone.

These studies indicate that not only intensity, but also duration of exercise is an important factor for the bone’s response to exercise. The tibia is one of site where fractures occur frequently in adolescents and young adults, especially in the diaphyseal region. Very few reports exist with regard to metaphyseal or epiphyseal fractures. Similarly, these fractures are rare in the distal femur; most studies refer to fractures of the axis or neck of the femur.

**Prevention of osteoporosis**

Studies consistently confirm the role of certain types of physical exercise as a means for preventing osteoporosis. Prevention programs focus primarily on two populations: adolescents, in order to optimize their bone mass at the end of growth, and female adults and postmenopausal women, in order to reduce the slope of bone loss. The most important period for bone gain certainly is the peripubertal period, as shown in young tennis players in whom the increase in bone mass—and even more importantly in bone size—in the playing arm, can exceed 10%. Even if the effects of exercise on the BMD in postmenopausal women are modest, epidemiological studies suggest that physical activity and levels of dietary calcium are capable of reducing the risk of fracture. Walking alone is not enough to prevent bone loss. Consequently, exercise—even if it is dynamic and based on feedback of the limbs on the ground—must also achieve a certain level of frequency and intensity to be effective on bone tissue. More recent studies using peripheral tomography have shown, in this population, a positive association between physical activity scored over several years and cortical bone geometric parameters related to the radius, tibia, or femur. These data are invaluable since a minimal diaphyseal expansion induces a substantial improvement in flexural strength. It is possible that dual-energy x-ray absorptiometry (DXA) is not sufficiently powerful for the visualization of these changes.

**In elderly subjects**

As we saw in the previous section, the knowledge we have of the effects of exercise on bone tissue is essentially what high-level athletes have taught us. These effects are much more difficult to identify in a vast population. In the elderly, it remains unclear whether exercise programs or the fact of having been active offers protection from osteoporotic fractures. At a certain age, exercising (gymnastics, walking) does not always provide significant bone gain. It could, however, possibly prevent rapid bone loss and thus reduce the risk of fracture, while also improving muscle tone, cardiovascular function, balance, and posture, thereby limiting the risk of fractures from falls. Recent reviews conclude that there is a need for better-targeted randomized controlled trials to evaluate the true effectiveness of exercise. In other words, this subject is not closed (Figure 3).

Instead of talking about physical activity, particularly when it comes to the elderly, one could speak of mechanical systems that are aimed at generating an effective stimulus to the skeleton. Hope is permitted, because it has been shown that use of vibration programs could be osteogenic. However, given all that has been said previously, this seems somewhat unlikely. Indeed, we know that very-high-amplitude (>2000 µε) and low-frequency (<2 Hz) signals, which exist during strong-impact physical activities, are osteogenics until a threshold beyond which deleterious effects occur, based on Frost’s mechanostat theory.

Since a pioneering study from 1990 and the early 2000s, it has been shown that low-amplitude signals, well below the amplitudes that can cause fractures, can also, when applied at high frequencies, induce an osteogenic response. Several studies using strain gauges attached to the limbs of different animals report that mechanical stimuli generated during motion (walking, running), or in static position (subject standing, sitting), induced signals of amplitudes around 500 to 2000 µε occurring at low frequencies (<2 Hz), but also signals of low amplitude (<300 µε) occurring at higher frequencies (10 to 50 Hz). It should be noted that the lower the amplitude of the signals, the higher their frequency and the more they are...
represented during daily activities (thousands of times) in contrast to high-amplitude signals, which are weakly represented, and this, regardless of the species or the bone site studied. Moreover, studies have shown that a force applied at high frequency (10 to 20 Hz) was more osteogenic than the same force applied at a lower frequency, as the motion frequency (1 Hz). This property of high frequencies as well as the balance of low-amplitude signals during everyday activities, has provided further insights into the understanding of their roles on bone tissue.

In 70 postmenopausal women, a prospective, randomized, double-blind study during a period of 1 year showed that episodes of less than 20 minutes with subjects standing on vibrating tables (<0.3 g; 20 to 90 Hz) were able to reduce bone loss in the lumbar and femoral regions. Compliance was increasingly high for increasingly frail subjects. Another interesting study was carried out in postmenopausal women undergoing three sessions per week on vibrating tables during 6 months (35 to 40 Hz; 2.28 to 5.09 g), who were asked to carry out knee bending exercises.

The origin of these signals is unclear. The observed high frequencies could be harmonics of large-amplitude signals (which occur at low frequency). The signals could also originate from muscle activity. A sarcopenia of type II fibers is observed in the elderly, which causes a decrease in muscle strength, as well as a decrease in muscle activity, with frequencies ranging from 30 at 50 Hz. This sarcopenia also causes an alteration of mechanical signals that regulate bone. Thus, muscle wasting is an etiologic factor in osteoporosis.40

Various studies have therefore investigated the effect of low-amplitude/high-frequency signals on bone. Those of Rubin et al are the most illustrative.41,42 These researchers showed that sessions of 20 minutes of low-amplitude (0.3 g, 5 με), high-frequency (30 Hz) signals, applied for 1 year to the hindlimbs of adult ewes were able to increase the density and volume of the trabecular bone in the proximal femur.13

Furthermore, a signal simulating a physical activity (sinusoidal signal; 3 N; 2 Hz) coupled to low-amplitude (0.3 N) and high-frequency (0 to 50 Hz) signals applied during two consecutive days, 30 s/day, on mouse ulna in vivo has been shown to raise the rate of bone formation by approximately a factor of 4, as compared to a signal simulation exercise on its own.43 In humans, one can easily understand the relevance of employing this type of noninvasive, nonpharmacological mechanical system in frail or disabled individuals, who are incapable of carrying out regular physical exercise.

Results showed a gain of proximal femur bone and an increase in isometric and dynamic muscle strength.46 Gusi et al reported improved balance and reduced body fat following a gain in the neck of the femur in postmenopausal women after 8 months of training (3 times/week, 12.6 Hz, 3 cm displacement amplitude of the pad).46

In contrast, another group of postmenopausal women who carried out vibration exercises of 30 to 40 Hz 3 times/week in addition to resistance training during 8 months did not achieve any additional gain in bone mass or muscle strength, as opposed to those carrying out solely resistance training.47

Studies on a larger scale are now required to not only confirm the benefits with regard to bone and muscle in different populations, but also to assess micro- and macro-scale changes in bone architecture at the and the impact on the risk of fracture. Other potential circulatory, postural, and neurovestibular effects should also be studied in parallel. A dosage effect needs to be established, not only with regard to time of use, but also with regard to the acceleration transmitted to different segments based on various postures when standing on the aforementioned vibrating tables.

**Conclusion**

Optimal response to loading appears to occur during the prepubertal stage, at least in girls (the window might be larger in boys). According to estimates, an increase in peak bone mass

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**Figure 3. Exercise and prevention of bone fragility.**

Exercise plays a pivotal role in prevention of bone fragility, and falls in the elderly population. Exercise regimens chosen for bone or balance are diverse and not all exercise regimens are effective. The optimal type, intensity, frequency and duration of exercise to maximize prevention of fractures remain incompletely characterized.
of 10% (depending on the type of sport) would delay the onset of osteoporosis by 13 years,\(^4\) suggesting that this period of 10% (depending on the type of sport) would delay the onset of osteoporosis by 13 years.\(^4\) This period of 10% (depending on the type of sport) would delay the onset of osteoporosis by 13 years.\(^4\)

Data from short-term prospective studies indicate a positive association between areal BMD and physical activity, but bone benefits may be lost if the practice of sports is stopped. In the elderly, physical activity may also reduce fracture risk through other mechanisms than those affecting BMD. Decreased bone mass, muscle strength, tissue perfusion, systemic hormones, and articular cartilage are common in elderly individuals. Whole-body vibration therapy may be efficient in alleviating these deteriorations, but its use for therapeutic purposes is far from being standardized. Although areal BMD measured by DXA is a common surrogate for bone strength, it is now possible to measure other aspects of bone strength such as bone geometry and volumetric BMD, using three-dimensional imaging techniques. Evaluation of bone macro- and micro-architectural parameters is gaining widespread acceptance and will improve our understanding of human skeletal adaptation to mechanical loading.

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tions, but its use for therapeutic purposes is far from being standardized. Although areal BMD measured by DXA is a common surrogate for bone strength, it is now possible to measure other aspects of bone strength such as bone geometry and volumetric BMD, using three-dimensional imaging techniques. Evaluation of bone macro- and micro-architectural parameters is gaining widespread acceptance and will improve our understanding of human skeletal adaptation to mechanical loading. □


Keywords: sports; leisure; bone; whole body vibration therapy; puberty; menopause; cortical bone; mechanotransduction

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**Activité physique et qualité de l’os**

L’exercice physique agit directement sur le système osseux par l’intermédiaire d’un stress mécanique, et indirectement par des changements des paramètres cardio-vasculaires, ventilatoires, métaboliques et hormonaux. Les études menées chez les athlètes montrent que les activités comme la course, la gymnastique et l’haltérophilie induisent un gain osseux, tandis que le cyclisme et la nage sont faiblement ostéogènes. Le gain osseux est principalement observé sur les parties de l’organisme sollicitées par l’exercice. L’arrêt de l’exercice au cours de l’âge adulte peut avoir un effet négatif sur le gain osseux. Au cours des premiers stades de la puberté, l’exercice physique augmente la masse osseuse, tandis que chez les femmes ménopausées et chez les personnes âgées, si l’exercice n’apporte pas toujours un gain osseux, il permet néanmoins de prévenir l’ostéopénie et d’améliorer le tonus musculaire, la fonction cardio-vasculaire et l’équilibre, réduisant ainsi le risque de chute. Comme c’est le cas chez les personnes plus jeunes, un entraînement excessif peut être dangereux, comme le montre l’amincissement de l’os cortical observé chez les cyclistes âgés qui s’entraînent plus de 6 heures par semaine. Il a été mis en évidence un effet non linéaire de l’exercice physique sur le squelette. Des exercices entraînant des impacts importants sont difficilement applicables aux sujets fragilisés. Les plateformes vibrantes peuvent avoir un potentiel ostéogène. Dans des modèles animaux de perte osseuse, les plateformes vibrantes améliorent la masse et la qualité de l’os. Chez l’homme, certaines études ont montré les bénéfices potentiels des plateformes vibrantes en ce qui concerne le système musculaire, le système osseux et la posture. L’utilisation thérapeutique des plateformes vibrantes n’est pas standardisée, et l’impact et le cadre de leur application restent à définir en ce qui concerne la fréquence, l’amplitude, la durée, etc. Cela nécessitera de personnaliser les plateformes vibrantes afin de les adapter aux caractéristiques des utilisateurs et d’évaluer leurs effets sur le corps entier ainsi que sur les différents compartiments individuels (cartilage, circulation périphérique, tendons).
The overwhelming majority of fractures occur after a fall, and fall rates increase with age and poor muscle strength or function. Furthermore, after a first fall, about 30% of persons develop a fear of falling, and as a result restrict their activities and suffer from decreased quality of life. Thus, the benefit of vitamin D in terms of fall and fracture prevention has significant clinical implications, all the more so as there is a growing number of epidemiologic studies linking low vitamin D status with an increase in the risk of colon and possibly other cancers, as well as in the risk of hypertension, myocardial infarction, cardiovascular and overall mortality, infections, and diabetes. Several recent meta-analyses have addressed the benefit of vitamin D on fracture reduction, with conflicting findings. This article will first summarize the findings from double-blind randomized trials of oral vitamin D supplementation with respect to antifracture efficacy. It will then address why meta-analyses using alternative approaches, including open-design trials and trials that tested intramuscular vitamin D, have reported discordant findings. Finally, as vitamin D modulates fracture risk in two ways, by decreasing falls and increasing bone density, the efficacy of vitamin D on fall prevention will be reviewed, and the optimal 25-hydroxyvitamin D level to achieve these benefits will be discussed.

Medicographia. 2010;32:384-390 (see French abstract on page 390)
Vitamin D supplementation in seniors aged 65 and above

We now look at the available evidence from double-blind randomized controlled trials of oral vitamin D supplementation in seniors aged 65 and older, and its efficacy in terms of fall and fracture prevention. Two 2009 meta-analyses of double-blind randomized controlled trials came to the conclusion that vitamin D reduces the risk of falls by 19%,13 the risk of hip fracture by 18%,13 and the risk of any nonvertebral fracture by 20%.13 However, this benefit was dose-dependent. Fall prevention was only observed in trials with a treatment dose of at least 700 IU vitamin D per day, and fracture prevention required a received dose (treatment dose*adherence) of more than 400 IU vitamin D per day. Lower doses failed to reduce fracture or fall risk, while the benefit of fall prevention and fracture prevention was present in all subgroups of the senior population at the higher dose of vitamin D. Primary prevention based on received dose (treatment dose*adherence) as opposed to treatment dose in double-blind randomized controlled trials (RCTs), made it possible to assess antifracture efficacy using a dose that accounted for the low adherence in several recent large trials.14,15

2009 meta-analysis on fracture prevention

The 2009 meta-analysis on fracture prevention included 8 double-blind RCTs with predefined fall assessment throughout the trial period (n=2426) and found significant heterogeneity by dose (low-dose: <700 IU/day versus higher dose: 700 to 1000 IU/day; P-value 0.02) and achieved 25-hydroxyvitamin D level (<60 nmol/L versus ≥60 nmol/L; P-value = 0.005).12 Higher-dose supplemental vitamin D reduced fall risk by 19% (pooled relative risk [RR], 0.81; 95% confidence interval [CI], 0.71-0.92; n=1921 from 7 trials) versus a lower dose did not (pooled RR=1.10, 95% CI, 0.89-1.35 from 2 trials), also achieved serum 25-hydroxyvitamin D concentrations less than 60 nmol/L did not reduce the risk of falling (pooled RR=1.35, 95% CI, 0.98-1.84). Notably, at the higher dose of 700 to 1000 IU vitamin D, this meta-analysis documented a 38% reduction in the risk of falling with treatment duration of 2 to 5 months and a sustained significant effect of 17% fall reduction with treatment duration of 12 to 36 months, and the benefit was independent of type of dwelling and age. Thus, benefits of 700 to 1000 IU vitamin D per day on fall prevention are rapid and sustained and include all subgroups of the senior population.

Further support for a dose-response relationship of vitamin D and fall reduction comes from a multidose double-blind RCT in 124 nursing home residents receiving 200, 400, 600, or 800 IU vitamin D compared with placebo over a 5-month period.16 Participants in the 800 IU group had a 72% lower rate of falls than those taking placebo or a lower dose of vitamin D (rate ratio, 0.28; 95% CI, 0.11-0.75).16

2009 meta-analysis on fracture prevention

This meta-analysis on fracture prevention included 12 double-blind RCTs for nonvertebral fractures (n=42 279) and 8 RCTs for hip fractures (n=40 886), and, similar to the meta-analysis on fall prevention, it found significant heterogeneity for received dose of vitamin D and achieved level of 25-hydroxyvitamin D in the treatment group for hip and any nonvertebral fractures (Figures 1 and 2, page 386).13,15,17-26 No fracture reduction was observed for a received dose of 400 IU or less per day or achieved 25-hydroxyvitamin D levels of less than 75 nmol/L. Conversely, a higher received dose of 482 to 770 IU supplemental vitamin D per day reduced nonvertebral fractures by 20% (pooled RR, 0.80; 95% CI, 0.72-0.89; n=33 265 from 9 trials) and hip fractures by 18% (pooled RR, 0.82; 95% CI, 0.69-0.97; n=31 872 from 5 trials). Notably, subgroup analyses for the prevention of nonvertebral fractures with the higher received dose suggested a benefit in all subgroups of the older population, and possibly better fracture reduction with vitamin D3 compared with vitamin D2, while additional calcium did not further improve antifracture efficacy (Table I, page 386).13

Results from meta-analyses having included double-blind and open-design trials in their primary analysis

In August 2007, a review and meta-analysis commissioned by the US Department of Health and Human Services (HHS) addressed the effect of vitamin D supplementation on all fractures in postmenopausal women and men ages 50 and older.27 The pooled results for all fractures included 10 double-blind and 3 open-design trials (n=58 712) and did not support a significant reduction of fractures with vitamin D (pooled odds ratio [OR], 0.90; 95% CI, 0.81-1.02). The report suggested that the benefit of vitamin D may depend on additional calcium and be primarily seen in institutionalized individuals, which is consistent with the meta-analysis of Boonen et al.28

**SELECTED ABBREVIATIONS AND ACRONYMS**

- NHANES III: Third National Health And Nutrition Examination Survey
- RCT: randomized controlled trial
- VDR: vitamin D receptor gene
- WHI: Women’s Health Initiative [trial]
A 2010 patient-based meta-analysis of a subgroup of 7 large trials of vitamin D included 68,500 individuals age 47 and older. The authors defined alternative criteria that permitted the inclusion of two open-design trials, one trial with intramuscular vitamin D, and 4 of the 12 double-blind RCTs of oral vitamin D included in the 2009 meta-analysis described above (one RCT using intermittent vitamin D2 without calcium, one RCT with 400 IU vitamin D3 without calcium, one trial with 800 IU vitamin D3 per day with and without calcium and less than 50% adherence, and one trial with 400 IU vitamin D with calcium). The authors did not account for adherence to treatment. Based on these criteria, their findings showed a reduced overall risk of fracture (hazard ratio [HR], 0.92; 95% CI, 0.86 to 0.99) and a nonsignificant reduction of hip fractures (HR, 0.84; 95% CI, 0.70 to 1.01) for trials that used vitamin D plus calcium. Vitamin D alone, irrespective of dose, did not reduce fracture risk. The authors concluded that vitamin D, even in a dose of 400 IU vitamin D per day, reduces the risk of fracture if combined with calcium. Notably, this regimen was tested in 36,282 postmenopausal women in the Women’s Health Initiative (WHI) trial over a treatment period of 7 years and did not reduce the risk of fracture.

Table 1. Nonvertebral fracture reduction with vitamin D based on evidence from double-blind RCTs.

<table>
<thead>
<tr>
<th>Subgroups by received dose of vitamin D</th>
<th>Fracture reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled analysis from 3 trials with low-dose vitamin D (340-380 IU/day)</td>
<td>+2% 0</td>
</tr>
<tr>
<td>Pooled analysis from 9 trials with higher-dose vitamin D (482-770 IU/day)</td>
<td>-20% Sig.</td>
</tr>
<tr>
<td>Pooled subgroup analysis from trials higher-dose vitamin D (482-770 IE/Tag):</td>
<td></td>
</tr>
<tr>
<td>Vitamin D2</td>
<td>-10% 0</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>-23% Sig.</td>
</tr>
<tr>
<td>Age 65-74</td>
<td>-33% Sig.</td>
</tr>
<tr>
<td>Age 75+</td>
<td>-17% Sig.</td>
</tr>
<tr>
<td>Institutionalized 65+</td>
<td>-15% Sig.</td>
</tr>
<tr>
<td>Community-dwelling 65+</td>
<td>-29% Sig.</td>
</tr>
<tr>
<td>Vitamin D plus calcium</td>
<td>-21% Sig.</td>
</tr>
<tr>
<td>Vitamin D main effect</td>
<td>-21% Sig.</td>
</tr>
</tbody>
</table>

Sig. = significant

Figure 1. Nonvertebral fracture prevention by received daily dose of 25(OH)D.

Triangles indicate trials with D3, circles trials with D2. Line = Trend line. All 12 high-quality trials were included for the received dose metaregression (n=42,729 individuals). For any nonvertebral fractures, antifracture efficacy increased significantly with higher received dose (meta-regression: Beta = -0.0007; P=0.003).


Figure 2. Nonvertebral fracture prevention by achieved 25(OH)D levels.

Triangles indicate trials with D3, circles trials with D2. Line = Trend line. For achieved 25(OH)D levels, 2 trials (out of the 12 trials) did not provide serum 25(OH)D levels measured in the study population during the trial period. For any nonvertebral fractures, antifracture efficacy increased significantly with higher achieved 25-hydroxy-vitamin D levels (meta-regression: Beta = -0.005; P=0.04).

In all three reports reviewed under this section, heterogeneity by dose may have been missed due to the inclusion of open-design trials plus a dose evaluation that did not incorporate adherence. Biologically, the exclusion of heterogeneity by dose seems implausible even if a formal test of heterogeneity is not statistically significant. A dose-response relationship between vitamin D and fracture reduction is supported by epidemiologic data showing a significant positive trend between serum 25(OH)D concentrations and hip bone density, lower extremity strength, and trial data for fall prevention.

In addition, greater antifracture efficacy with higher achieved 25(OH)D levels was documented in an earlier meta-analysis of high-quality primary prevention trials with supplemental vitamin D. Factors that may obscure a benefit of vitamin D are low adherence to treatment, low dose of vitamin D, or the use of less potent D<sub>2</sub>. Furthermore, open-design trials may bias results toward nil, because vitamin D is available over the counter.

Notably, the 2009 meta-analyses on fall and fracture prevention from double-blind RCTs performed sensitivity analyses that included 4 open-design trials for fracture prevention and 3 open-design trials for fall prevention. Both analyses found significant variation in results between open-design and double-blind trials at any dose of vitamin D, the lower and the higher dose suggesting that trial quality introduces heterogeneity.

Finally, the consistency of the results for both received dose and achieved 25(OH)D levels in the treatment group across all 12 masked trials lends support to the presence of a dose-response relationship between supplemental vitamin D and fracture reduction (Figures 1 and 2).

**Optimal 25-hydroxyvitamin D levels for bone and muscle health**

The threshold for optimal 25(OH)D and hip BMD was investigated in 13,432 individuals of NHANES III (Third National Health And Nutrition Examination Survey), including both younger (20 to 49 years) and older (50+ years) individuals of various ethnic backgrounds. In the regression plots, higher serum 25(OH)D levels were associated with higher BMD throughout the reference range of 22.5 to 94 nmol/L in all subgroups. In younger whites and younger Mexican-Americans, higher 25(OH)D was associated with higher BMD, even beyond 100 nmol/L.

The threshold for optimal 25(OH)D and lower-extremity function was evaluated in the same survey (NHANES III) in 4,100 ambulatory adults age 60 years and older and a Dutch cohort of older individuals. Results from the smaller Dutch cohort suggested a threshold of 50 nmol/L for optimal function, while a threshold beyond which function would not further improve was not identified in the larger NHANES III survey, even beyond the upper end of the reference range (>100 nmol/L). In NHANES III, a similar benefit of higher 25-hydroxyvitamin D status was documented by gender, level of physical activity, and level of calcium intake.

The threshold for optimal 25(OH)D and fracture and fall prevention was assessed in a recent benefit-risk analysis and is illustrated in Figure 3. Based on these data, 75 or better 100 nmol/L (30 or better 40 ng/mL) is suggested as the optimal threshold of 25-hydroxyvitamin D for fall and fracture prevention.

![Figure 3. Threshold for optimal fall and fracture prevention based on double-blind randomized controlled trials. Data points show the relative risk of falls and the relative risk of sustaining any nonvertebral fracture from double-blind RCTs, by achieved 25-hydroxyvitamin D levels in the treatment groups. Data were extracted from two 2009 meta-analyses and summarized in a recent benefit-risk analysis of vitamin D. Based on these data, 75 or better 100 nmol/L (30 or better 40 ng/mL) are suggested as an optimal threshold of 25-hydroxyvitamin D for fall and fracture prevention. Modified from reference 39: Bischoff-Ferrari HA et al. Osteoporos Int. 2009. Dec 3. [Epub ahead of print]. © 2009, © Springer.](image-url)
er doses of vitamin D beyond 2000 IU per day are safe or require downward adjustment.43 If dietary calcium is a threshold nutrient, as suggested by Heaney,44 then that threshold for optimal calcium absorption may be at a lower calcium intake when vitamin D supplementation is adequate.

**Other potential benefits of vitamin D supplementation**

Many lines of evidence also suggest that low vitamin D status increases the risk of colon45 and possibly other cancers,46 as well as the risk of hypertension,47 myocardial infarction,48 cardiovascular49 and overall mortality,50 infections51 and diabetes.52 The development of mice lacking the receptor for vitamin D (VDR) has provided insight into the physiological role of vitamin D. These mice express phenotypes that are consistent with epidemiologic studies of 25-hydroxyvitamin D deficiency in humans.10

**Are current recommended vitamin D intakes sufficient for optimal bone and muscle health?**

The recommended intake of vitamin D as defined by the Institute of Medicine in 1997 is 200 IU per day for adults up to 50 years of age, 400 IU per day for adults between age 51 and 70, and 600 IU per day for those aged 70 years and above. These recommendations are insufficient to meet the requirements for optimal fall and nonvertebral fracture prevention. The current intake recommendation for older persons (800 IU per day) may bring most individuals to 50-60 nmol/L, but not to 75-100 nmol/L.33

Studies suggest that 700 to 1000 IU of vitamin D per day may bring 50% of younger and older adults up to 75-100 nmol/L.53,55 Thus, to bring most older adults to the desirable range of 75-100 nmol/L, vitamin D doses higher than 700-1000 IU would be needed. According to a recent benefit-risk analysis on vitamin D, mean levels of 75 to 110 nmol/L were reached in most RCTs with 1800 IU to 4000 IU vitamin D/d without risk.39 In a recent trial among acute hip fracture patients, 70% reached the 75 nmol/L threshold with 800 IU vitamin D3 per day, and 93% with 2000 IU vitamin D3 per day, at 12 months follow-up and with over 90% adherence.56

Heaney and colleagues, in a study of healthy men, consistently estimated that 1000 IU cholecalciferol per day is needed during the winter months in Nebraska to maintain a late summer starting level of 70 nmol/L, while baseline levels between 20 and 40 nmol/L may require a daily dose of 2200 IU vitamin D to achieve and maintain 80 nmol/L.54,57 These results indicate that individuals with a lower starting level may need a higher dose of vitamin D to achieve desirable levels, while relatively lower doses may be sufficient in individuals who start at higher baseline levels.

**Due to seasonal fluctuations in 25(OH)D levels, some individuals may be in the desirable range during summer months. However, these levels will not be maintained during the winter months even in sunny latitudes.55,57 Thus, winter supplementation with vitamin D is needed even after a sunny summer.**

Furthermore, several studies suggest that many older persons will not achieve optimal serum 25(OH)D levels during summer months, which suggests that vitamin D supplementation should be independent of season in older persons.50,52

Even in younger persons, the use of sunscreen or sun-protective clothing may prevent a significant increase in 25-hydroxyvitamin D levels.52

The persons most vulnerable to low vitamin D levels include older individuals,60,63 individuals living in northern latitudes with prolonged winters,58,64 obese individuals,65 and individuals of all ages with dark skin pigmentation living in northern latitudes.33,66,67 In healthy outdoor workers, naturally elevated 25-hydroxyvitamin D levels are observed: 135 nmol/L in farmers and 163 nmol/L in lifeguards. The first sign of toxicity, hypercalcemia, is only observed with serum levels of 25(OH)D above 220 nmol/L.70,71

**In summary**

Evidence from double-blind randomized-controlled trials shows that vitamin D supplementation reduces both falls and nonvertebral fractures, including hip fractures. However, this benefit is dose-dependent. According to two 2009 meta-analyses of double-blind RCTs, no fall reduction was observed at doses of less than 700 IU per day, while a higher dose of 700 to 1000 IU vitamin D per day reduced falls by 19%.12 Similarly, no fracture reduction was observed for a received dose of 400 IU or less per day, while a higher received dose of 482 to 770 IU vitamin D per day reduced nonvertebral fractures by 20% and hip fractures by 18%. Of note, the antifracture benefit was present in all subgroups of the older population and was most pronounced among community dwellers (~29%) and those age 65 to 74 years (~33%).

Fall prevention and nonvertebral fracture prevention increased consistently and significantly with higher achieved 25-hydroxyvitamin D levels in the 2009 meta-analyses. Fall prevention started at 25-hydroxyvitamin D levels of 60 nmol/L,12 while at least 75 nmol/L is required for nonvertebral fracture prevention.13 Optimal fall and fracture prevention was observed with 25-hydroxyvitamin D levels of close to 100 nmol/L.9 Given the absence of available data beyond this beneficial range, these recent meta-analyses do not preclude the possibility that higher doses or higher achieved 25-hydroxyvitamin D concentrations may be even more effective in reducing falls and nonvertebral fractures.
References

41. Aurier P, Gandhi S. Vitamin D supplementation and total mortality: a meta-analysis.

Contribution of vitamin D to bone health – Bischoff-Ferrari

MULTIPLE CONNECTIONS: NEW CONCEPTS IN BONE HEALTH

MEDICOGRAPHIA, Vol 32, No. 4, 2010 389
Les chutes constituent la principale cause des fractures, et leur fréquence augmente avec l’âge et la diminution de la résistance ou de la fonction musculaire. En outre, après une première chute, environ 30 % des personnes développent une crainte de tomber, et par conséquent restreignent leurs activités et souffrent d’une diminution de leur qualité de vie. Par conséquent, les bénéfices de la vitamine D sur le plan de la prévention des chutes et des fractures ont des conséquences cliniques significatives, d’autant plus qu’un nombre croissant d’études épidémiologiques indiquent l’existence de liens entre une carence en vitamine D et l’augmentation du risque de cancers, notamment du côlon, ainsi qu’avec le risque d’hypertension, d’infarctus du myocarde, de mortalité cardio-vasculaire et globale, d’infections et de diabète. Plusieurs méta-analyses récentes ont porté sur le bénéfice de la vitamine D sur la réduction des fractures, avec des résultats contradictoires. Cet article commence par passer en revue les résultats d’études randomisées et en double aveugle portant sur une supplémentation orale en vitamine D et son efficacité dans la prévention des fractures. Il traitera ensuite des raisons pour lesquelles les méta-analyses utilisant d’autres approches, notamment des études ouvertes ou des études ayant exploré l’administration intramusculaire de vitamine D, ont montré des résultats discordants. Enfin, puisque la vitamine D module le risque de fractures de deux façons, en diminuant les chutes et en augmentant la densité osseuse, nous discuterons de l’efficacité de la vitamine D sur la prévention des chutes, ainsi que la concentration optimale de 25-hydroxyvitamine D permettant d’obtenir ces bénéfices.
Osteoporosis (OP) and osteoarthritis (OA) are two major health burdens in our modern societies. Both are complex musculoskeletal diseases and although OA affects different tissues, they both affect bone. Although these diseases affect more women than men and were suggested to be mutually exclusive, mechanisms leading to them may overlap. Indeed, a number of factors involved in OP pathophysiology also seem to be involved in OA subchondral bone; however the mechanisms may be different in both conditions. The present review explores these two diseases from a perspective of how bone/subchondral bone tissue is modified, and which mechanisms could be responsible for the alterations.

Pathophysiology of osteoporosis

According to the World Health Organization, osteoporosis (OP) is the most common metabolic bone disorder.\(^1,2\) Osteoporosis is characterized by low bone mass due to an imbalance in favor of bone resorption over bone formation, leading to altered bone remodeling. Indeed, OP is not solely the result of bone loss since bone loss occurs in both women and men with age, and the failure to attain an optimal (ie, peak) bone mass during childhood and adolescence is one of the most important factors leading to OP without any evidence of accrued bone loss. There are abnormalities in the amount and architecture of bone tissue leading to altered strength and an increased susceptibility to fractures.\(^3\) Risk factors for postmenopausal OP include, in addition to female gender, ethnicity with white women being more affected than any other group, estrogen deficiency, repeated fractures during adult life and/or in first-degree relatives, low body weight or low body mass index (BMI), smoking, and use of oral corticosteroid therapy for more than 3 months.\(^4,5\)

At the tissue level, OP can be described as a thinning of bony rod-like trabeculae due to the net loss of calcium and bone structure, eventually leading to overt perforation. This is due to an imbalance in the sequence of events between bone resorption and bone formation. Each sequence is composed of a bone resorption period that cre-
ates resorption cavities, followed by osteoblast activation and formation of the osteoid matrix filling the cavity. Upon completion, the osteoblasts are embedded in the matrix and they become osteocytes.

During physiological bone remodeling, there is a close relationship between cortical and trabecular bone. Chemical/mechanical factors permitting the cortical and trabecular bone to adapt to each other control the expansion of the cortical compartment downregulating the cancellous bone compartment and vice-versa. These mechanisms appear to fail during post-menopause, with aging, and obviously in OP women, leading to higher mechanical constraints imposed on the skeleton, loss of bone mineral, and microstructural deteriorations.

Bone remodeling occurs through osteoblast activity for bone formation via the synthesis of bone matrix, and through osteoclast activity for the degradation of bone matrix. The equilibrium between the activities of these two cells maintains the mineral homeostasis. Osteoblasts, which synthesize the protein matrix, originate from local mesenchymal stem cells (MSCs). These cells form a layer of organic matrix called osteoid, whose thickness depends on the time interval between matrix formation and its subsequent calcification. The plasma membrane of the osteoblast is rich in alkaline phosphatase, which initiates the bone mineralization process. Later in the process, osteoblasts are progressively transformed into osteocytes; they become flat lining cells and are embedded in an organic bone matrix, which becomes mineralized. Osteocytes have long cell processes that form thin canaliculi, which connect them to each other as well as to active osteoblasts and flat lining cells, and carry circulating bone extracellular fluid. Osteoclasts are giant multinucleated cells originating from stem cells of the mononuclear/macrophage lineage and are responsible for bone resorption. Parathyroid hormone (PTH), 1,25-dihydroxyvitamin D3 (calcitriol), sex hormones, and cytokines such as tumor necrosis factors (TNFs) and interleukins (ILs) within the bone marrow control the formation of osteoclasts.

During OP, the osteoclast removes more bone than the osteoblast is able to form, which translates into a reduction in total bone mass. Indeed, less osteoid matrix is produced in OP bone due to both a reduced organic matrix and less inorganic content.

Pathophysiology of osteoarthritis
Osteoarthritis (OA) has been characterized by progressive articular cartilage loss and osteophyte formation. Despite major progress in the last few decades, we still have much to learn about the etiology, pathogenesis, and progression of this disease. The slowly progressive and multifactorial nature of OA, its cyclical course, where a period of active disease is followed by a period of remission, has limited our comprehension of this disease. Although OA was long considered to be due only to an imbalance between loss of cartilage and an attempt to repair cartilage matrix, it is now known that OA, at least in the knee, is a heterogeneous disease involving all the articular tissues including cartilage, subchondral bone, menisci, and periarticular soft tissues such as the synovial membrane. Synovitis is often present and is considered to be secondary to the alterations in other joint tissues. Yet, findings indicate that synovial inflammation could be a component of even the ear-
ly events leading to the clinical stage of the disease. In addition, emerging evidence suggests that changes in subchondral bone and menisci are closely involved in the disease progression.

Subchondral bone is suggested to be the site of the causally most significant pathophysiological events occurring in cartilage (Figure 2A). Although OA is not considered a generalized bone metabolic disease, data suggest that the subchondral bone alterations may precede cartilage changes. Indeed, it was long believed that OA subchondral bone underwent only appositional new bone formation and sclerosis; however, it is now understood that there are also phases of resorption in this diseased tissue. Inasmuch as early bone resorption features can be observed in OA, patients with progressive knee OA show increased indices of bone resorption, whereas, in general, nonprogressing OA patients do not show altered resorption.

Of importance, subchondral bone plate and trabecular bone do not show the same architecture or the same abnormal cell metabolism during OA. Indeed, as indices of bone resorption indicate loss of trabecular tissue, the increase in collagen type I cross-linked N-telopeptide (NTX) and C-telopeptide (CTX) observed in subsets of OA patients suggests a progressive loss of trabecular bone, not subchondral bone, which actually shows sclerosis. In addition, in those patients showing sclerosis, recent evidence using microcomputed tomography (microCT) indicates that bone sclerosis is due to an altered microarchitecture of the bone with trabeculae showing more plate-like structures than rod-like structures.15,16 Such alterations in the microarchitecture of bone tissue would also likely alter bone stiffness.

Morphologically, one of the hallmarks of knee OA is the presence of bone marrow lesions (BMLs) consisting of edema-like lesions and cysts in subchondral bone as seen with magnetic resonance imaging (Figure 2B). These BMLs were found to be strong indicators of bone turnover indices as well as progressive structural changes in knee OA patients. Moreover, BMLs are associated with poorly mineralized sclerotic bone tissue in OA patients.

### Osteoporosis vs osteoarthritis

The prevalence of both OP and OA is higher in women than men. Risk factors for OA include age, gender (female), genetic predisposition, mechanical stress and/or joint trauma, and obesity (high BMI). Some of these risk factors are also associated with OP, yet the opposite weight conditions in the two diseases and the presence of fractures in OP vs OA are some of the conditions that distinguish the two diseases (Table I).

Although it is well established that in OP the low bone mass is due to an imbalance in favor of bone resorption over bone formation (Figure 3, page 394), new hypotheses about OA pathophysiology have been put forward. Hence, OA was re-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Osteoporosis</th>
<th>Osteoarthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineral density</td>
<td>Reduced</td>
<td>Increased</td>
</tr>
<tr>
<td>Bone mineral content</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Mineralization</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Osteoid matrix</td>
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<td>Type I collagen α1/α2 ratio</td>
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<td>Yes</td>
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</table>

Table I. Comparison of osteoporosis and osteoarthritis bone parameters.
cently suggested to be related to an inappropriate attempt at subchondral bone formation leading to cartilage remodelling/degeneration and synovitis. Moreover, Aspden proposed an alternative theory, in which OA could be a pathological growth, not decay, problem showing excessive and poorly regulated growth of musculoskeletal tissues, with cells possibly reverting to an abnormal developmental phenotype with a loss of proper function. Hence, (a) mechanism(s) leading to normal tissue formation could be altered in such a way that tissue integrity is never attained. However, although the latter hypothesis is very attractive and deserves consideration, many questions still remain to be answered.

Another interesting thought is that, as bone resorption is now considered to be centrally controlled via leptin, an adipokine produced by adipocytes that plays a role in bone homeostasis and is locally modulated by adrenergic β2 receptors in osteoblasts, this regulation via leptin may be a key element, whereas leptin levels are different in OP and OA patients.

**Morphological level**
Compared with OP, which is a systemic skeletal disorder characterized by a decrease in BMD with alterations in bone microstructure and a reduction in the bone mineral component, OA does not seem to be a systemic bone disorder, as it shows increases in BMD, yet reduced bone mineral content and increased osteoid, as well as alterations in subchondral bone microstructure. In this disease, the progression of joint cartilage degeneration is associated with intensified remodeling of the subchondral bone and increased subchondral bone stiffness, whereas in OP bone remodeling and bone stiffness decrease.

A number of studies suggest that OA patients should have better bone mass. Indeed, data revealed that these patients have a better preserved bone mass, and primary OA and OP rarely coexist. Indeed, hip and spine BMD were found higher in women with radiographically defined knee OA. However, low hip BMD levels have also been associated with a greater risk of progression of OA, and a significant percentage of women with OA undergoing hip replacement met the criteria for OP. Furthermore, there is an association between osteophytes and the pathophysiology of OA, whereas osteophytes are not observed in OP.

In addition to thicker trabeculae, trabecular microfractures are also observed in OA bone tissue at a greater frequency, especially in the hip. This in turn could lead to BML formation, as such lesions may be the result of microfractures. Healing of microfractures in OA subchondral bone could generate a stiffer bone, which is no longer an effective shock absorber. Conversely, subchondral bone stiffness may be part of a more generalized bone alteration leading to an apparent increase in BMD or volume. However, subchondral bone thickening reflects osteoid volume increases, but not necessarily an increase in this tissue’s mineralization. In the knee, BMLs have not been reported in OP, yet in the hip they can be observed in both OP and OA. Stiffness and BMD are not uniform in OA subchondral bone.

The bone closest to the articular cartilage has the greatest effect on cartilage integrity, with variations in stiffness and BMD probably causing more damage to cartilage than any other parameters. Although OA is associated at a later stage with a thickening of subchondral bone as opposed to a progressive thinning of bone in OP, explants of the femoral head of OA patients at autopsy showed a low mineralization pattern compared with normal. Hence, the apparent increase in BMD in OA may be due to an increase in material density, not an increase in mineral density. Indeed, bone tissue mineralization in OA has been reported to be lower than normal (Figure 4) and, very surprisingly, even lower than in OP. Although there is an increase in type I collagen production, the
undermineralization could be related to an abnormal increase in the ratio of type I collagen $\alpha_1$ to $\alpha_2$ chains in OA compared with normal tissue.\cite{48,49} Indeed, data showed a 2- to 3-fold increase in the expression of COL1A1 chains of type I collagen, with no variations in COL1A2 expression in OA subchondral bone osteoblasts, leading to an increase in the production of type I collagen $\alpha_1$ chains. Together with the reduced number of crosslinks in OA bone tissue,\cite{44} this could explain the reduction in bone mineralization. OA osteoblasts also show increased levels of osteocalcin and alkaline phosphatase.\cite{50,51} Hence, both the terminal differentiation and the mineralization of OA subchondral bone osteoblasts are altered.

**Cellular level**

The hypercellularity observed in OA subchondral bone tissue may be linked with the increased rate of osteoblast proliferation observed in these cells\cite{52} or with reduced apoptosis of OA osteoblasts.\cite{52,53} In contrast, OP osteoblasts proliferate at a slower rate and show more pronounced apoptosis.\cite{54,55} Increased cell numbers and more collagen production per cell would suggest that OA individuals should have better bone mass as noted above. The molecular mechanisms locally involved in the bone remodeling process include the coupling between osteoblasts and osteoclasts. Among the factors of importance are the membrane-bound intercellular adhesion molecules-1 (mICAM-1 or CD54) and the molecular triad receptor activator of nuclear factor $\kappa$light (RANKL)/RANK/osteoprotegerin (OPG), which have emerged as essential role players, not only in bone formation, but also in bone resorption processes. Cellular interactions between osteoblasts and preosteoclasts mediated through adhesion molecules such as mICAM-1 have been recognized as important modulators of osteoclast recruitment and differentiation.\cite{56,57} RANKL, a member of the TNF ligand family and produced by the osteoblasts, binds to its specific receptor RANK on osteoclast precursors, promoting their differentiation and fusion, and eventually the formation of mature osteoclasts. RANKL also binds to RANK on the mature osteoclasts and induces their activity. OPG, also produced by the osteoblasts, is a decoy receptor that binds to RANKL, thus inhibiting osteoclastogenesis. From a clinical standpoint, studies reported progressively higher mICAM-1 levels in the synovium of OA, rheumatoid arthritis (RA), and OP patients, respectively, compared with healthy individuals, and in bone from hip or knee OA patients undergoing primary arthroplasty or patients with a hip fracture secondary to OP.\cite{58,60}

The equilibrium between OPG and RANKL also plays a crucial role in the physiology of bone.\cite{59} Under normal conditions the ratio of OPG to RANKL produced by osteoblasts favors bone formation by keeping bone resorption under strict control. In OP, the OPG/RANKL ratio decreases, favoring bone resorption by activating osteoclasts and apoptosis of osteoblasts.\cite{62,63} Currently, potential drugs for OP target a reduction in RANKL or an increase in OPG levels. In contrast to OP, ex vivo studies performed on human OA subchondral bone osteoblasts revealed two distinct subgroups of patients based on these cells’ low (L) or high (H) endogenous prostaglandin (PGE$_2$) levels,\cite{64} which otherwise demonstrate no different phenotypic features. Interestingly, differences in OPG and RANKL levels also exist between the two OA subpopulations. In short, both the L-OA and H-OA subchondral bone osteoblasts demonstrated an abnormal OPG/RANKL mRNA ratio, yet it was reduced in the L-OA, suggesting increased subchondral bone resorption, and increased in H-OA, indicating a shift toward subchondral bone formation.\cite{65} This observation was further strengthened by data showing that L-OA osteoblasts induced a significantly higher level of mature osteoclasts compared to the H-OA and higher bone resorption activity.\cite{65} Such findings suggest that each human OA subchondral bone subpopulation has reached a different metabolic state; L-OA being enriched with factors promoting bone resorption and H-OA having reduced resorptive properties, with the metabolism of the latter cells favoring bone formation. Thus, in humans, the OA subchondral bone osteoblast subpopulation could reflect different stages or attempts to repair this damaged tissue: an increase in bone resorption followed by bone formation.

Another family of signal proteins, the Wnt/LPR5 (a Wnt receptor)/$\beta$-catenin canonical signaling pathway, was also identified as a crucial role player in bone formation. Recent studies suggested the potential direct contribution of mature osteoblasts/osteocytes to the recruitment and fate of MSCs via the Wnt signaling pathway. Indeed, the control of adipogenesis, osteogenesis, and chondrogenesis in bone marrow appears to be regulated locally, at least in part, by Wnt agonists and antagonists produced by the mature osteoblast/osteocytes.\cite{66,67} Such antagonists include members of the dickkopf family (DKK1 and DKK2). Osteocytes also contribute to local control of bone resorption via the production of the Wnt antagonist, sclerostin (SOST).\cite{68}
It is proposed that the alterations in Wnt/LRP5 expression and/or activity could be implicated in the pathogenesis of OP, as this system appears to be an important transduction mechanism by which mechanical loading increases bone mass. Interestingly, recent evidence also showed that low estrogen levels diminished the skeletal response by downregulating the transcriptional activity of β-catenin. DKK-1 was suggested to be directly involved in the pathophysiology of OP, but as DKK-1 may have opposite effects on early and late osteoblast development, this could complicate the development of DKK antagonists for the treatment of OP. In addition, recent data on humans and mice delineated SOST as a compelling target for the development of OP therapeutics. With regard to OA, data on the Wnt signaling system are only emerging, and contradictory data have been published, even by the same authors. This could be due to the fact that this system does not have a similar involvement in cartilage and subchondral bone. However, this system is involved in the pathophysiological process of this disease, at least in the subchondral bone, as human OA subchondral bone osteoblasts were shown to produce abnormal levels of DKK-2 and SOST. Therefore, a strong rationale exists for therapeutic approaches that target improving bone quality in both diseases by inhibiting subchondral bone resorption and/or promoting matrix quality in subchondral bone in OA and reducing bone resorption in OP. However, therapeutics that would reduce only bone resorption would be more beneficial for the subchondral bone of the L-OA patients as this tissue is in a regenerative phase, but in the H-OA patients, anti-erosive agents are expected to be less effective since the subchondral bone was shown to be in a regenerative phase. Nonetheless, more clinical trials exploring the effects of an anti-bone remodeling agent on the evolution of OA structural changes are required.

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Osteoporosis and osteoarthritis are the common battleground – Lajeunesse and others


Multiple Connections: New Concepts in Bone Health


Keywords: osteoporosis; osteoarthritis; osteoid matrix; mineralization; microfracture; bone marrow lesion; bone stiffness; bone remodeling; bone turnover; subchondral bone

L’OSTÉOPOROSE ET L’ARTHROSE : L’OS, UN CHAMP DE BATAILLE

L’ostéoporose (OP) et l’arthrose (OA) sont deux maladies dont la prévalence est importante dans nos sociétés modernes. Ces dernières appartiennent au système musculosquelettique et malgré le fait que l’OA affecte plusieurs tissus de l’articulation, toutes deux présentent des altérations osseuses. Bien que ces maladies affectent plus les femmes que les hommes et ont été suggérées de s’exclure mutuellement, certains mécanismes responsables de ces dernières sont similaires. Ainsi, de nombreux facteurs impliqués dans la physiopathologie de l’OP semblent être présent au niveau de l’os sous-chondral lors de l’OA, cependant les mécanismes sous-jacents apparaissent être différents pour chacune de ces pathologies. La présente revue explore ces deux maladies, discute des facteurs impliqués dans l’os/os sous-chondral et des mécanismes responsables de leur altération.
What is the goal of antiosteoporotic therapy: improve bone health or only prevent fractures?

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Osteoporosis is a major public health concern in adults over age 55, resulting in billions of euros/dollars in costs. Over the past 20 years, antiresorptive drugs have been the treatment of choice for osteoporosis. Most of these drugs are derived from the bisphosphonate molecule. Large, placebo-controlled trials generally show that these drugs can indeed increase bone mineral density (BMD) and reduce the risk of vertebral, hip, and other nonvertebral fractures in women with osteoporosis—at least in the short run. The main potential problem is that antiresorptive drugs not only directly—and unnaturally—inhibit osteoclastic bone resorption, they also indirectly inhibit the flip side of the bone-building coin, osteoblastic bone formation. What does this mean for bone health in the long term? This is a crucial question, because there is no such thing as short-term treatment with these drugs.

Bone remodeling is a physiological process that replaces old bone with new and preserves the mechanical integrity of the skeleton. During aging, an increase in the rate of remodeling is observed, together with incomplete filling of individual bone remodeling units by osteoblasts, resulting in bone loss and increased risk of fractures. Most treatments for osteoporosis act predominantly by inhibiting the osteoclasts, hence decreasing bone resorption. While clinical trials, generally performed over 3 years, have shown these drugs to be effective in reducing fractures, concerns have been expressed about the potential for long-term suppression of bone remodeling to produce adverse effects on bone strength and fracture risk. Recent reports of atypical fractures in patients receiving bisphosphonates, the most commonly used treatment for osteoporosis, have attracted much attention in this respect.

During the past few years, remarkable advances in molecular biology and genetics have led to deeper understanding of the bone remodeling cycle and the implications with regard to this biologic process for the concept of bone quality. Bone quality is difficult to define and includes aspects such as toughness, strength, resistance to failure, load-bearing capacity, etc. More recent definitions include a number of aspects that are part of a single concept that includes bone intrinsic material properties, bone remodeling, bone microarchitecture, and bone mass. This has led to the definition of new therapeutic targets. New drugs have or are being developed, which reduce the risk of fracture in patients with osteoporosis and, at the same time, seek to improve structural and material parameters of bone quality. This ultimately translates into enhanced bone health and long-term efficacy and safety.

Strontium ranelate (SR) is a novel antiresorptive agent approved for the treatment of postmenopausal osteoporosis that appears to be going in the right direction. In contrast to other available treatments for osteoporosis, SR induces antiresorption and bone-forming effects. SR reduces bone resorption by decreasing osteoclast differentiation and activity, and stimulates bone formation by increasing replication of preosteoblast cells, leading to increased matrix synthesis. It is suggested that strontium ranelate exerts its dual mechanism of action, at least in part, through the calcium-sensing receptor (CaSR), thereby activating osteoblastic cell replication, and by reducing osteoclastogenesis and bone resorption through the modulation of the RANKL/OPG ratio (= receptor activator of nuclear factor-kappaB ligand/osteoprotegerin ratio). Preclinical studies have shown that this dual effect results in increased bone mass and improves bone microarchitecture and strength. In clinical trials, strontium ranelate reduces vertebral fractures in women with osteopenia, osteoporosis, and severe osteoporosis. Reduction in nonvertebral and hip fractures has been documented in elderly subjects with low femoral density. Histomorphometry and microcomputed tomography (mCT) of bone biopsies from these osteoporotic patients have also highlighted the capacity of SR to promote bone quality and improve bone microarchitecture and strength.

In summary, we are now looking to drugs that are real bone health builders and not only bone hardeners.

References
Antiresorptive therapy seeks to prevent fragility fractures and improve bone quality. While the effects of available antifracture treatments on fracture risk have been relatively well established, the effect of many of them on bone quality is relatively unknown. Current agents used in the treatment of osteoporosis are classified either as antiresorptive or bone-forming agents. Thus, their mechanism of action involves only one of the aspects of bone remodeling.

Antiresorptive drugs, particularly bisphosphonates, reduce bone turnover, resulting in an increase in bone mineralization and homogeneity of mineralization. It is suggested that most of the change in bone mineral density induced by antiresorptive agents is a consequence of the increase in mineralization. Aging also increases bone mineralization, like antiresorptive therapy, which seems contradictory. Greater mineralization seems to be beneficial, at least up to a certain extent, since excessive mineralization may result in poor bone quality. There is concern that prolonged therapy with bisphosphonates leads to oversuppression of bone remodeling and overmineralization of bone. This results in impaired ability to repair microfractures and increased bone fragility. Increased rates of microfractures have been reported in dogs treated with high doses of bisphosphonates. Although this finding does not appear to be common among postmenopausal women with osteoporosis treated with bisphosphonates, increased numbers of cases with atypical subtrochanteric femur fractures have been reported under bisphosphonate therapy. Awaited data on the material properties of bone and data on the prevention of fractures after long-term bisphosphonate therapy should help clarify this issue. Bone-forming agents, such as parathyroid hormone, reduce fracture risk by stimulating the formation of new bone and increasing bone turnover in favor of bone formation, thus increasing bone mass and improving bone architectural properties, and by reducing fracture rates. Parathyroid hormone also influences bone mineralization, leading to decreased mean mineralization of bone and increased heterogeneity of mineralization.

Strontium ranelate has been shown to be effective in reducing the risk of vertebral and nonvertebral fractures, including hip, in postmenopausal women with osteoporosis. In contrast to other available treatments for osteoporosis, strontium ranelate induces a dual effect on bone resorption and formation: it increases bone formation and reduces bone resorption, thereby rebalancing bone remodeling in favor of bone formation. In addition to its effect on fracture reduction, strontium ranelate has also been shown to improve bone quality. Bone biopsies obtained from both the SOTI (Spinal Osteoporosis Therapeutic Intervention) and TROPOS (Treatment Of Peripheral OSteoporosis) studies have shown that patients treated with strontium ranelate have a significant increase in trabeculae number, a significant decrease in trabecular separation, and a significant increase in cortical thickness when compared with placebo.

Although antiresorptive agents such as bisphosphonates also increase mean bone volume and preserve trabecular microarchitecture, they have no effect on cortical bone. On the other hand, bone-forming agents such as strontium ranelate and parathyroid hormone improve trabecular microarchitect and increase cortical thickness. While strontium ranelate has a positive effect on bone quality, mean bone mineralization remains unchanged, regardless of dosage and duration of treatment.

In conclusion, the aim of antosteoporotic therapy should be not only to prevent fractures, but also to improve bone quality. With its unique dual mode of action, strontium ranelate both improves bone health and prevents fractures, and should be considered as a first-choice treatment in the prevention of osteoporotic fractures.

References
What is the goal of antiosteoporotic therapy: improve bone health or only prevent fractures?

The goal of any treatment for osteoporosis is to improve bone strength, thereby decreasing fracture risk. In the past several years, a number of therapies have been developed that are effective in achieving this goal, but do not treat bone loss. These therapies, eg, the bisphosphonates, largely target bone remodeling and increase bone mass by significantly suppressing bone resorption and also bone formation, resulting in an overall suppression of bone turnover.

Another approach has been to stimulate bone formation and decrease bone resorption, resulting in an overall stimulation of bone turnover, by using anabolic agents such as parathyroid hormone, fluoride, and, recently, strontium ranelate.

These two diametrically opposed ways of treating osteoporosis (the antiresorptive and the anabolic approaches) have been shown to significantly decrease the risk of fracture by improving the mechanical properties of bone.

Antiresorptive treatment avoids the elimination of bone that should be reabsorbed chiefly because it is no longer functional (ie, bone that is not deformed as usual by mechanical usage, because of the presence of microcracks), though they may protect some mechanically useful elements, too. Among these agents, bisphosphonates have recently been associated with atypical femoral shaft fractures in long-term treated patients, which could be a consequence of excessive overall bone remodeling suppression.1

In addition, bisphosphonates seem to improve some little-known aspects of the mechanical quality of bone tissue. In some cases, the positive effects eventually produced on bone architecture could be optimized, provided that the drug has a positive interaction with the bone’s mechanostat, and the mechanical stimulation of that system is maintained through adequate control of the patient’s physical activity. The impact of the positive effects of some of these treatments on bone strength does not necessarily correlate with the relatively small improvements (if any) in densitometric bone mass.

The fact that current antiresorptive therapeutic agents produce only modest increases in bone mineral density would appear to stress the need for anabolic strategies, in order to produce larger increases in bone mass and strength. One such strategy is intermittent treatment with anabolic agents such as parathyroid hormone (PTH) and sodium fluoride.

Anabolic treatments enhance bone mass chiefly by inducing peritrabecular apposition, with small evidence (if any) of improvement in bone architectural design. Some of these agents may even deteriorate the mechanical quality of bone material because of crystal contamination (fluoride) or excessive haversianization (PTH).2

Strontium ranelate, for its part, possesses a novel and unique dual mode of action, which rebalances bone turnover in favor of bone formation. It activates the calcium-sensing receptor, and increases the expression of osteoprotegerin, while decreasing RANKL (receptor activator of nuclear factor-kappaB ligand) expression by the osteoblast. In addition, micro-CT analysis of bone biopsies from strontium ranelate–treated patients has evidenced an improvement in intrinsic bone tissue quality, as shown by an increase in trabecular number, a decrease in trabecular separation, a lower structure model index, and an increase in cortical thickness.3

Our growing knowledge of the cellular and molecular pathways involved in the maintenance of bone homeostasis and of the disturbances in these pathways caused by osteoporosis has permitted better understanding of the mechanisms through which antiosteoporotic agents work, and opens up perspectives for the development of ever-more effective therapeutic options.

References
The primary aim of osteoporosis therapy is the prevention of fragility fractures in patients at increased risk of fracture. The ability of a drug to significantly reduce fracture risk is judged by comparison versus placebo over a 3-year period. Such randomized placebo-controlled trials have become the golden standard of regulatory approval and prescriber and consumer acceptance. Although this approach was acceptable in the initial registration of new modalities in bone therapeutics, it can be questioned presently for various reasons.

The ethics of conducting placebo-controlled trials are challenged in view of the availability of several approved antifracture agents. This may have a negative impact on the procedure for registration of any new agents. Also, agents registered under the present rules can be questioned regarding the effects on bone health over periods longer than 3 years.

The longest placebo-controlled antifracture data available are for alendronate (4 years) and strontium ranelate (5 years). Even here where the data exceed the compulsory 3 years, interpretation of data beyond 3 years is fraught with statistical pitfalls. Thus, smaller numbers in both the placebo and treated groups compound covariates that influence fracture outcomes in not being equally distributed in the remaining population. Also, the removal of subjects from the study after fractures usually involves more patients from the placebo group, which may leave patients at lower risk of fracture in the placebo group. It is unlikely that any antifracture study will extend the placebo arm beyond 5 years. Typically, studies longer than 5 years drop the placebo group and compare fracture incidence over periods of time to detect a trend of sustained efficacy. The numbers involved in these extension studies become small and the ability to detect changes in efficacy is compromised.

Why is it important to know the effect of drugs on bone health for periods longer than 3 to 5 years? Clinicians are treating patients for longer than 5 years in view of lack of evidence as to the optimal duration of treatment. Patients are being treated from a younger age because of increased osteoporosis awareness and wider availability of diagnostic tools such as dual x-ray absorptiometry (DXA) and integrated risk factor tools. In the UK, the lower price of generic alendronate has liberalized intervention thresholds. All these factors, combined with an ever-increasing life expectancy, increase the likelihood that patients will be exposed to antifracture drugs for periods exceeding 5 years.

The possibility of sustained long-term suppression of bone turnover causing poor bone health has been raised by recent observations. Alendronate-induced osteonecrosis of the jaw is an example of a possible negative influence on bone health by a drug with proven fracture efficacy when given over longer periods of time at higher dosages. Atypical low-trauma subtrochanteric fractures have likewise been implicated as the result of long-term effects of bisphosphonates, although this has not been proven.

It is clear that long-term bone health in patients on antifracture therapy is of cardinal importance. Available diagnostic tools for this purpose are limited. Biochemical markers of bone turnover, DXA, and ultrasound have limited application. Transiliac bone biopsies yield more information, but are limited by technical and practical considerations.

It is thus with great interest that the study of bone microstructure and changes induced by drugs over time as recorded by high-resolution peripheral computed tomography (HR-pQCT) on the radius and tibia is followed. This in vivo technique is noninvasive and produces brilliant images of the trabecular and cortical structures. The technique is presently being limited by cost, availability, restriction to peripheral sites, and limited validation of the outcomes measured. It is the opinion of the author that development of HR-pQCT and other techniques will lead to new insights into the effect of drugs on long-term bone health. This will complement knowledge of antifracture efficacy in determining not only the choice of drug, but also the duration of treatment in osteoporosis based on bone health.

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5. J. Lains, Portugal

At first glance, this definition of osteoporosis implies that the goal of any antiosteoporotic treatment is to decrease fracture risk by increasing bone resistance. However, bone resistance is dependent on its health and quality, and bone quality is dependent on its architecture, degree, and age of mineralization, and the accumulation of damage. In turn, all of these depend on turnover and the possibility of maintaining the youth of all the components of bone tissue.²

All antosteoporotic drugs act on bone turnover, on the activation frequency of the BMU, either by inhibiting resorption (estrogens, bisphosphonates, calcitonin, and raloxifene), or by stimulating formation (parathormone [rhPTH 1-84] and its fragment [rhPTH 1-34]), or by a simultaneous dual action resulting in stimulation of bone formation and inhibition of bone resorption (strontium ranelate).³ Most probably, these drugs act not only on bone mineral density (BMD), but also on bone quality. Again most probably, it is not a coincidence that osteonecrosis of the jaw and a particular type of fractures in the shaft of the femur are reported with (prolonged) use of antiresorptives, perhaps in relation with the inhibition of bone turnover, leading to the so-called “frozen bone.” In contrast, drugs promoting bone formation are proven to ameliorate microarchitecture.² Interestingly, to my knowledge, there is no published article or research mentioning any negative interference with bone health with strontium ranelate.

To conclude, when considering treatment with an antosteoporotic drug, we should take into account both bone safety and bone health, and not only the prevention of fractures.

References
Bone is a specialized connective tissue endowed with three functions: mechanical, protective, and metabolic. Bone development and function are dictated by the activity of the osteoblasts and osteoclasts. These include growth, modeling, repair, and remodeling. Bone remodeling is a renewal process geared to removing damage in order to maintain bone strength. This cellular machinery is effective during the period of adolescence, but fails with advancing age as the remodeling balance grows negative. The crucial window for bone accrual during the third decade of life and the critical transition of postmenopausal bone loss are key determinants of skeletal mass in the elderly. However, bone strength—the maximal load that can be applied before a fracture occurs—is also influenced by factors other than bone mass. For instance, sex differences in bone width with greater periosteal bone formation in boys and higher endocortical apposition in girls result in a wider bone in boys, conferring greater resistance to bending. Bone tissue quality, which is related to the degree of bone mineralization and the characteristics of bone matrix also exerts important role in determining bone strength.

The triad of antiosteoporotic therapy includes: (i) enhancing peak bone mass during adulthood; (ii) preventing bone loss after menopause; and (iii) preventing falls in the elderly. Most antiosteoporotic medications used in advanced age to prevent bone loss can be categorized into three main groups: antiresorptives, bone-formative agents, and “the others.” Most of the available antiosteoporotic drugs, particularly the bisphosphonates, have been shown to exert their antifracture efficacy by retarding osteoclast maturation and inhibiting the cascade of resorbing activities. Bone-formative agents, which are fewer in number, play a greater role in osteoblastic bone formation, in particular intermittent parathyroid hormone (PTH).

Strontium ranelate, a recently developed agent, claims a dual action on bone resorption and bone formation. Vitamin K2 is a key coenzyme critical for the maturation of osteocalcin, which seems to play a crucial role in osseous and nonosseous systems.

Fractures have devastating consequences in terms of physical, economic, and psychosocial outcomes. One of the major goals of antosteoporotic therapy is to prevent fractures in order to minimize morbidity and maximize quality of life. However, bone health is an issue that goes far beyond the quantifiable repercussion of fractures, since bone functions are multiple. In addition to antifracture efficacy, long-term safety should be taken into account when considering antosteoporotic therapy. The risk-benefit ratios of the short- and long-term safety and efficacy outcomes of each treatment option should be thoroughly examined. This would include the cardiovascular and cancer risks in elderly users of hormone replacement therapy, the long-term risk of cerebrovascular accident in raloxifene users at high cardiovascular risk, the unresolved issue of osteonecrosis of the jaw in patients using high-dose intravenous bisphosphonates, and the frozen bone debate around the long-term use of bisphosphonates.

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“The doctor has been taught to be interested not in health, but in disease. What the public is taught is that health is the cure for disease.”
Ashley Montagu

Osteoporosis is the paradigm of impaired bone health, as it is a condition of reduced bone mass and impaired bone architecture caused by perturbed bone physiology (bone remodeling), resulting in bone fragility and fracture. Several classes of antiosteoporotic treatment are available, and their effects on the determinants of bone strength differ—hence the potential conflict raised in this question.

A bone that fractures in a low-trauma injury is “fragile.” Can we recognize, and therefore treat, impaired bone health prior to this first fracture? Our most reliable single tool is the measurement of areal bone mineral density (BMD) in the hip and spine. However, the majority of those who fracture have a normal or only modestly impaired BMD.

Bone architecture is assessed on bone biopsy or high-resolution imaging, which is certainly not applicable clinically. Data suggest that BMD loss may explain only 20% to 30% of the microarchitectural deterioration seen in an osteoporotic population with prevalent fractures. Clinical risk factors can predict the risk of fracture independently of BMD measurements, and the presence of these BMD-independent risk factors correlates with the deterioration in measures of bone microarchitecture. Finally, bone turnover markers also support more accurate prediction of fracture than BMD alone. The purpose of having thus identified impaired bone health is to prevent fragility fractures. While it is logical to assume that improving bone health will do so, this may be a fallacy—an improvement in bone health is a means to an end, not an end in itself. This does not preclude the argument that a treatment that best restores bone health should be the preferred choice when fracture risk reduction is comparable.

Each of the existing treatment options alter one or more determinants of bone strength: (i) tissue properties, such as hardness, maximal strength; (ii) bone architecture such as trabecular number, thickness and connectivity, cortical porosity, trabecularization, and transformation between plate and rod-like trabecular structures; and (iii) dynamic measures such as mineral apposition rate, activation frequency, and resorption surfaces.

Interpretation of these comparative data is complex—for instance, while raloxifene has the most pronounced effects on tissue quality, as assessed by nanoindentation, teriparatide reduces tissue hardness in trabecular bone, but has the greatest effect on bone volume. Bisphosphonates increase stiffness, but not hardness, and do not alter bone volume significantly. The significance of these findings can only be interpreted in regard to the clinical utility of these treatment options, ie, in their ability to protect a patient from fracture.

Strontium ranelate is notable in adjusting bone formation and bone resorption in a way that most closely resembles pre-osteoporotic bone health, with desirable effects on trabecular and cortical bone and without adverse effects on tissue quality such as stiffness. This allows a restoration of bone quality, mineral properties, and, most crucially normal bone remodeling.

To paraphrase the architect Leon Battista Alberti, this treatment can “adjust all the parts proportionally so as not to impair the harmony of the whole,” achieving the combined, not conflicting goals of reduced bone fragility through optimizing every aspect of bone health.

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The principal functions of the skeleton are mechanical support, maintenance of calcium homeostasis, and hematopoiesis in the bone marrow. These functions can be disturbed in a variety of metabolic bone diseases of which osteoporosis is the commonest. Metabolic bone disease is a rather loose term that encompasses diseases of bone in which abnormal bone remodeling results in a reduced volume of mineralized bone and/or abnormal bone architecture. These processes in turn usually give rise to an increased risk of fracture. For this reason, the most important complication of osteoporosis is often considered to be fracture, although other manifestations can have significant effects on patient quality of life.

Osteoporotic fractures result from a combination of decreased bone strength and increased incidence of falls. Bone mineral density (BMD), because it is easy to measure and has an excellent precision, was initially the favored end point in most clinical trials and remains the best noninvasive assessment of bone strength available in routine clinical practice. Prevention of fractures subsequently became the more relevant end point for clinical trials with a view to satisfying regulatory authorities about the efficacy of a particular drug.

However, it is now recognized that bone strength (and hence fracture risk) depends on many properties including the shape and size of the bone as well as the strength of the material inside. Material strength is influenced by architectural abnormalities and microdamage as well as BMD. Assessment of these other end points (often referred to as "bone quality") in the past, is now considered a more appropriate reflection of overall bone health. Architectural abnormalities occur particularly in the trabeculae of vertebral bodies. A loss of trabecular connectivity (density of connections between trabeculae) especially with horizontal loss, results in increased loads on remaining trabeculae resulting in a weakened structure. Loss of trabecular connectivity has been demonstrated in individuals with vertebral crush fractures compared with controls, even when matched for bone volume. Prior fracture, an independent risk factor for further fracture, may reflect these existing architectural abnormalities. Measurement of microarchitecture is possible in the research setting, but is more problematic in clinical practice. Nevertheless, it is now considered an important end point in all recent major trials of antiosteooporotic therapies.

Biochemical bone markers have also been used as intermediate end points in most recent major studies of antiosteoporotic therapies. Bone resorption markers, in particular, may add an independent, predictive value to the assessment of bone loss and fracture risk. There are also potential advantages in monitoring antiosteoporotic treatment in the short term in addition to bone densitometry, to more quickly identify nonresponders to therapy, or noncompliance.

To summarize, while the clinical goal of antiosteoporotic therapy is to prevent fractures, understanding the mechanism of action of such benefit to the skeleton is enhanced when measures of bone health that include not just BMD, but also bone turnover and microarchitecture are included in trial endpoints.

References

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Estrogen depletion in postmenopausal women induces a rapid decrease in bone quality, bone quantity, and consequently, bone strength, resulting in an increase in fracture risk. Treatments addressing osteoporosis must be judged not only on their ability to decrease the risk of fractures, but also to improve the bone status of osteoporotic women and ensure efficacy and long-term protection, whatever the patient profile, including those patients most difficult to treat. Protelos (strontium ranelate) is an antosteoporotic agent indicated in the prevention of vertebral and hip fracture risk in postmenopausal women with osteoporosis. The efficacy of Protelos actually goes beyond this definition inasmuch as its range of efficacy extends from preventing fracture risk in osteopenic patients to those with the most severe forms of osteoporosis, and from the youngest patients to those most advanced in years. Protelos is the only among all other antosteoporotic agents with proven long-term efficacy against vertebral, nonvertebral, and hip fractures over 5 years, as established by a randomized, double-blind controlled trial. This efficacy is acknowledged by the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO), which published, in 2008, a European Guidance that ranked Protelos as a first-line treatment with proven efficacy in reducing the risk, not only of vertebral fractures, but also of nonvertebral fractures, among which specifically hip fractures. Protelos’s unrivalled efficacy results from its ability to build new bone and improve bone health in osteoporotic women. Three studies have shown that Protelos consistently improves both cortical and trabecular bone, which are the main determinants of hip and vertebral fractures, respectively. In osteoporotic women, the improvement in bone architecture with Protelos is already significant after 1 year of treatment and is maintained over 5 years. Protelos builds strong and healthy bone and provides full protection against fracture risk in osteoporotic postmenopausal women. Protelos heralds a new therapeutic approach that fills the bill as first-line treatment in the management of osteoporosis.

Better bone health for osteoporotic patients: Protelos decreases fracture risk and improves bone quality

by P. Halbout, France
was not effective enough to ensure a satisfactory outcome of the management of osteoporosis. First, these agents failed to provide complete efficacy in preventing hip and vertebral fractures; second, they proved unable to build new bone to counterbalance the bone loss induced by the menopause; third, their efficacy over the entire range of osteoporosis patient profiles and in the long-term is not established; fourth, these drugs stop bone remodeling, an effect that may be associated with rare, but severe, side effects on bone. Today's treatment of osteoporosis must aim to improve bone architecture while taking into account the living nature of bone tissue; this is particularly crucial in view of the long-term nature of osteoporosis treatment. Protelos is a modern treatment for postmenopausal osteoporosis with proven efficacy against vertebral and hip fractures, whatever the risk factors, both in the short and in the long-term. The antifracture efficacy of Protelos has direct benefits for bone architecture: there is robust evidence showing that, thanks to its unique dual mode of action, Protelos builds strong and healthy bone in postmenopausal osteoporotic women. This chapter focuses on Protelos' ability to ensure comprehensive antifracture efficacy against all types of osteoporotic fractures, while at the same time improving bone health in osteoporotic patients (Figure 1).

**Comprehensive efficacy: Protelos protects against vertebral, nonvertebral, and hip fractures**

Two types of fractures must be considered in the prevention of fractures in postmenopausal women with osteoporosis:
- **Vertebral fractures**: these are the most common type of fracture, which generally occur in the youngest osteoporotic patients; when associated with height loss or back pain they are designated as “clinical vertebral fractures”;
- **Hip fractures**: these are definitely the most serious type of fracture and have a major impact on morbidity and mortality, especially in elderly subjects.

Two pivotal trials have assessed the efficacy of Protelos: SOTI (the Spinal Osteoporosis Therapeutic Intervention trial) and TROPOS (TReatment Of Peripheral Osteoporosis Study). Both were multinational, randomized, double-blind, and placebo-controlled trials, involving a total of 6740 postmenopausal women, all of whom received concomitant calcium/vitamin D supplementation at a dose tailored to the degree of deficiency (calcium 500/1000 mg; vitamin D₃ 400/800 IU).

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**Figure 1. Key criteria for the treatment of osteoporosis.**

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**Figure 2. Effects of Protelos on vertebral fracture risk in women with postmenopausal osteoporosis.**


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**SELECTED ABBREVIATIONS AND ACRONYMS**

- **ARR** absolute risk reduction
- **BMD** bone mineral density
- **BMI** body mass index
- **DXA** dual-energy x-ray absorptiometry
- **ESCEO** European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis
- **FRAX®** Fracture Risk Assessment Tool
- **HR-pQCT** high-resolution peripheral quantitative computed tomography
- **NNT** number needed to treat
- **RRR** relative risk reduction
- **SOTI** Spinal Osteoporosis Therapeutic Intervention
- **TROPOS** TReatment Of Peripheral Osteoporosis Study
TROPOS assessed the efficacy of Protelos against nonvertebral and hip fractures in 5091 postmenopausal women with femoral neck BMD equivalent to a T-score below –2.5 SD (centralized normative data analysis: Dr D. O. Slosman, Geneva, Switzerland), and age ≥74 years or 70 to 74 years with an additional fracture risk factor. At 3 years, Protelos decreased the risk of nonvertebral fractures by 16% (RR, 0.84; 95% CI, 0.702–0.995; P<0.05) and the risk of major nonvertebral fractures (hip, wrist, pelvis, sacrum, ribs-sternum, clavicle, and humerus) by 19% (RR, 0.81; 95% CI, 0.66–0.98; P<0.05) (Figure 2). In the subgroup of patients with the highest risk of hip fracture, ie, those aged ≥74 years with femoral neck T score ≤–3 SD, Protelos decreased the risk of hip fractures by 36% (RR, 0.64; 95% CI, 0.412–0.83; P=0.046) over 3 years. TROPOS also confirmed the decrease in risk of new vertebral fractures over 1 and 3 years by 45% (RR, 0.55; 95% CI, 0.39–0.77; P<0.001) and 39% (RR, 0.61; 95% CI, 0.51–0.73; P<0.001), respectively, versus placebo.

In summary, SOTI and TROPOS confirmed the efficacy of Protelos against all types of osteoporotic fractures, including vertebral and hip fractures, and thus its comprehensive efficacy in postmenopausal women.

Place of Protelos in the treatment of osteoporosis

For clinicians treating patients with osteoporosis, it is difficult to judge the comparative efficacy of antosteoporotic treatments due to the fact that no comparative studies are available. This is because such studies are in practice not feasible, as they require an exceedingly high number of patients and long-term follow-up. In an attempt to obviate this difficulty, the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) published in 2008 the European Guidance for the Treatment and Management of Osteoporosis in Postmenopausal Women, which compared the efficacy of antosteoporotic treatments on the basis of the findings of available large trials. In Table 6 of the Guidance, the board of experts highlights the efficacy of Protelos against vertebral and nonvertebral—including hip—fractures in patients with osteoporosis and established osteoporosis, in comparison with other treatments (Table 1).

Full assessment of efficacy requires that the usual analysis of clinical trials and relative risk reduction (RRR) be complemented by the analysis of absolute risk reduction (ARR) and number needed to treat (NNT).

![Graph showing the decrease in relative risk of nonvertebral and major non-vertebral fractures with Protelos vs placebo in the TROPOS study.](image-url)
ber needed to treat (NNT, the reciprocal of ARR). Even if it is not possible to directly compare studies with different populations and different levels of risk, these parameters are fair indicators of the magnitude of the benefits a physician can expect from a treatment. In a very recent review, J. D. Ringe and J. G. Doherty showed that Protelos had very low NNTs, with only 9 patients needing to be treated in order to prevent, over 3 years, 1 vertebral fracture, vs 21 for ibandronate, and 48 patients needed to be treated to prevent 1 hip fracture, vs 91 for three of the bisphosphonates studied by the authors (Figure 4). These AAR and NNT figures confirm Protelos as a very effective treatment, and fully justify the ESCEO Guidance ranking Protelos as first-line treatment.

**Protelos: efficacy whatever the risk factor profile and whatever the type of patient**

Until recently, evaluation of risk of fracture in postmenopausal women was solely based on BMD measurement, with osteopenia being defined by a T-score between –1 SD and 2.5 SD, and osteoporosis by a T-score <–2.5 SD. However, epidemiological studies stress the key role played by other risk factors, the most important one being age, followed by prevalent fractures, steroid treatment, smoking, alcohol intake, maternal fracture history, and low body mass index (BMI).

The diversity of possible risk factor combination profiles makes each osteoporotic patient unique. An ideal treatment should be able to achieve antifracture efficacy independently of any given risk factor profile. This has been widely demonstrated to be the case with Protelos, well before the World Health Organization (WHO) Fracture Risk Assessment Tool (FRAX®) to predict fracture risk came into widespread use.

Protelos is effective from the youngest to the oldest and frailest osteoporotic patients

In the youngest age-group of postmenopausal women, ie, those aged 50-65 years, in whom osteoporosis, due to a dramatic increase in bone remodeling, is one of the most common disorders, Roux et al showed that Protelos reduced vertebral fracture risk by 43% (RR, 0.57; 95% CI, 0.36-0.92; P=0.019) over 3 years and that this effect was sustained over a further year, as shown by a 35% reduction in vertebral fracture risk at 4 years (RR, 0.65; 95% CI, 0.42-0.99; P=0.049). Efficacy was also independent of age in the pooled SOTI and TROPOS populations: 3-year vertebral fracture risk fell by 37% in women <70 years (RR, 0.63; 95% CI, 0.46-0.85; P=0.003) and by 42% in those aged 70 to 80 years (RR, 0.58; 95% CI, 0.48-0.68; P<0.001). Elderly osteoporotic women (80 years and more), because of the frequent combination of risk factors observed in that age-group, are especially prone to fractures. In these women, osteoporotic fractures have particularly debilitating consequences, characterized by delayed fracture healing, functional impairment, and loss of autonomy.

This results in increased mortality and consumption of nursing home and health care financial resources. In such patients, Protelos has been shown to have complete and sustained efficacy, reducing vertebral fracture risk by 59% (RR, 0.41; 95% CI, 0.22-0.75; P=0.002), clinical fractures by 37% (RR, 0.63; 95% CI, 0.44-0.91; P=0.012), and nonvertebral fractures by...
41% (RR, 0.59; 95% CI, 0.37-0.95; P=0.027) after 1 year, and by 32% (RR, 0.68; 95% CI, 0.50-0.92; P=0.013), 22% (RR, 0.78; 95% CI, 0.61-0.99; P=0.040), and 31% (RR, 0.69; 95% CI, 0.52-0.92; P=0.011) after 3 years. Protelos is the only antosteoporosis agent to have shown long-term efficacy in the elderly, with decreases of 31% in vertebral fracture risk (RR, 0.69; 95% CI, 0.52-0.92; P=0.010) and 26% in nonvertebral fracture risk (RR, 0.74; 95% CI, 0.57-0.95; P=0.019) over 5 years (Figure 5).10

A new approach, taking into account not only age, but a combination of health status factors (decreased strength, tiredness, involuntary weight loss, slowness, and inactivity), has recently been used to define the “frailty” of the most elderly patients and analyze the efficacy of antosteoporotic treatments.11 It was found that frail osteoporotic women were more vulnerable when exposed to stressors and more likely to fall, but also, as a result, to fracture. Another finding was that Protelos was shown to be the only treatment able to decrease vertebral fracture risk by 66% (RR, 0.34; 95% CI, 0.12-0.92; P=0.02) and by 58% (RR, 0.42; 95% CI, 0.23-0.73; P=0.002) over 1 and 3 years, respectively.12

The above confirms that Protelos has complete antifracture efficacy, from the youngest to the most elderly patients. In the youngest patients, the earlier Protelos is introduced at menopause onset, the greater the anticipated benefit. In the oldest patients, clinical trials show a significant reduction in osteoporotic fractures after only 1 year of treatment, indicating that it is never too late to prevent fractures in such patients.

**Figure 5. Decrease in nonvertebral fracture risk in elderly patients with Protelos.**

**Figure 6. Decrease in hip fracture risk with Protelos. Results at 3 and 5 years.**

**Protelos: proven efficacy whatever the type of patient**

In osteoporotic women with a hip/lumbar spine T-score ≤–2.5 SD, Protelos decreases vertebral fracture risk by 39% (RR, 0.61; 95% CI, 0.53-0.70; P<0.001).13 Importantly, this decrease is observed whatever the patient’s risk factor profile. In addition, Protelos is the only treatment able to achieve a reduction in vertebral fracture risk in osteopenic women (hip/lumbar spine T-score between –1 and –2.5 SD) by as high as 72% (RR, 0.28; 95% CI, 0.07-0.99; P=0.045).13 The efficacy of Protelos is also independent of the number of prevalent fractures: in osteoporotic women with 0, 1, or 2 prevalent fractures, Protelos decreased vertebral fracture risk by 48% (RR, 0.52; 95% CI, 0.40-0.67; P<0.001), 45% (RR, 0.55; 95% CI, 0.41-0.74; P<0.001), and 33% (RR, 0.67; 95% CI, 0.55-0.81; P<0.001), respectively.11 Similarly, in osteopenic women with and without prevalent fractures, Protelos decreased vertebral fracture risk by 38% (RR, 0.62; 95% CI, 0.44-0.88; P=0.008) and 59% (RR, 0.41; 95% CI, 0.17-0.99; P=0.039), respectively.13

With regard to bone markers, Protelos decreased vertebral fracture risk by 33% (RR, 0.67; 95% CI, 0.47-0.95; P=0.023) and 49% (RR, 0.51; 95% CI, 0.37-0.70; P<0.001) in postmenopausal osteoporotic women with low- and high-turnover, respectively.11

Finally, the efficacy of Protelos has been shown to be independent of family history of osteoporosis, bone mass index, and smoking.15

**Abbreviations:** ARR, absolute risk reduction; CI, confidence interval; RR, relative risk; TROPOS, TRreatment Of Peripheral Osteoporosis Study.


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</table>

*Patients aged 74 years or more and with femoral neck and lumbar spine BMD T-score ≤–2.4 SD.*

*Abbreviations:* ARR, absolute risk reduction; BMD, bone mineral density; CI, confidence interval; RR, relative risk; TROPOS, TRreatment Of Peripheral Osteoporosis Study.

Protelos: comprehensive efficacy over 5 years, sustained over 8 years

In Western societies, in which life expectancy is growing year after year, only an antosteoporotic treatment with long-term efficacy will be able to guarantee effective protection against the consequences of osteoporotic fractures and preserve the patients’ quality of life. To date, there is no evidence showing that conventional antosteoporotic treatments, even the bisphosphonates, are able to decrease vertebral or nonvertebral fractures beyond 3 to 4 years of treatment, probably because these treatments fail to create a new and healthy bone in osteoporotic patients. In contrast, Protelos has robust evidence derived from studies with the most stringent design (randomized, double-blind, placebo-controlled trials) that it is effective against vertebral, nonvertebral, and hip fractures over 5 years. This efficacy was even shown to persist after 8 years of treatment, as demonstrated in an open-label extension study of TROPOS.

Randomized, double-blind, multicenter, placebo-controlled studies with a preplanned analysis are the gold standard to assess the efficacy of a treatment: to date, Protelos is the only antosteoporotic treatment for which long-term efficacy has actually been investigated in such a trial, namely, TROPOS, which was conducted over 5 years. In this trial, Protelos was found to have comprehensive efficacy: nonvertebral fracture risk was reduced by 15% (RR, 0.85; 95% CI, 0.73-0.99; P=0.032); new major nonvertebral osteoporotic fracture risk was reduced by 18% (RR, 0.82; 95% CI, 0.69-0.98; P=0.025), and vertebral fracture risk was reduced by 24% (RR, 0.76; 95% CI, 0.65-0.88; P<0.001) versus placebo, over 5 years.

In a subgroup of patients at high risk of hip fractures (n=1128; ≥74 years and lumbar/femoral neck T-score ≤−2.4 SD), Protelos reduced hip fracture risk by 43% versus placebo over 5 years (RR, 0.57; 95% CI, 0.33-0.97; P=0.036) (Figure 6). Finally, only 21 patients needed to be treated with Protelos to prevent 1 new osteoporotic fracture. The long-term efficacy of Protelos was confirmed in a 3-year open-label extension of TROPOS, including 893 patients followed for a total of 8 years. Cumulative incidence of new vertebral fractures over the 3-year extension period (13.7%, 5-8 years) was fully comparable with that in the first 3 years of TROPOS (11.5%, 1-3 years), thus showing that Protelos’ efficacy extended for as long as over 8 years. The same conclusion can be drawn for nonvertebral fractures, for which the cumulative incidence was similar at the beginning (12%, 1-3 years) and at the end (9.6%, 5-8 years) of the 8-year follow-up of the patients treated with Protelos. Last but not least, assessment of the long-term safety of Protelos showed that it was safe and very well tolerated. 16

Protelos treats osteoporosis by building new strong and healthy bone

Although mandatory, it is not enough for an antosteoporotic treatment today to merely prevent osteoporotic fractures: it should also, concomitantly, be able to treat osteoporotic bone itself. Indeed, since it is the decrease in bone quantity and quality occurring after menopause that is at the origin of the fracture risk in osteoporotic patients, improving bone status is a strong requirement, as only this can ensure strong and rapid protection against all osteoporotic fractures, both in the short and long term. Conventional treatments, including antiresorptive treatments, have not been able to prevent fracture risk in the long term, nor have they shown effectiveness in patients displaying the most severe risk factor profiles, probably due to the fact that these treatments are unable to build new bone. It is now well established that the increase in BMD observed in bisphosphonate-treated patients is due to the hypermineralization of bone resulting from the marked decrease in bone remodeling induced by these agents. The consequence of this negative impact on bone remodeling is illustrated by the rare—but dangerous—occurrence of severe side effects on bone leading to atypical fractures—an issue of severe

**Figure 7.** The unique mode of action of Protelos.

Protelos increases bone formation through an increase in osteoblast replication, differentiation, and activity. In parallel, Protelos decreases bone resorption via a decrease in osteoclast differentiation and activity and the upregulation of the OPG/RANK ratio in osteoblasts.

**Abbreviations:** CaSR, calcium-sensing receptor; OPG, osteoprotegerin; RANK(L), receptor activator nuclear factor-κB ligand.

growing concern for the European Medicines Agency (EMA) and US Food and Drug Administration (FDA). The situation is quite the reverse with Protelos. Protelos provides a new approach to the management of osteoporosis thanks to its unique dual mode of action, which enables it to build new and strong bone (Figure 7, page 413). It shows comprehensive and long-term efficacy whatever the type of osteoporotic fracture, whatever the patient’s profile, and whatever the patient’s risk factors. With Protelos, it is now possible not only to prevent fractures, but also to treat osteoporotic bone, as consistently confirmed in the literature.

Bone biopsies from SOTI and TROPOS patients were the first to establish the efficacy of Protelos in osteoporotic patients. Treatment for 3 years resulted in marked bone microarchitecture benefits, as evidenced by an 18% increase in cortical bone thickness ($P=0.008$) and a 14% increase in trabecular number ($P=0.05$), together with a 16% decrease in trabecular separation ($P=0.004$). These beneficial effects of Protelos on bone architecture result from increased osteoblast activity, as reflected by an increase in mineral apposition rate (+9%; $P=0.019$) and osteoblast surface (+38%; $P=0.047$), and a 10% trend toward a decrease in osteoclast surface. These improvements were associated with a change in bone structure from “rod-like” on placebo to “plate-like” on Protelos, signaling more resistant bone (Figure 8).

Further demonstration is provided by a comparison of the effects of Protelos and alendronate in a recent head-to-head randomized, double-dummy, double-blind study in osteoporotic women. In this study, a high-resolution-peripheral quantitative computed tomography (HR-pQCT, SCANCO Medical) was used to compare the effect on bone microarchitecture after 1 year of treatment with Protelos versus alendronate. Results showed that cortical thickness was increased by 5.3% ($P<0.001$) and trabecular bone volume/tissue volume ratio by 2.0% ($P=0.002$) with Protelos; the changes in each parameter were significant as early as by 3 months of treatment ($P=0.012$ and $P=0.042$, respectively) (Figure 9); and remained so after 2 years. No improvement occurred in the alendronate group, confirming alendronate’s inability to build new bone.

Analysis of hip architecture in TROPOS patients is yet another demonstration of the benefits of Protelos on bone. The relationship between hip geometry and bone strength was studied in 483 TROPOS patients (Protelos, n=251; placebo, n=231) after 5 years of treatment, by using the dual-energy x-ray absorptiometry (DXA)-derived hip structure analysis (HSA) program devised by Thomas Beck. In this study, Protelos was shown to increase cortical thickness at the femoral neck, intertrochanteric region, and proximal shaft (+5.2±9.8% vs –3.6±7.9%, $P<0.001$ vs placebo). This improvement in bone microarchitecture was independent of the increase in BMD and resulted in improved bending strength, with an increase in section modulus of +8.6±14.3% vs –2.3±11.6% vs placebo ($P<0.001$; Figure 10).


Figure 9. Comparison of changes in cortical thickness and ratio of bone volume to tissue volume for Protelos and alendronate. Abbreviations: Ct.Th, cortical thickness; BV/TV, bone volume/tissue volume. Modified after reference 20: Rizzoli et al. Rhumatol Int. 2010;30:1341-1348. © 2010, Springer.
Finally, four recent studies performed in nonclinical models—including osteoporotic models—have shown that the effects of Protelos on bone architecture improved fracture healing and osseointegration. Thus, Ly et al\(^\text{23}\) and Haberman et al\(^\text{24}\) evidenced a consistent improvement in both bone callus architecture and bone strength with Protelos, but not with teriparatide. In parallel, two other studies\(^\text{25,26}\) showed that treatment with Protelos improved osseointegration by increasing the resistance of implants in bone.

In summary, three independent clinical studies have consistently demonstrated the benefits of Protelos on bone in postmenopausal osteoporotic patients. The improvement in cortical bone—which is the main determinant of hip fracture—is undoubtedly the basis for Protelos’ efficacy in reducing the risk of hip fractures, while the efficacy of Protelos in improving trabecular bone—which is the main determinant of vertebral fracture—accounts for its ability to reduce the risk of vertebral fractures. Importantly, these studies also illustrate the consistency of the relationship between the improvement in bone health with Protelos, which has been established by randomized clinical trials after 1, 3, and 5 years of treatment, and the efficacy of Protelos in decreasing the risk of osteoporotic fractures, likewise established after 1 to 5 years of treatment.

The fact that Protelos is able to treat the osteoporotic bone explains its comprehensive efficacy against osteoporotic fractures, whatever the patient profile, whatever the type of fracture, and that this efficacy extends to the long term. Finally, no abnormalities in bone structure or mineralization have been reported after 5 years of treatment with Protelos, indicating that, besides its obvious benefits in terms of bone architecture, Protelos is totally safe for bone, as well as for patients.\(^\text{27}\)

**Conclusion**

Consistent and robust evidence supports the efficacy of Protelos in improving cortical and trabecular bone, the main determinants of hip and vertebral fractures, respectively. Protelos treats the bone defects responsible for the increased risk of fractures in postmenopausal women by building new strong and healthy bone. This effect accounts for the comprehensive efficacy of Protelos in reducing vertebral, nonvertebral, and hip fracture risk, no matter what the patient profile. Protelos’ efficacy develops quickly and is sustained in the long-term. Studies consistently show that the efficacy of Protelos is superior to that of other antosteoporotic agents, both on bone architecture and on reduction of fracture risk. By building strong and healthy bone and providing comprehensive protection against fractures in osteoporotic postmenopausal women, Protelos ushered in a new era as first-line treatment in the management of osteoporosis.

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**Figure 10.** Protelos improves hip geometry and bone strength compared with the placebo group. Abbreviations: CSA, cross-sectional area; CSMI, cross-sectional moment of inertia. Modified after reference 22: Briot et al. Ann Rheum Dis. 2006;65(suppl 3)665. Abstract SAT0375. © 2009, BMJ Publishing Group.
Protelos: better bone health for osteoporotic patients – Halbout

Keywords: strontium ranelate; osteoporosis; treatment guidelines; Protelos

Un os plus sain pour le patient ostéoporotique :
Protelos diminue le risque fracturaire tout en augmentant la qualité de l’os

Le déficit en ostrogène chez la femme post-ménopausée se traduit par une détérioration rapide de la qualité de l’os, de sa quantité, et par conséquent de sa solidité, aboutissant ainsi à l’augmentation du risque fracturaire. Les traitements antioestropérotiques doivent être évalués non seulement sur leur capacité à diminuer ce risque chez la femme post-ménopausée, mais aussi à améliorer l’état global de l’os en assurant une efficacité et une protection à long terme, quel que soit le profil des patientes, y compris celles pour qui le traitement est le plus difficile à mettre en œuvre. Protelos (ranélate de strontium) est un agent antioestropérotique indiqué dans la prévention du risque de fracture vertébrale et de hanche chez la femme post-ménopausée ostéoporotique. À ce titre, l’efficacité de Protelos va même au-delà de cette définition dans la mesure où il prévient le risque fracturaire tant chez la patiente ostéopérotique que dans les formes les plus sévères d’ostéoporose, et ce quel que soit l’âge des patientes, pour qui le traitement est le plus difîcile à mettre en œuvre. Protelos diminue le risque fracturaire tout en augmentant la qualité de l’os.
Bone health is also for men

Interview with M. Audran, France

Osteoporosis is a major health issue in men: 1 in 8 men older than 50 years has a risk of sustaining an osteoporotic fracture. Fractures of the hip and vertebrae are associated with the greatest morbidity and mortality. Sexual differences exist in skeletal bone metabolism. Boys have larger bones, thicker cortices, whereas trabecular pattern appears similar at the end of adolescence. Aging in men is mainly characterized by trabecular thinning and a decrease in trabecular number. A decrease in cortical volumetric bone mineral density (BMD) due to an increase in midcortical and endocortical porosity has been described. Significant associations between bone loss and estrogen levels have been found in men. BMD measures are effective to define the risk of future fractures and should be performed in patients with risk factors. A careful assessment of secondary osteoporosis as well as of mineralization disorders due to malignant diseases is mandatory in men. The FRAX® tool is a significant advance in clinical care and should prove useful in appropriate targeting of osteoporosis therapy. US NOF (National Osteoporosis Foundation) Guidelines recommend treating men older than 50 years with a history of hip or vertebral fracture, or with a 10-year probability of hip fracture of ≥3%, or a 10-year probability of major fractures ≥20% as calculated by FRAX®. Yet, men rarely receive osteoporosis treatment, despite the availability of a variety of agents (bisphosphonates, teriparatide, and maybe in future strontium ranelate) with proven efficacy in women, and which are presumed to be as efficient in men with equivalent fracture risk.

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Could you describe the epidemiological data of osteoporosis in men? Is it a common disease?

Osteoporosis-related fractures constitute a major health concern in men. Fracture incidence is even higher in men than in women below the age of 50, but they are very often related to high-energy trauma events.

After the age of 50, women tend to have a higher incidence of fractures than men; differences in bone mass and strength, the type and frequency of trauma, the fact that elderly women appear to have an increased frequency of falls relative to men may explain this inversion of the curves. Nonetheless, in aging men, fractures may also occur after minimal trauma; fractures of the hip and vertebrae are associated with the greatest morbidity and mortality.

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A recent review on the annual worldwide incidence of fractures showed that 39% occur in men and that 1 in 8 men older than 50 years has a risk of sustaining an osteoporotic fracture. In 42% of cases it will be a vertebral fracture, in 30% of cases a hip fracture, in 20% a wrist fracture, in 25% a fracture of the humerus. Fragility fractures in aging men may also occur at other sites, including the pelvis, ribs, and collarbone. With the increasing life expectancy of men, osteoporosis in men will become a greater burden to society. In 2000, in France, the medical cost of male osteoporosis was estimated at €197.5 million.

It is also important to take into account that the risk of a subsequent fracture is the same in male patients as in osteoporotic women after a low-energy fracture.

The mortality and morbidity associated with hip fractures are greater in men than in women. Men are twice as likely to die in hospital after a hip fracture. Comorbid conditions might contribute to this increased mortality risk. In men aged 60 to 69, the life expectancy after a hip fracture is 7.9 years, versus 19.4 in controls.¹ Loss of physical function and autonomy results in up to 50% of men having to be institutionalized after a hip fracture.

What is the pathophysiology of osteoporosis in men? Are there differences with that in women?

Sexual differences exist in skeletal development and peak bone mass acquisition. Boys have larger bones and thicker cortices, whereas trabecular pattern appears similar at the end of adolescence. Age-related changes in bone mass have been studied by means of several independent techniques (histomorphometry, x-ray microtomography [micro-QCT], high resolution QCT [HR-QCT], micro-MRI, synchrotron, finite element analysis). Our group and others have shown that parameters of trabecular microarchitecture are a major and independent determinant of vertebral fractures in middle-aged men with osteopenia. Increased cortical porosity has been observed in patients with severe osteoporosis.²,³ Using a different approach, significant decreases in trabecular volumetric BMD at the vertebrae have been shown in cross-sectional as well as in longitudinal studies. Aging in men is therefore mainly characterized by trabecular thinning and to a lesser extent by a decrease in trabecular number. In cortical bone, a decrease in cortical volumetric BMD due to an increase in the mid-cortical and endocortical porosity is observed. In contrast, cortical volumetric BMD shows little changes in men or in women.⁴ Changes in bone geometry in aging men are defined by an increase in cross-sectional area at different axial and peripheral sites of the skeleton, mainly due to continued periosteal apposition.

In men, as in women, sex steroids are important for skeletal development during growth as well for maintenance of peak bone mass. Their role in fracture risk in men has been recently extensively studied. Sex steroids, estrogens, and androgens circulate either free or bound to sex-hormone binding globulin (SHBG). Significant associations between BMD, bone resorption, and bone loss have now been found with estrogen levels in men. Our group showed that serum SHBG may predict the risk of future fractures. The respective roles of sex steroids and SHBG have been recently confirmed in the Osteoporotic Fractures in Men (MrOS) studies. In the Swedish arm of the cohort, elderly men with low serum E2 and high SHBG levels had an increased risk of fractures. In the US cohort, men with lowest bioavailable estradiol or highest levels of SHBG had greater risk of all nonvertebral fractures.⁵ Non-skeletal effects of testosterone, on muscle mass and reduced risk of falls, might also play a role on fracture risk in elderly men.

Vitamin D deficiency is common among older adults and may result in secondary hyperparathyroidism and increased bone resorption. In a US prospective cohort study of community-dwelling men aged 65 or older, the annualized average rate of loss in total hip BMD was twice higher among men with 25(OH)D levels below 15 ng/mL than among men with 25(OH)D levels of at least 30 ng/mL, suggesting that low 25(OH)D levels are detrimental to BMD in older men.

Declining levels of insulin-like growth factor–1 (IGF-1), possibly mediated by alterations of IGF binding proteins with age, may alter bone microarchitecture, with a thinning of bone trabeculae. The decrease in IGF-1 activity, which is an inhibitor of SHBG synthesis by the hepatocytes, might also indirectly influence bone metabolism through an increase in SHBG levels.⁴

Are risk factors for osteoporosis similar in men and women?

Low BMD is a major risk factor of osteoporotic fractures in men. BMD measures are therefore effective to define the risk of future fractures, regarding low-trauma fractures, but also high-trauma fractures.

Cessation of estrogen secretion is the main causal factor of osteoporosis in women. In men, in the absence of such a cause, osteoporosis is described as secondary in up to 40% of cases. The causes are heterogeneous and may be combined.
Bone density (BMD) predicts fracture risk in men as it does in women, but the prevalence of osteoporosis depends on the reference population. The World Health Organization (WHO) definition of osteopenia and osteoporosis (BMD measured by dual-energy x-ray absorptiometry (DXA) that is 2.5 or more standard deviations (SD) below that of a young normal adult, that is, a T-score of -2.5 or below) applies to white postmenopausal women and there is no consensus on the densitometric diagnosis of osteoporosis in men. Nonetheless, using the WHO criteria to define osteopenia and osteoporosis, two cut-offs have been proposed for men, based either on the young normal male or female reference groups.

Osteoporosis in men was defined as a BMD value 2.5 SD below the mean of either white men or women aged 20 to 29 years; low BMD or osteopenia was characterized as a BMD value between 1 and 2.5 SD below the respective young male and female reference means. Based on data from the Mayo Clinic, when bone density at any of the total hip, spine, or wrist sites was used, the prevalence of osteoporosis in men over age 50 was 19% using male reference ranges, and only 3% using the female reference ranges. The prevalence of osteoporosis in men, using sex-specific normal values, is therefore more substantial and may provide a better estimate for the proportion of men at risk for an osteoporotic fracture. The current ISCD recommendation is to use a male database for T-score derivation in men.

Spinal degenerative changes are common after the age of 65 years and have to be taken into account because they may falsely elevate the measured spine BMD.

It should also be underlined that, in the Rotterdam study, only 21% of all nonvertebral fractures occurred in men with a T-score below -2.5.

**How is osteoporosis in men handled by health authorities? What are the main recommendations and guidelines? Are doctors aware enough of the risk of osteoporosis in their elderly male patients?**

In 2008, the American College of Physicians, the US NOF, and the ISCD made recommendations relative to BMD measurement in men. French guidelines have been also released regarding the indications of the measure by DXA. The 2008 US NOF Guidelines warrant a recommendation for treatment in men: (i) older than 50 years with a history of hip or vertebral fracture; or (ii) with a T-score between -1 and -2.45; or (iii) a T-score between -1 and -2.5 and a 10-year probability of hip fracture of \( \geq 3\% \) or a 10-year probability of major fractures (spine, forearm, hip, humerus fracture) \( \geq 20\% \) as calculated by FRAX\(^\text{®}\). The French Health Authorities ("Haute Autorité de Santé") recommended in 2007 to treat male patients suffering from osteoporosis characterized by a T-score less than -2.5 with other risk factors or with T-score less than -3.

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**Table I. Main causes of secondary osteoporosis in men.**

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<th>Cause</th>
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<td>- Primary or secondary hypogonadism (hormonal suppressive therapy for prostate cancer)</td>
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<td>- Glucocorticoid treatment</td>
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<td>- Alcoholism and cigarette smoking</td>
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<td>- Hyperparathyroidism, hyperthyroidism, Cushing’s disease</td>
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<td>- Inflammatory bowel disease, gluten enteropathy, malabsorption syndromes, gastrointestinal disorders</td>
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<td>- Primary biliary cirrhosis, hemochromatosis</td>
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<td>- Chronic obstructive pulmonary disease</td>
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<td>- Hypercalcemia</td>
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<td>- Organ transplant</td>
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<td>- Rheumatoid arthritis and systemic diseases</td>
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<td>- Mastocytosis</td>
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<tr>
<td>- Neuromuscular disorders, anticonvulsants</td>
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<td>- Immobilization</td>
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<td>- Proton pump inhibitors</td>
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Three major causes have been identified: (i) prolonged glucocorticoid therapy; (ii) hypogonadism (sometimes induced by gonadotropin-releasing hormone [GnRH] treatment in patients suffering from prostate cancer); (iii) excessive alcohol intake.

Some others factors have also been consistently documented to be associated in men with a significant increase in fracture risk (Table I). Osteoporosis may be defined as primary or idiopathic when no cause or risk factor is identified; some cases might be due to genetic factors in the acquisition of peak bone mass. A careful assessment of secondary osteoporosis as well as of mineralization disorders due to malignant diseases (myeloma, lymphomas) is mandatory in men.

How and when are patients diagnosed? Is it only after fractures or before? What are the diagnostic criteria?

Prevention and treatment of bone loss and fractures are often underestimated priorities. In many clinical situations it might be useful to perform a careful evaluation because they represent a significant risk factor of osteoporosis (Table I). Risk factors may interfere with bone fragility in different ways: (i) by decreasing bone mass; (ii) by qualitative alterations of cortical or trabecular bone; (iii) by increasing the risk of falls. Both the National Osteoporosis Foundation (NOF) and the International Society for Clinical Densitometry (ISCD) recommend performing BMD measurement after 70 years of age (but a cost-effectiveness analysis showed this measure to be effective only over 80 years or in men aged 65 or more with a prevalent vertebral fracture), after a prior vertebral or nonvertebral low-trauma fracture, and when secondary causes (including medications) have been identified. Several different factors may be associated and should be considered in the future risk of osteoporosis.
Is FRAX® useful in the diagnosis of male patients?

A normal BMD measurement is no guarantee that a fracture will not occur. The use of risk factors may in this way add useful information on fracture risk independently of BMD.

FRAX® is a computer-based algorithm derived from data obtained in 11 independent cohorts (http://www.shef.ac.uk/FRAX) that provides models for the assessment of 10-year probability of fracture risk (hip, clinical spine, humerus, or wrist fracture) and the 10-year probability of hip fracture alone in men and women using clinical risk factors. The tool can be used alone or with femoral neck BMD to enhance fracture risk prediction. The presence of more than one risk factor increases fracture probability in an incremental manner.

FRAX® has limitations: (i) it has largely been validated in women and additional evaluation of FRAX® in men is needed; (ii) some risk factors are described as dichotomous variables (yes or no), despite data clearly showing a dose–response relationship; (iii) silent, radiological vertebral fractures are not taken into account. It appears nonetheless as a significant advance in clinical care and should prove useful in appropriate targeting of osteoporosis therapy.

What are the main bases of the management of osteoporosis in men (pharmacologic, nonpharmacologic treatments)?

In the Framingham osteoporosis study, the proportion of men meeting the 2008 NOF criterion increased with advancing age (1.7% of men aged 50 to 65 and 37.9% of men aged >75 years). In total, one sixth of men aged over 50 years would be recommended for osteoporosis treatment. Nonetheless, the loss of potential years of life in younger age-groups suggests that preventive strategies for fracture should not only focus on older patients at the expense of younger high-risk men.

Although hypogonadism in men leads to bone loss, deterioration of trabecular architecture, loss of muscle mass, and increased risk of fracture, androgen treatment remains controversial. Testosterone therapy has been shown to increase BMD in hypogonadal men, but clinical trials concerned a small number of patients, were of short duration, without any definitive evidence of fracture risk reduction. The issue of the long-term safety of testosterone treatment in older men, (increased risk of prostate cancer, adverse cardiovascular effects), must be taken into account.

Because of the presumed role of estrogens on bone in men, the effects of selective estrogen receptor modulators (SERMs) has been studied. Raloxifene reduced bone turnover in men with low estradiol concentrations and increased BMD in men treated with GnRH agonists for prostate cancer. Toremifene reduced the risk of vertebral fractures in patients on androgen deprivation therapy for prostate cancer.

Calcitonin has received limited evaluation in men and no conclusions may be drawn from the small short-term clinical trials.

Most studies with alendronate or risedronate in men have shown a beneficial effect on BMD at lumbar and femoral sites, when compared with placebo. Intravenous zoledronic acid increased BMD in men after hip fracture and in patients with androgen-deprivation treatment for prostate cancer. Few clinical trials clearly proved a significant reduction in fracture risk. Because vertebral and nonvertebral fracture risk reduction has been well documented in women at risk of fractures receiving bisphosphonate therapy, it has been suggested that such treatment interventions would have a similar efficacy in men with equivalent fracture risk, and therefore bisphosphonates are considered to be first-line therapy for men with osteoporosis.

The effects of daily SC teriparatide appear similar in men and women. The induced lumbar and femoral increase in BMD was of the same magnitude as in women, with similar changes in bone remodeling. Teriparatide appears to reduce the risk of vertebral fracture, but not of nonvertebral fracture.

Strontium ranelate induces an increase in bone formation and a decrease in bone resorption. It has been shown to decrease vertebral and nonvertebral fracture in women at different ages, for different levels of risk. This dual-effect bone agent may represent an interesting alternative to bisphosphonates in men. A large clinical trial (MALEO) is under way.

Long-term vitamin D daily supplementation (800 IU) is often required. A daily calcium intake of 1000 to 1200 mg has been recommended.

Men rarely receive osteoporosis treatment. Following a hip fracture, less than 10% of patients are treated and only one third of men receiving androgen deprivation therapy for prostate cancer receive osteoporosis evaluation or treatment.

In 2008, the recommendations of the American College of Physicians (ACP) were that pharmacologic treatment should be offered to men with known osteoporosis and those having sustained a fragility fracture, as well as to patients with BMD T-scores below -2.5, but at risk due to clinical factors. A cost-effectiveness analysis conducted by NOF found pharmacologic treatment to be cost-effective for both men and women provided the 10-year estimated fracture risk exceeded approximately 20% for major osteoporotic fracture or 3% for hip fracture, based on a US-adapted FRAX® model. In future, health and economic considerations, not simply fracture risk, will influence treatment recommendations, based

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on the resources dedicated to health care. Nonpharmacological measures are useful in the management of male osteoporosis. Increasing physical exercise may be considered, but the way to optimize its effects on skeleton is not well defined. Prevention of falls is mandatory in elderly patients, with different measures: correction of functional disability, treatment of comorbidity known to facilitate gait disorders; reduction of drug consumption or alcohol abuse; action on architectural or environmental factors. The interest of hip protectors is still controversial.

References

Keywords: osteoporosis; fracture; men; FRAX®; morbidity; mortality
Fracture risk prediction can be enhanced by the concurrent assessment of clinical risk factors in addition to measurements of bone mineral density (BMD). FRAX®, a combination of four algorithms, can calculate the 10-year probability of hip or major osteoporotic fracture, with or without the input of femoral neck BMD. A number of studies have now examined the efficacy of osteoporosis treatments across a range of fracture probabilities and are contributing to a body of evidence demonstrating that treatments can generally reduce fracture risk in women identified to be at high risk by FRAX®. A community-based study of oral clodronate clearly demonstrated a reduction in nonvertebral fractures, and the treatment was equally or more effective in women with higher FRAX® probabilities. The original studies of bazedoxifene and alendronate demonstrated significant reductions in vertebral fractures, but required post hoc subgroup analyses to demonstrate significant reductions in nonvertebral fractures. For bazedoxifene, while the interaction with treatment was not statistically significant, the efficacy was clearly more obvious in patients with higher baseline FRAX® probabilities. The interpretation of the alendronate study (Second Fracture Intervention Trial [FIT2]) is somewhat more problematic. One conclusion is that the drug is simply not effective in reducing nonvertebral fractures in this study population and that this is equally true across a wide range of baseline FRAX® probabilities. The evidence base will continue to expand as a number of other studies will shortly be examined to determine the interaction between treatment efficacy and baseline FRAX®.

A number of agents are available for the treatment of osteoporosis, all of which have been shown to significantly reduce fracture risk in at least one skeletal site.1-11 In the very near future, several new agents that have shown reductions in fracture risk will also be available for clinical use.12-14

Treatment efficacy, fracture probability, and FRAX®

The efficacy of osteoporosis therapies has usually been characterized in individuals with low bone mass, such that the bone mineral density (BMD) thresholds published by the World Health Organization (WHO) in 199415 are widely accepted as both a diagnostic and an intervention threshold. Indeed, to date most pivotal antifracture studies have reported on the use of these agents in individuals selected to be at high risk for fracture usually by the presence of low BMD and/or a prior fragility fracture, most commonly at the spine. A problem with the predominant use of BMD to direct
interventions is that BMD alone is not optimal for the detection of individuals at high risk of fracture. Indeed, the majority of osteoporotic fractures will occur in individuals without osteoporosis.16-17

In the past decade, other factors have been identified that contribute to fracture risk, partially or wholly independent of BMD, which improve fracture prediction and the selection of individuals at high risk for treatment.18-22 A series of meta-analyses using individualized data from 12 global population cohorts23-30 has identified clinical risk factors for use in the assessment of fracture risk with or without the use of BMD. The adequacy of the risk factors has been validated in a further 12 independent population-based cohorts.31 The risk factors identified formed the basis for the development of the WHO algorithms that calculate fracture probability in an individual, expressed as the 10-year fracture probability (FRAX®).31 Unlike many previous algorithms, the FRAX® tool takes into account the relationship between individual risk factors and both fracture and death hazards.31 The risk factors in the FRAX® tool include age, sex, glucocorticoid use, secondary osteoporosis, parental history of hip fracture, prior fragility fracture, low body mass index (BMI), current smoking, excess alcohol consumption (3 or more units daily) and femoral neck BMD selected on the basis of their international validity.32

A critical question in proposing the use of clinical risk factors for patient risk assessment relates to the reversibility by pharmacological intervention of the risk so identified. The risk factors in FRAX® were also selected on the basis of having at least indirect evidence that the risk was likely to be modified by subsequent intervention (modifiable risk). This was validated from clinical trials (BMD, prior fracture, glucocorticoid use, secondary osteoporosis), or partially validated by excluding interactions of risk factors on therapeutic efficacy in large randomized intervention studies (eg, smoking, family history, BMI). It is important to note that risk factors for falling were not considered for inclusion in the FRAX® tool, since there is some concern that the risk identified would not be modified by a pharmaceutical intervention targeted at the skeleton.7 It is notable that in this latter study, the precise criteria for inclusion were not documented, and further work is required to determine whether risk factors for falls or a history of falls would identify a risk that was modifiable by pharmacological intervention.33 A number of studies have now addressed the interaction between treatment efficacy and fracture probabilities assessed by FRAX®.

Clodronate
Daily oral clodronate 800 mg has been shown to decrease vertebral fracture risk in women with postmenopausal or secondary osteoporosis.1 More recently, it has been demonstrated to reduce clinical and osteoporotic fracture risk in elderly women unselected for osteoporosis.34 The latter study was a double-blind, prospective, randomized, placebo-controlled, single center study in elderly community-dwelling women aged 75 years or more. Treatment was associated with a significant reduction in all clinical fractures (hazard ratio [HR], 0.80; 95% confidence interval [CI], 0.68-0.94)34 and clinical osteoporotic fractures (HR, 0.76; 95% CI, 0.63-0.93, P=0.006).35

The interaction between efficacy and FRAX® probabilities was conducted in a cohort comprising 76% of the women recruited to the main part of the study, in whom complete data on clinical risk factors required for the computation of 10-year fracture probability were available.35 The following clinical variables were used to compute the 10-year probability of a major osteoporotic fracture (hip, clinical vertebral, wrist or humerus) by FRAX®, age, BMI, history of prior fragility fracture after the age of 50 years, maternal history of hip fracture (father’s history of hip fracture was not documented), rheumatoid arthritis (yes, if patient self-reported ever being told they probably had or did have rheumatoid arthritis), oral glucocorticoid use (yes, if ever used) and smoking (yes, if current), information on alcohol intake was not captured in the study. The 10-year probability was calculated with and without input of femoral neck BMD.

The mean±SD 10-year probability of a major osteoporotic fracture calculated by clinical risk factors alone was 20%±7%. When femoral neck BMD was added to the FRAX® calculation, the mean 10-year probability was slightly lower at 18%±9%.36 This suggests that the mean femoral neck BMD in the study population was slightly higher than expected for age and a healthy selection bias had already been noted in the study.34

The effects of clodronate to reduce fracture incidence at various 10-year probabilities of fracture, calculated with and without femoral neck BMD, are shown in Figures 1 and 2 (page 424). In the absence of BMD, there was a borderline statistically significant interaction (P=0.043) with a better effect of clodronate at higher probabilities (Figure 1). For example, at a probability of 15% (25th percentile), the relative risk for fracture was reduced by 8% (NS) whereas at a probability of 24% (75th percentile) the reduction was 27% (95% CI 8% to 42%). The interaction between efficacy and probability of fracture was not statistically significant when BMD was used in the calculation of probability (P=0.10), but the pattern of efficacy was very similar with more evident fracture reductions at higher probabilities of fracture (Figure 2).

**Selected abbreviations and acronyms**

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BMD</td>
<td>Bone mineral density</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>FIT1 and 2</td>
<td>Fracture Intervention Trial (First; Second)</td>
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<td>FLEX</td>
<td>Fracture intervention trial Long-term EXTension</td>
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<td>NHANES</td>
<td>National Health And Nutritional Examination Survey</td>
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Bazedoxifene

Bazedoxifene acetate is a new agent within the class of drugs known as selective estrogen receptor modulators (SERMs). Its fracture efficacy has been examined in a phase 3 study designed to determine the primary effect of this agent on vertebral fracture risk in postmenopausal women with osteoporosis. In brief, the study was a double-blind, randomized, placebo- and raloxifene-controlled trial including 7492 postmenopausal women with osteoporosis. The latter observation is supportive of the systematic differences between machine manufacturers due to the use of a machine specific Z-score was calculated by age to remove energy x-ray absorptiometry (DXA) equipment used so that machine specific Z-score was calculated by age to remove the systematic differences between machine manufacturers and permit the computation of 10-year probabilities with the FRAX® tool. Medicinal Products for Human Use (CHMP), an analysis was undertaken to test the hypothesis that the combined data for the two doses of bazedoxifene would demonstrate a reduced fracture risk in women with the higher fracture probabilities. Baseline data were used to calculate 10-year fracture probabilities with the FRAX® tool in placebo- and bazedoxifene-treated patients. The risk factors at baseline were further clarified in the following ways; for a prior fracture, data on self-reported peripheral fractures was combined with the finding of a grade 2 or greater morphometric vertebral fracture on baseline spine radiographs. No information was available on parental history of hip fracture, so that this variable was simulated resulting in a prevalence of 6%—in a sensitivity analysis, a more conservative position assumed that no patient had a family history of hip fracture. Three different types of dual-energy x-ray absorptiometry (DXA) equipment were used so that a machine specific Z-score was calculated by age to remove the systematic differences between machine manufacturers and permit the computation of 10-year probabilities with the FRAX® tool. The mean±SD 10-year probability of a major osteoporotic fracture calculated by clinical risk factors alone was 11%±8% and was similar when femoral neck BMD was added to the FRAX® calculation. The probability is somewhat lower than that observed in the population-based cohort recruited to the clodronate study above and reflects the younger age of the present study population (mean age 66 years vs 80 years in the clodronate study), despite the selection criteria based on BMD and prior fracture. The latter observation is supportive of the need to use of age-dependent intervention thresholds as requested for new phase 2 studies by the Committee for Medicinal Products for Human Use (CHMP), an analysis was undertaken to test the hypothesis that the combined data for the two doses of bazedoxifene would demonstrate a reduced fracture risk in women with the higher fracture probabilities. Baseline data were used to calculate 10-year fracture probabilities with the FRAX® tool in placebo- and bazedoxifene-treated patients. The risk factors at baseline were further clarified in the following ways; for a prior fracture, data on self-reported peripheral fractures was combined with the finding of a grade 2 or greater morphometric vertebral fracture on baseline spine radiographs. No information was available on parental history of hip fracture, so that this variable was simulated resulting in a prevalence of 6%—in a sensitivity analysis, a more conservative position assumed that no patient had a family history of hip fracture. Three different types of dual-energy x-ray absorptiometry (DXA) equipment were used so that a machine specific Z-score was calculated by age to remove the systematic differences between machine manufacturers and permit the computation of 10-year probabilities with the FRAX® tool. The mean±SD 10-year probability of a major osteoporotic fracture calculated by clinical risk factors alone was 11%±8% and was similar when femoral neck BMD was added to the FRAX® calculation. The probability is somewhat lower than that observed in the population-based cohort recruited to the clodronate study above and reflects the younger age of the present study population (mean age 66 years vs 80 years in the clodronate study), despite the selection criteria based on BMD and prior fracture. The latter observation is supportive of the need to use of age-dependent intervention thresholds as
adopted by the UK. Overall, bazedoxifene was associated with a significant 39% decrease in incident morphometric vertebral fractures ($P=0.005$) and a 16% decrease in the incidence of all clinical fractures ($P=0.14$). While there was no significant interaction between baseline FRAX® probability and treatment efficacy ($P>0.3$), the reduction in fracture risk increased progressively at higher baseline fracture probabilities (Figures 3 and 4). For morphometric vertebral fractures, treatment with bazedoxifene was associated with a significant decrease in the risk at probability values above 7%, corresponding to the 41st percentile of the study population. In patients with fracture probabilities above 16%, the 80th percentile, bazedoxifene was associated with a significant decrease in all clinical fractures. When BMD was not used in the FRAX® tools to compute fracture probabilities, similar results were observed, but with wider confidence estimates.

**Alendronate**

The pivotal studies for the clinical use of alendronate in postmenopausal osteoporosis were the two arms of the Fracture Intervention Trial (FIT). The first (FIT1) examined the efficacy of alendronate over 36 months in approximately 2000 women aged 55 to 81 years with low femoral-neck bone mineral density (BMD), and the efficacy of bazedoxifene to reduce morphometric vertebral fracture risk (hazard ratio with 95% confidence intervals). The black horizontal line represents the overall treatment efficacy and the dashed horizontal line a hazard ratio of 1. The diamonds correspond to the 10th, 50th, and 90th percentiles of probability in the population studied.

A pre-planned analysis of the two arms of FIT combined was subsequently published, though this deviated from the original plan as it concentrated solely on women with BMD T-scores $<-2.5$ or at least one vertebral fracture. This further post hoc analysis suggested that alendronate treatment was
An analysis of the interaction between alendronate efficacy and baseline FRAX® probabilities in the clinical fracture arm of the FIT (FIT2) has also recently been presented, but full publication is still awaited.39 The analysis used Cox proportional hazards models with interaction terms to analyze whether the effect of alendronate on risk of nonvertebral and major osteoporotic fracture risk (hazard ratio with 95% confidence intervals).

**Abbreviations:** FN BMD, femoral neck bone mineral density; HR, hazard ratio; LCL, lower confidence limit; UCL, upper confidence limit.


Figure 5. Alendronate and reduction of clinical osteoporotic fracture risk (clinical risk factors + femoral neck BMD).

Relationship between 10-year probabilities of major osteoporotic fracture, calculated with clinical risk factors combined with femoral neck bone mineral density, and the efficacy of alendronate to reduce clinical osteoporotic fracture risk (hazard ratio with 95% confidence intervals).

Forest plots show similar nonvertebral fracture efficacy as 10 years, so it should be borne in mind though that the overall results from the two individual arms of FIT suggested an overall nonsignificant 12% to 20% decrease in nonvertebral fractures.

An analysis of the interaction between alendronate efficacy and baseline FRAX® probabilities in the clinical fracture arm of the FIT (FIT2) is somewhat problematic. One conclusion is that the drug is simply not effective at reducing nonvertebral fractures in this study population and that this is equally true across a wide range of baseline FRAX® probabilities. The Fracture intervention trial Long-term EXtension (FLEX) trial, a randomized extension to the FIT, may also be consistent with the lack of efficacy at nonvertebral sites.45 In this study, women randomized to continue alendronate 10 mg daily for a further 5 years after the original study showed no difference in nonvertebral fracture rates compared with those randomized to receive placebo during the extension. This “lack of offset” has been widely interpreted to suggest that 5 years of therapy with alendronate shows similar nonvertebral fracture efficacy as 10 years, so that patients may be able to get “treatment-free” windows. An alternative interpretation is that it is not possible to show an offset of effect if one has not demonstrated an onset of effect.

The studies reviewed here are the first to contribute to the body of evidence demonstrating that treatments can generally reduce fracture risk in women identified to be at high risk by FRAX®. Certainly the analysis of clodronate is consistent with this hypothesis. The original studies of bazedoxifene and alendronate demonstrated significant reductions in vertebral fractures, but required post hoc subgroup analyses to demonstrate significant reductions in nonvertebral fractures. There are obvious difficulties with post hoc analyses that are particularly acute when undertaken on subgroups, especially subgroups that may be difficult to justify on clinical grounds. The post hoc nature, the change in the significance of the primary outcome, and the way of categorizing the high-risk group, all weaken the validity of these analyses. Against this background, examination of the interaction of treatment efficacy with baseline FRAX® probabilities, as a continuous variable, while not avoiding post hoc status, aims to avoid subgroup analysis and the associated loss of statistical power. For bazedoxifene, while the interaction with treatment was not statistically significant, the efficacy was clearly more obvious in patients with higher baseline FRAX® probabilities. The interpretation of the alendronate study (FIT2) is somewhat more problematic. One conclusion is that the drug is simply not effective at reducing nonvertebral fractures in this study population and that this is equally true across a wide range of baseline FRAX® probabilities. The Fracture intervention trial Long-term EXtension (FLEX) trial, a randomized extension to the FIT, may also be consistent with the lack of efficacy at nonvertebral sites.45 In this study, women randomized to continue alendronate 10 mg daily for a further 5 years after the original study showed no difference in nonvertebral fracture rates compared with those randomized to receive placebo during the extension. This “lack of offset” has been widely interpreted to suggest that 5 years of therapy with alendronate shows similar nonvertebral fracture efficacy as 10 years, so that patients may be able to get “treatment-free” windows. An alternative interpretation is that it is not possible to show an offset of effect if one has not demonstrated an onset of effect.

A number of other studies will shortly be examined to determine the interaction between treatment efficacy and baseline FRAX® probabilities.
References


Keywords: fracture; FRAX®; osteoporosis; bone mineral density; treatment; alendronate; clodronate; bazedoxifene
FRAX® et efficacité du traitement de l’ostéoporose

La prévision du risque de fracture peut être améliorée par l’évaluation des facteurs de risque cliniques complétée par des mesures de la densité minérale osseuse (DMO). FRAX®, qui associe quatre algorithmes, permet de calculer la probabilité à 10 ans d’une fracture de la hanche ou de fractures ostéoporotiques majeures, avec ou sans l’utilisation de la DMO du col fémoral. Un certain nombre d’études ont désormais établi l’efficacité des traitements de l’ostéoporose en relation avec différentes probabilités de fracture, et ont permis de constituer un ensemble de données démontrant que les traitements réduisent généralement le risque de fractures chez les femmes présentant un risque élevé calculé avec FRAX®. Une étude sur des patients de ville portant sur l’utilisation du clodronate par voie orale a clairement mis en évidence une réduction des fractures non vertébrales, et a fait apparaître que le traitement était au moins aussi efficace chez les femmes présentant un risque élevé calculé avec FRAX®. Les études originales sur le bazédoxifène et l’alendronate ont indiqué des réductions significatives des fractures vertébrales, mais ont nécessité des analyses de sous-groupes post hoc pour déceler des réductions significatives des fractures non vertébrales. Pour le bazédoxifène, si l’interaction avec le traitement n’a pas été statistiquement significative, l’efficacité a été nettement plus manifeste chez les patients présentant un risque élevé avec FRAX®. L’interprétation de l’étude sur l’alendronate (Second Fracture Intervention Trial, FIT2) est un peu plus problématique. L’une des conclusions est que le médicament est simplement inefficace pour réduire les fractures non vertébrales dans cette population, et que cela est avéré dans un large éventail de valeurs initiales de probabilités FRAX®. La base de données continuera à s’enrichir lorsqu’un certain nombre d’autres études seront prochainement examinées afin de déterminer l’interaction entre l’efficacité du traitement et la valeur initiale FRAX®.
Peripheral quantitative computed tomography is a new technique for the in vivo approximation of bone microstructure parameters such as geometry, structural parameters, and distal radial and tibial density. It determines the structural and material properties of peripheral bone, at the cost of acceptable whole-body radiation, within a few minutes, image processing included. The resulting data extend beyond DXA-BMD measurement and provide an estimate of bone strength that is grounded in fundamental mechanics.

Peripheral and vertebral fractures are the greatest hazard facing patients with osteoporosis, reducing their physical mobility, quality of life, and life expectancy. There is increasing evidence, supported by fundamental physics, that more than dual energy x-ray absorptiometry is required to estimate individual fracture risk and monitor treatment response. The determinants of bone strength are geometry, structural properties (bone distribution), material properties, and direction of force. It is therefore essential to develop and implement more sophisticated techniques such as in vivo microcomputed tomography. Scanco Medical AG’s peripheral quantitative computed tomography system, XtremeCT, is a relatively new device for the in vivo approximation of geometry, structural parameters, and distal radial and tibial density. Structural parameters include trabecular thickness, trabecular separation, structural model index, connectivity, anisotropy, and cortical thickness. Other calculations include bone volume/tissue volume ratios and subregional and cortical bone mineral densities, expressed in mg/cm³. Finite element analysis based on 3-D reconstructions of 110 slices is used for stress mapping. The technique’s main limitations are movement artifacts and the fact that calculation of the structural parameters is density-based.

Since bone is a multifunctional tissue, assessment of its health involves multiple parameters. This paper reviews the latest techniques for evaluating two of these parameters, bone strength and fracture risk, based on the material and structural properties of bone and the direction of force. Mass does not come into it, although physicians persist in ignoring the basic mechanics involved, using terms such as bone quality to simplify a complex system.

According to Dalzell et al,¹ the material properties of bone cannot currently be studied noninvasively. Of course there is dual energy x-ray absorptiometry (DXA), which determines bone mineral density (BMD), but the latter is very different from actual physical density. DXA scanners mainly measure bone mass, which the World Health Organization (WHO) has deemed important for classifying the clinical impact of osteoporosis treatments. However, we know from the experience of major pivotal studies that DXA is of very limited use in monitoring treatment effect, largely because it fails to separate measures for trabecular and cortical bone. Other limitations include the absence of geometric data for calculating cross-sectional moment of inertia (CSMI) and of structural and material property data.

New techniques for assessing bone health by D. Felsenberg, Germany

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Bone strength is dependent on a material property, expressed by the elastic (Young’s) modulus (E), and the CSMI. The elastic modulus is given by the slope of the stress/strain curve (stress on the y axis and strain on the x axis) during the linear elastic phase (Figure 1). It is constant for a given material and is expressed in N/mm² (1 newton/ mm² = 1 megapascal [MPa], and 1 kilonewton/mm² = 1 gigapascal [GPa]). Bone has an E value of 18 to 21 GPa. For comparison, glass has a very steep slope, with an E value of 50 to 90 GPa, whereas the E value of silicone rubber is 0.01 to 0.1 GPa. In other words, the higher the E value the more rigid the material. However, E values also depend on temperature, humidity, and speed of deformation.

Fracture risk is a function not only of bone’s material properties, but also of its geometry and the direction of force. The CSMI reflects the dependence on geometry. These calculations are mostly important in long bones (humerus, radius, ulna, femur,ibia, fibula, femoral neck, etc) where the direction of force is not uniaxial, along the long axis, but in all the other directions for which the bone is not adapted. The CSMI depends mostly on the distance of the bone mass to the neutral surface: \( I = \int y^2 \Delta A \) (Figure 2). Bone strength, expressed as bending stiffness (EI), is given by the product of E and CSMI.

Peripheral computed tomography (pCT) systems perform these calculations routinely. Beck et al.2 devised an interactive hip structure analysis (HSA) program that derives femoral neck geometry from raw bone mineral image data in order to estimate hip strength using single plane engineering stress analysis. The purpose of the program was to improve the predictive value of hip bone mineral data for osteoporosis fracture risk assessment. The authors reported a series of experiments with an aluminum phantom and cadaver femora designed to test the accuracy of derived geometric measurements and strength estimates. HSA-computed femoral neck cross-sectional areas (CSA) and CSMI on an aluminum phantom agreed closely with actual values (r>0.99). HSA-computed cross-sectional properties of three human cadavers were compared with measurements derived from sequential CT cross-sectional images. Discrepancy between the two methods averaged less than 10% along the length of the femoral neck. The breaking strengths of 20 femora showed better agreement with HSA-predicted strength (r=0.89) than with femoral neck BMD (r=0.79).2

It takes more sophisticated devices to calculate the trabecular network in vitro and answer another question of general interest: how does the most typical fracture in osteoporosis, vertebral compression fracture (often referred to as “sintering” in German-language osteoporosis literature, a term taken from metallurgy), relate to microarchitectural deterioration in other

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**Selected abbreviations and acronyms**

- BMD: bone mineral density
- CSA: cross-sectional areas
- CSMI: cross-sectional moment of inertia
- DXA: dual energy x-ray absorptiometry
- FEA: finite element analysis
- FPT: Fracture Prevention Trial
- HSA: hip strength analysis
- mCT: microcomputed tomography
- MORE: Multiple Outcomes of Raloxifene Evaluation [study] 
- SMI: structural model index
- WHO: World Health Organization
- WISE: Women International Space simulation for Exploration [study]
New techniques for assessing bone health – Felsenberg

Update

Epidemiological studies have shown that osteoporotic (vertebral and nonvertebral) fracture incidence relates not only to vertebral fracture prevalence, but also to prevalent vertebral fracture severity, suggesting that vertebral fracture severity is a marker for increased bone fragility at all skeletal sites. Bone architecture, defined as the distribution of bone mass in a trabecular network, can be directly assessed by histomorphometry or microcomputed tomography (mCT) analyses of invasively obtained bone biopsy samples or by in vivo assessment of the distal forearm or distal tibia.

Since no in vivo measurements are available we have to focus on in vitro data to determine the relationship between vertebral compression fracture and microarchitectural deterioration elsewhere. Genant et al conducted a semiquantitative analysis of baseline vertebral fracture severity on spinal radiographs from 190 postmenopausal women with osteoporosis. Bone structure indices were obtained by 2-D histomorphometry and 3-D mCT analyses in transiliac bone biopsy samples taken at baseline in a subset of patients from the Multiple Outcomes of Raloxifene Evaluation trial (MORE) and the teriparatide Fracture Prevention Trial (FPT). After adjustment for age, height, and spinal DXA-BMD, there were significant trends for 3-D bone volume, trabecular number, trabecular separation, and connectivity density: mCT bone volume was significantly lower ($P<0.05$) in women with mild (-23%), moderate (-30%), and severe fractures (-51%) than in women with no fractures. Trabecular number was lower ($P<0.05$) in women with mild (-14%), moderate (-18%), and severe (-28%) vertebral fractures compared to women without vertebral fractures, while trabecular separation was higher ($P<0.05$) in those with mild (33%), moderate (42%), and severe (55%) vertebral fractures. These data show a clear relationship between vertebral fracture severity and microstructural deterioration in transiliac bone biopsies. The task for the future is to determine how closely these data match the structural deterioration of the distal forearm and tibia as assessed by in vitro mCT.

**The device**

The only in vivo mCT system currently available for human measurements is the XtremeCT (Scanco Medical, 8303 Basersdorf, Switzerland [www.scanco.ch/systems-solutions/preclinical-systems/xtremect.html]). It scans the distal radius or tibia in 2.8 minutes, acquiring a 9 mm-high stack of 110 slices at a resolution of 82 µm. Its ability to accommodate specimen sizes up to 150 mm (height) × 126 mm (diameter) provides scope for clinical applications (Figures 3 and 4).

**Measurement**

Each measurement takes about 2.8 minutes, plus another couple of minutes for calculations that include analysis of the regional or subregional BMD data: total bone, cortical bone, trabecular bone, and some trabecular bone subregions (Figure 5, page 432). Measurement is standardized to a defined distance from the joint endplates of the radiocarpal junction.

**Figure 3.** XtremeCT peripheral quantitative computed tomography scanner at the Charité Hospital, Benjamin Franklin Campus, Berlin. The scanner can be used for both basic research (mouse, rat) and intervention clinical studies.

**Figure 4.** Microcomputed tomography (mCT) scan of a Russian cosmonaut at the Charité before leaving for the International Space Station (ISS).

During measurement the forearm or tibia is fixed in a cast. The structural parameters measured include trabecular number, trabecular thickness, trabecular separation (Figure 6), structural model index (Figure 7), connectivity, anisotropy, and cortical thickness (Figure 5). All structural parameter calculations are based on density measurements.

The mCT methodology has been used in bending tests in rats to determine the relevance of geometry and cortical thickness. It provides data that accurately describe cortical bone geometry and parallel cortical bone strength results obtained by the 3-point bending method. These data meet the criteria of providing quick, reproducible, and accurate answers regarding cortical bone geometry as a predictor of cortical bone strength.
Reference values and reproducibility

The first population-based normative data for in vivo measurements of bone microstructure, published in 2006, were obtained using a prototype of the current mCT system. The results may therefore be less robust than subsequent data. The first reference data obtained with the current device were reported by Dalzell et al. In 2005, Boutroy et al. published mCT precision values of 0.7% to 1.5% for total, trabecular, and cortical densities and 2.5% to 4.4% for trabecular architecture. Postmenopausal women had lower density, trabecular number, and cortical thickness than premenopausal women (P<0.001) at both radius and tibia. Osteoporotic women had lower density, cortical thickness, and increased trabecular separation than osteopenic women (P<0.01) at both sites. Furthermore, although spine and hip BMD were similar, fractured osteopenic women had lower trabecular density and more heterogeneous trabecular distribution (P<0.02) at the radius than nonfractured osteopenic women.

Clinical applications

Recent clinical applications of in vivo mCT include space research (effect of bed rest) and therapeutic trials with various bisphosphonates (risedronate, ibandronate), denosumab, odanacatib, and strontium ranelate, most of which have just been completed and hence are only available as posters or abstracts.

Bed rest studies have measured the effect of weightlessness on bone density and structure in young healthy female and male volunteers. In the Women International Space simulation for Exploration (WISE) study, subjects remained in bed at 6° head down tilt for 60 days. The mCT data showed a clear tendency to a decline in all structural parameters and an increase in trabecular separation, but at 60 days the values did not differ significantly from baseline. We found no significant differences between the control group (no exercise), the exercise group (resistive exercise plus endurance training), and the nutrition group (specific amino acid-enriched diet). In the Berlin Bed Rest-2 (BBR2) study, mCT revealed significant tibial cortex loss in the control group. The bone loss observed in both studies showed high interindividual variation, with no conclusive pattern. A randomized double-blind prospective study compared strontium ranelate (SrR) and alendronate (70 mg once weekly) in postmenopausal women with osteoporosis (spine and/or total hip T-scores ≤–2.5 SD) over 24 months. Preplanned interim analysis of the mCT data at 12 months documented a 5.3% increase in cortical thickness in...
the SrR group compared to the alendronate group, which did not differ from baseline \((P=0.001)\). The blood volume/tissue volume (BV/TV) ratio increased by 2.1\% over baseline compared with the alendronate group \((P=0.002)\), which again did not differ significantly from baseline.

At 24 months, cortical thickness increased by 6.3\% (±9.5\%) in the SrR group and by 0.9\% (±6.2\%) in the alendronate group \((P=0.004)\). The comparative increases in BV/TV ratio [2.5\% (±5.1\%) vs 0.8\% (±3.8\%)] also differed significantly in favor of SrR \((P=0.040)\), as did those in trabecular and cortical BMD: 2.5\% (±5.1\%) vs 0.9\% (±4.0\%) \((P=0.048)\) and 1.4\% (±2.8\%) vs 0.7\% (±2.1\%) \((P=0.045)\). The marker of bone formation, bone alkaline phosphatase, showed an 18\% increase over baseline \((P<0.001)\), while the marker of bone resorption, serum C-telopeptide crosslinked collagen type I, decreased −16\% vs baseline \((P=0.002)\), thereby confirming the dual mode of action of SrR. The results point to significant structural benefit in the distal tibia in women with postmenopausal osteoporosis treated for 2 years with SrR compared to alendronate.

Finite element analysis

Scanco provides specific finite element analysis (FEA) software for their image format. (FEA is a mathematical technique that originated from the need to solve complex elasticity and structural problems in civil and aeronautical engineering.) It is used to simulate tests and measure mechanical and elasticity properties such as stiffness, estimated failure load, trabecular/cortical load distribution, and changes in mechanical properties. For example, the “von Mises stress distribution calculation” generates important data about stress risers in the trabecular network, sites of treatment effect, and increases/decreases in structural strength (Figure 8).1,12

The Pros and Cons

Pros

In vivo mCT determines the structural and material properties of peripheral bone at the cost of acceptable whole-body radiation (<15 µSv) within a few minutes, image processing included. The resulting data extend beyond DXA-BMD measurement and provide an estimate of bone strength that is grounded in fundamental mechanics. The mCT images can be processed by FEA to simulate mechanical testing and derive estimates of stress distribution and failure load. The technique measures cortical bone separately from trabecular bone. Physical density (mass/volume) reflects the material properties of bone. However, we are not yet able to match structural information to mechanical strength tests.
Cons

The local radiation dose is quite high. In the event of procedural error (wrong positioning, movement artifacts, etc.), measurement can be repeated only twice, to a total of three measurements of the same region. Despite the short scan time (2.6 minutes), the very high resolution, visualizing structures down to 82 μm in diameter, produces multiple movement artifacts (MA) (Figure 7). We have identified MA in 38% of a total of several thousand mCT scans, 79% of which were in the forearm and 21% in the tibia. Even after repeating the forearm measurements twice, only 40% were MA-free. An MA grading system may help to increase the quality of forearm analyses. We have detected no pattern to the MA seen in repeated measurements. The problem is less evident with the tibia but still present. It can be decreased by shortening the scan time even further.

Another limitation is the peripheral measurement region. To measure bending stiffness together with cortical thickness and density, measurements should ideally be taken at the midshaft of radius and tibia. But the design of the device allows only very distal measurements. Reference values are not yet robust because of the relatively few normal subjects who have been scanned. A final limitation is that most of the programs for analyzing structural parameters are density based (Figure 9, page 433).

Keywords: osteoporosis; fracture; vertebral fracture; fracture risk; bone strength; dual-energy x-ray absorptiometry; microcomputed tomography; peripheral quantitative computed tomography

Nouvelles techniques d‘évaluation de la santé osseuse

Les fractures périphériques et vertébrales constituent le risque le plus important auquel ont à faire face les patients atteints d’ostéoporose, dans la mesure où elles réduisent leur mobilité physique, leur qualité de vie et leur espérance de vie. Un nombre croissant de données, fondées sur les notions fondamentales de la physique, indiquent que l’absorptiométrie biénérgetique à rayons X n’est pas suffisante pour estimer le risque individuel de fractures et contrôler la réponse thérapeutique. Les déterminants de la résistance osseuse sont la géométrie, les propriétés structurales (distribution osseuse), les propriétés matérielles et la direction des forces. Il est par conséquent essentiel de développer et de mettre en œuvre des techniques plus sophistiquées, par exemple la microdensitométrie. La tomodensitométrie quantitative périphérique de Scanco Medical AG, XtremeCT, est un dispositif relativement nouveau permettant une approximation en vivo de la géométrie, des paramètres structuraux et de la densité du radius et du tibia distaux. Les paramètres structurels comprennent l’épaisseur trabéculaire, la séparation trabéculaire, l’indice de modèle structurel, la connectivité, l’anisotropie et l’épaisseur corticale. D’autres calculs comprennent les rapports entre le volume osseux et le volume tissulaire et les densités minérales osseuses subrégionales et corticales, exprimées en mg/cm². L’analyse par éléments finis (FEA, finite element analysis) basée sur des reconstructions tridimensionnelles de 110 coupes est utilisée pour une cartographie des contraintes. Les principales limitations techniques sont les artefacts dus aux mouvements et le fait que le calcul des paramètres structuraux est basé sur la densité.
New life for old bones

Giving a face to Lucy, King Tut, and an 18th-century shipwrecked scientist

by É. Daynès, France

The eternal life of bones

Tidbits of French history through the trials and tribulations of relics of the illustrious

by C. Portier-Kaltenbach, France
New life for old bones
Giving a face to Lucy, King Tut, and an 18th-century shipwrecked scientist

by É. Daynès, France

Trained as painter and sculptor, Élisabeth Daynès has, since the 1990s, combined scientific research, technological innovation, and art to bring the latest anthropological discoveries back to life. She discovered a passion for prehistory, when in 1988 the Thot Museum in Montignac, France, commissioned her to create a life-size mammoth and a group of Magdaleniens. Her meeting with Dr. Jean-Noël Vignal, a forensic anthropologist, was a turning point in her career as a prehistoric sculptress. He brought her technological skills, as she deepened her knowledge of anatomy. “Lucy,” the Australopithecus, often described as her finest work, is one of the hundreds of her anthropological sculptures scattered around the world in leading museums. She gained international fame in 2006 with her bust of Tutankhamen, depicted on the cover of the 25 international issues of National Geographic, on the occasion of the “Tutankhamen” exhibition devoted to the young Egyptian pharaoh, which attracted huge crowds in Los Angeles and Chicago.
Pensive in the half-light, plump-cheeked and rubicund, the child is the cynosure of the exhibition room. "How cute! He's lovely. And so lifelike." A single glance at the reconstruction of the three-year-old Neanderthal is enough to dispel any lingering thoughts museumgoers may have that his people, those "also-rans" of human evolution, were brutish halfwits on the wrong (the losing) side of humanity.

Beyond feelings of tenderness towards this child from the past, besides an empathy that overcomes latent speciesism, what is expressed here is the promise of another way of seeing these people who are no longer with us (except, it would seem, among our genes: it has recently been claimed that up to 1% to 4% of the present-day Eurasian genome comes from Neanderthal DNA). And it is this new vision of our ancestors that I strive to transmit through my sculptures, where art and science meet.

In my studio in the Belleville neighborhood of Paris, I have for twenty years been recreating australopithecines like the famous Lucy, as well as Homo habilis, Homo georgicus from Dmanisi (Georgia) Homo erectus, and more recent examples of Homo sapiens, like Tutankhamen and Albert Einstein. My clients are museums in France and elsewhere seeking to offer visitors a glimpse into the world of some of our forefathers.

A consuming passion
Looking back there was nothing hinting that one day I would, so to speak, bring back to life our great family of ancestors. After courses in painting and sculpture, at the age of 21 I started to create make-up and masks for the theater and cinema. As fate would have it, in 1988 I received a commission from the Thot Museum at Montignac (near the famous Lascaux caves) to recreate a campsite with a few Magdalenians (the first people to produce cave art). And a mammoth, which was a big challenge: 4.5 meters high at the withers! But in the end it wasn’t this prehistoric pachyderm that fascinated me, rather it was the fossil skulls that the museum’s sci-
Lucy in the Sky with Diamonds...

One of our most famous and distant cousins lived more than 3.2 million years ago in what is present-day Ethiopia, where her osseous remains were discovered on 24 November 1974 by two American anthropologists, Tom Gray and Donald Johanson, the members of an international team that also comprised two Frenchmen, Maurice Taieb, a geologist, Yves Coppens, a paleontologist, and several others.

More than 50 bones and bone fragments were uncovered in a ravine situated in the Afar Depression. The skeleton, 40% complete, was that of an individual of female sex, measuring 1.07 m. Her weight was estimated at 29 kg, and she boasted a brain volume of a mere 450 cm³ vs today’s circa 1200 cm³. At the time of her discovery, AL 288-1—as the diminutive hominid was immediately named for the scientific record—was the earliest known hominid and provided the first proof that bipedalism preceded the increase in brain size in the long path of evolution leading to present-day humans.

As an exhilarated team gathered at the base camp in the evening to celebrate the discovery with the help of well-deserved refreshments and a tape recorder playing the Beatles’ song “Lucy in the Sky with Diamonds,” nonstop, the group unanimously bestowed the nickname “Lucy” upon AL-288-1, a name that was to stick and very rapidly gain universal and affectionate recognition by the public. Lucy later was given her formal scientific name, *Australopithecus afarensis*. The skeleton was reconstructed by Owen Lovejoy of Kent State University, Ohio. In 1997, Élisabeth Daynès was commissioned by the National Institute of Anthropology and History (INAH), in Mexico City, to undertake the reconstruction of Lucy. This was to prove to be one of her most challenging works—which took a full 8 months to finish—particularly as only several small fragments of Lucy’s skull had been found, and she had to extrapolate by using the cranial bones of another *Australopithecus* (cranium AL 417) found later at the same site. The original skeleton of Lucy is preserved at the National Museum of Ethiopia in Addis Ababa.

entists showed me. My enthusiasm was immediate. I knew little of prehistory, but there and then started delving into anthropology and anatomy: I scoured scientific publications, attended major congresses, met the world’s most renowned anthropologists and anatomists. I needed to convince anthropology departments around the world of the rigor and seriousness of my plans, so as to solicit their help and support.

It was far from easy, but I persisted and won them over, and for the last 15 years recognition by the scientific community has enabled me to work with exceptional fossils, the essential basis of my work, access to which would otherwise have been impossible. Moreover, without relations of trust that I have forged with the researchers, without the dialogue and permanent exchanges I have set in place with them, I would never be able to take up the scientific and artistic challenge of facial reconstruction. For recreating from skull fragments the facial shapes and traits of a human being is a long and delicate operation based notably on methods developed for use in forensic medicine.

A little history

These methods go back to the work of the French anatomist Paul Broca (1824-1880) who was the first to consider the human face scientifically and to show the relations between bone...
structures and soft parts. He rigorously described the different proportions of the skulls and faces of several ethnic groups. His results are still valid and confirmed in the anthropology laboratory every day, as anthropologists realize that the skulls of each species have particular features, such as those of the facial bones, that distinguish them from the skulls of other species. For the form of the skull shapes the face: we may all have two eyes, a nose, lips, and a chin, but it is their interrelations and relative proportions that make faces different.

From this principle stems the technique of facial reconstruction, which uses bone fragments to recreate the face they once formed. The soft tissues (fat, muscles, skin), between the skull and the face, define facial contours and topography. The German anatomist Hermann Welcker measured soft tissue thicknesses in 1883, using nine median points in 30 male cadavers. Twelve years later, the Swiss anatomist Wilhelm His examined 28 cadavers using a needle introduced at nine median points and six lateral points. The distance between the surface of the skin and the surface of the bone was calculated by measuring the space separating the point of the needle from a rubber washer pressed against the skin. Wilhelm His was an innovator and used his results to recreate the face of Johann-Sebastian Bach from a skull found during renovation work at the Johanniskirche (St John’s Church) in Leipzig. Many names are associated with the development of facial reconstruction—Kollmann and Buchly, Merkel, Czekanowski, Henri-Martin—but it was unquestionably Mikhail Gerasimov in the Soviet Union who pioneered forensic sculpture. Anthropologist, archeologist, ethnologist, Gerasimov experimented with forensic facial reconstruction using skulls, and in 1935 used his skills in the first facial reconstruction in a criminal case, to enable witnesses to recognize the victim. In 1950, the Soviet Union set up a Laboratory for Plastic Reconstruction where Gerasimov continued his work, recreating, for example, the faces of Ivan the Terrible and the German poet Friedrich Schiller. As he later wrote in his autobiography, *The Face Finder*, Gerasimov was fascinated by the opportunity to “gaze upon the faces of the long departed.”

From the early 1980s, the Americans J. S. Rhine, H. R. Campbell, and C. E. Moore revisited Gerasimov’s work and established tables of soft tissue thicknesses as a function of sex, ethnic group, and build. These values are often still used by some research teams, even if new medical imaging techniques are now able to visualize the inside of the body and distinguish soft tissues from bone.

**Methods**

Whether the commission is for a reconstruction of a Neanderthal boy, a young *Australopithecus* girl, or a Cro-Magnon man, the first step is to make a cast of a skull. This presupposes that the original is complete, or almost, or at least that the researchers have been able to reconstruct the missing parts (often the jawbone) using similar skulls, for the more complete the cast, the more accurate the reconstruction. The proportions and shape of the cast enable me to reconstruct the most logical likeness closest to the original. In my studio I make two copies of the skull. One serves as a support for the sculpture and I keep the other constantly in view so as to stick closely to the bone structures. The same method is then always applied. Using the skull, a veritable identity map of the subject must be drawn by observing the principles used in a criminal investigation. For this I have collaborated since 1996 with Dr Jean-Noël Vignal, forensic anthropologist and paleopathologist, the erstwhile director of the Department of Anthropology of the *Institut de Recherche Criminelle de la Gendarmerie Nationale* (Police Forensic Research Institute) at Rosny-sous-Bois. He uses the latest technologies to uncover a skull’s secrets. To someone who knows how to examine it, a skull speaks volumes. Its shape, for instance, can be used to determine which hominid family it belongs to, but also to estimate age at the time of death, sex (especially if other—postcranial [= all save the skull]—bones are available, notably the pelvis), diseases, deficiencies, and diet. Armed with this information, Jean-Noël Vignal can calculate the values of 18 craniometric points (soft tissue thicknesses) and generate the curve of the forehead, the slope of the chin, and precious indications for the reconstruction of the nose.
The method is spectacularly reliable for *Homo sapiens* and the Neanderthals. Older skulls pose greater problems: no anatomist has ever examined the cadaver of a *Paranthropus* or an *Australopithecus*, and the farther back in time we go the greater the role of informed guesswork, whence the importance of working directly on the bony structures.

It’s all in the look

Once these calculations are materialized using short sticks pushed into the cast to indicate the range of soft tissue thicknesses, I use clay to model the muscle masses for the whole skull. Far from being an artist’s mannerism, this step is essential to visualize the relative proportions of the face and check its self-consistency. It is at this point that I see the face beginning to emerge: the lacrimal punctum gives the position of the eye, the opening of the corner of the mouth, between the first and second premolar, indicates the width of the smile, eye orbits with downturned or upturned ends will determine whether the look is sad or happy. The shape of the nasal spine, when there is one, indicates whether the nose was straight, hooked, or upturned. The width of the nasal fossa provides an estimate of the width of the nose, and so forth. I then add the thickness of the skin and that of the subcutaneous fat. Here too interpretation plays a part: it is impossible know for certain whether a subject was plump or lean, had full or hollow cheeks. I stay close to the bone structures without adding too much fat, but the amount of muscle mass will depend on the indications gleaned from the skull and the postcranials.

Still to be defined are the wrinkles, the grain of the skin, and the last absolutely crucial touch: the eyes. For when completely covered by soft parts, the skull reveals a face that is lifeless, soulless. To breathe life into the reconstruction, I seek to invest it with character, personality, an air of goodness, a spark of intelligence, a moment of fear—an emotion however fleeting must animate the eyes and look. I spend hours working and refining the effect until I find the right expres-
sion. When I was reconstructing Paranthropus, an African hominin (2.6 to 1.3 million years ago), Yoël Rak, Professor of Anatomy at the Tel-Aviv Faculty of Medicine, author of a thesis on Paranthropus, told me many times: “Think that he wasn’t carnivorous, wasn’t aggressive.” That marked me. I gave the Paranthropus a soft look, to the point that when he was finished people passing through the studio could not stop themselves from caressing his head.

Some projects are an even greater challenge. The Science Museum in Barcelona asked me to represent a Neanderthal helping one of his fellows who is dying after a hunting accident. How could I show the wounded Neanderthal’s fear of dying, his friend’s compassion? In the end I found the answer in a photo in an old issue of LIFE magazine, showing a dying American soldier, staring into space, in the arms of a comrade who is looking at him with pain and powerlessness.

“Ecce Homo”

Now it remains to give a body to this head from the distant past. Here too collaboration with scientists is essential to acquire all the data on the postcranial bones (length of long bones, shape of the pelvis and rib cage, muscle insertions…).

For as we delve ever deeper into the past, we move further away from the anatomy of our contemporaries, and the data are scarcer, uncertain, debatable. There are, for instance, numerous hypotheses on the locomotion of our distant ancestors. To reconstruct the gait and postures of Australopithecines, I spent whole days in the Anvers Zoo observing bonobos, great apes that are our closest extant relatives (along with the common chimpanzee). I drew much inspiration from their powerful musculature. This work is important because I am not seeking to erect a static statue or to produce an archetype, but to give my sculpture movement, an attitude...
As he mounted steps leading to the guillotine, Louis XVI is said to have asked “Have we any news of Monsieur Lapérouse?” Such concern moments before death testifies to the immense importance that the King of France accorded to the expedition led by Lapérouse which he himself had ordered as France’s wish to complete the exploration of the Pacific Ocean started by James Cook. In 1788, the expeditions two frigates the Boussole and the Astrolabe ran aground and 220 crew and scientists perished. Forty years later, in 1827, the Irish captain Pierre Dillon found one of the wrecks on the Vanikoro reef, south of the archipelago of the islands of Santa Cruz, the easternmost part of the Solomon Islands, in the Pacific, west of New Guinea. The second wreck was only discovered in the early 1960s, less than one mile from the first. Since 1981, the Association Salomon has been trying to elucidate the circumstances in which Lapérouse and his men disappeared. In 2003, a skeleton was discovered at a depth of 15 meters, under a thick layer of sediment. These bones were sent for identification to the Institut de Recherche Criminelle de la Gendarmerie Nationale in Rosny-sous-Bois in France, and Élisabeth Daynès was commissioned by the French Navy to reconstruct the face of “the unknown man of Vanikoro.” The skeleton was remarkably preserved and virtually complete, which is extremely rare for a body found in seawater. Anthropological and paleopathological studies indicate a man of European type, aged 30 to 34, 1.65 to 1.70 m in height, with smallish muscle insertions suggesting that his musculature was not well developed. The left humerus had a deformation with an angle of 15°, most likely an old consolidated fracture without functional consequences. The right femur was shorter than the left, although this would not have a functional impact on walking. The right fibula had a fracture, but it was not possible to say whether this occurred perimortem or postmortem. The dentition was incomplete (teeth lost postmortem), but was remarkably healthy for the period. The cuspids showed severe abrasion suggestive of bruxism, type of diet, or regular use of teeth-cleaning twigs. The time since death, estimated by means of Nile blue staining, was 201±29 years, which is fully compatible with the disappearance of the two ships of Lapérouse’s expedition.

However, the anthropological and paleopathological characteristics of the skeleton did not match those of this illustrious navigator (who was about 47 years of age when he disappeared). But taking into account the state of the teeth, the weak musculature, the subject’s age and height (above the average for the time), it was hypothesized that the remains likely belonged to a royal navy officer or to a scientist. This was corroborated by the fact that the skeleton was found in the stern of the vessel, where officers were generally accommodated. Reconstruction has given a facial likeness to this unknown, the only latter-day witness to the fate of Lapérouse and his men.

Dr Jean-Noël Vignal
Forensic Anthropologist

Charles Darwin (1809-1882), as reconstructed by Élisabeth Daynès. © 2009 Photo S.Pally/Lookatsciences – Reconstruction Élisabeth Daynès Paris
My greatest pleasure then is to see the surprise and emotion of the researchers with whom I have worked as they contemplate the final result. They are face to face with an ancestor recreated using the latest scientific findings, an ancestor they thought they knew and who had peopled their most secret dreams. And suddenly, in the studio in Belleville, their dream takes shape.

A plea for our ancestors
My main aim is to give the museums or institutions that exhibit my sculptures a teaching tool that will encourage visitors to think about our origins through a face-to-face encounter with a representative of a prehistoric population. I hope in some small way to enhance understanding of the physical appearance of these prehistoric men and women from our past and to rehabilitate them, banishing forever an all-too-common perception of them as brutish and dull-witted. Through my work, I hope to change such attitudes and to help people recognize the extraordinary achievements of our hominid ancestors over millions of years.

Une nouvelle vie pour de vieux os : Lucy, Toutankhamon, et un scientifique naufragé du XVe siècle retrouvent leur visage
Dans son atelier de Belleville à Paris, Élisabeth Daynès exerce un métier rare : sculpteur en anthropologie. En collaboration avec des anatomistes, des anthropologues, des archéologues, et des préhistoriens elle donne une nouvelle vie à des ossements vieux de plusieurs milliers, voire plusieurs millions d’années en sculptant des australopithèques, des paranthropes, des néanderthaliens. Aux confins de l’art et de la science, la paléoartiste utilise des techniques de reconstruction faciale issues des méthodes mises au point pour la criminologie. Des méthodes devenues aujourd’hui très fiables avec les progrès de l’imagerie médicale mais qui s’enracinent dans les recherches du suisse Wilhelm His qui reconstituait à la fin du XVe siècle le visage de Jean-Sébastien Bach à partir d’un crâne présumé qui avait été exhumé de l’église Saint Jean de Leipzig (Johanniskirche), et dans celles plus récentes du russe Mikhail Gerasimov, premier anatomiste à reconstituer un visage dans le cadre d’une affaire criminelle en 1935. Travaillant essentiellement pour des musées dans le monde entier sourciers de montrer à leurs visiteurs d’anciens représentants de la grande famille humaine plus vrai que nature, chaque sculpture, dans un dialogue permanent avec les chercheurs, exige des mois de travail pour redonner un visage et une silhouette à l’un de nos aïeux disparus. Le résultat sert efficacement l’objectif de l’artiste : changer le regard de ses contemporains sur nos ancêtres.
Pharaoh Ramses II almost started a diplomatic row between Egypt and France in 2006—more than 3200 years after his death. Or rather his hair did. French police found a few tufts (of a rather fetching auburn, even after all these years) when they raided the home of postal worker Jean-Michel Diebolt in the Alpine town of Grenoble. It turned out that Diebolt had done research on the mummy in the 1970s and bequeathed the pharaonic keratin to Diebolt fils, who offered it for sale on the Internet, for 2000 euros.

Such relic-mongering for gain, monetary here, but also spiritual or secular, has a long and colorful history. A couple of Venetian merchants may have started the craze back in 828 when they stole Saint Mark’s bones from a church in Alexandria and took them back to Venice. From then onwards, human remains were on everyone’s wish list—Buddha’s teeth, Saint Matthew’s legs (all eleven of them), Voltaire’s heart, Napoleon’s hair—but especially bits of bone: bones are long-lasting, don’t stain, and can be fashioned into trinkets. But after centuries of bony prominence, the whole business became, well, ossified. Nowadays, there’s a more personal touch to commemoration. Why bother with body parts from people you’ve never met, however illustrious, when you need look no further than your nearest and dearest? Take a deceased loved one or pet, cremate, extract carbon, heat and compact for months, and voilà a diamond. Ashes to ashes, dust to diamond.

When Voltaire died, his admirers took his heart and brain, a heel bone and a few teeth, Descartes’ index finger was pocketed by the French ambassador to Sweden, rings were sculpted from his pelvic bone, and his skull, after many twists and turns, is now on display at the French National Museum of Natural History. Vivant Denon’s amazing reliquary contains bone fragments belonging to Abélard and Héloïse, Molière, La Fontaine, El Cid, and others. The bones of the famous live on for eternity in museums, churches, and even private homes..."
Earthly fame has seldom been rewarded with everlasting rest. Rather, peddlers of indulgences, collectors, articulators of bones have oft preyed upon the mortal remains of men and women famed in their lifetimes for righteousness or might, derring-do, or nimbleness of mind. Displayed for the curious and gullible, bought and sold, the “choicest morsels” among relics sacred and profane have always been bones.

From holy relics...

In Christianity
One of the most “encyclopedic” collections of relics of early Christian saints, in the form of little bits and chips of bone placed in ornate reliquaries, along with other larger holy remains, can be found in the Abbey of Saint-Victor in Marseilles.

Of the Abbey, one of the first on French soil, only the church remains after the destructions wrought by the French Revolution in the 18th century. The squat square-shaped two-towered crenellated edifice, which looks more like a toy castle than a religious building, overlooks the Old Port (Vieux-Port) of Marseilles and is a must visit. It was named after the eponymous saint martyred in Marseilles in 304 and founded in the 5th century by John Cassian (ca 360-435), who “imported” oriental monastic spirituality to Europe from the deserts of Palestine and Egypt. Saint-Victor exemplifies the importance for the faithful of being able to relate to their illustrious predecessors by enshrining their remains in their churches. Preserving relics of the saints and martyrs is a tradition that goes back to the dawn of the Church. However, the cult of holy relics properly flourished throughout the Middles Ages and reached its apogee in 13th-century Europe. Every saint was reputed to have touched God, so the least splinter of his bones was deemed sacred and, it was believed, had the miraculous capacity to protect its owner against all manner of ills. And so any good Christian would hope to procure one.

Louis IX (1214-1270), King of France, was one of the greatest collectors in the West and bought up everything linked, however tenuously, with the Passion of the Christ. The centerpiece of his collection was Christ’s Crown of Thorns, which he bought in 1238 from a Venetian merchant to whom it had been pawned for 135,000 livres by Baldwin II of Courtenay, the last and impecunious emperor of the Latin Empire of Constantinople. Louis IX’s precious acquisition cost three times more than the Sainte-Chapelle built to house it on the Île de la Cité in Paris. Today the relic in the treasure of Notre-Dame cathedral, a 2-minute walk from the Sainte-Chapelle, and is presented on the first Friday of every month to the veneration of the faithful at 3PM (the purported time of the Crucifixion), as well as on Good Friday. Its official custodians still are the Knights of the Equestrian Order of the Holy Sepulcher of Jerusalem, whose current Grand Master is John Patrick Foley, an American Cardinal.

When he died, Louis IX, who was considered a living saint, was promptly transmuted into relics: his body was boiled in wine and his bones were held in a silver casket. But not for long, since from 1308 the bones were shared among vari-
ous churches, a fate that was perhaps to be expected following his canonization as Saint Louis at the close of the 13th century. On the eve of the French Revolution of 1789, various Parisian churches still possessed one of his ribs, a finger, a bone from his hand, as well as his skull and a jawbone. Curiously, his heart is preserved at the cathedral of Monreale, in Sicily.

In 16th-century Europe there was such a glut of saintly bones in circulation that in 1543 the Protestant theologian John Calvin denounced their proliferation and the accompanying unbridled trade in his *A Treatise on Relics*. Calvin thought it behooved him to point out to guileless believers that supposed relics were often held in more than one place at the same time. Leaving aside Christ’s hair, chin whiskers, and milk teeth, and the Virgin Mary’s breast milk, was it reasonable to suppose that there were also three foreskins of Christ, eleven legs of Saint Matthew, thirty-two fingers of Saint Peter, ten heads of Saint Léger, and three bodies of Saint Agnes? Calvin’s treatise doubtless dampened the enthusiasm of collectors, but failed to stop the veneration of holy relics, to the point that even today the Vatican unblinkingly admits that it owns two heads of Saint Peter: one within the Vatican City in Rome, in Saint Peter’s Basilica, and one without, at the Papal Archbasilica of Saint John Lateran. Pilgrims fond of the Apostle Peter are therefore spoilt for choice, and can collect their thoughts alongside either head, with the benediction of the Holy See.

In other religions

Rest assured, Christianity is not the only religion to prize such collections. Although Muslims have none of Mohammed’s bones, hairs from his beard are on display in the Topkapi Museum in Istanbul. As for Buddhists, in Sri Lanka they have one of Buddha’s teeth, and the ashes from his funeral pyre.

More surprisingly, a country as “modern” as the United States is a venue of choice for relics, albeit in an “ecumenical” spirit. The relics of Saint Louis travel regularly to Louisiana, for display in the Saint Louis Cathedral in New Orleans (note that the name Louisiana has nothing to do with Saint Louis; rather this vast territory, which originally extended to the Great Lakes, was named by the French explorer René Robert Cavelier de La Salle in 1682 in honor of the Sun King Louis XIV). As to the Buddha, his ashes are held at the United Nations headquarters in New York City, donated by Thailand, Sri Lanka, and Burma in thanks for the international recognition of the Day of Vesak, commonly equated with the Buddha’s day of birth, but in fact encompassing his birth, enlightenment (nirvana), and death.
Relics then can play a political role and further friendship between peoples: in 2006, as a gesture of goodwill and to express desire for dialogue and cordial relations with Orthodox Russians, the Vatican lent Russia the hand that Saint John the Baptist is reputed to have used to baptize Christ.

...to the bones of the famous

◆ Man, this admirable creature

The Age of Enlightenment in the 18th century changed the way holy relics were viewed. The existence of God was called into question, and if God does not exist then clearly man is the most fascinating creature in this lowly world, and if there is no resurrection of the flesh or soul, no eternal life, all that remains of him after death is his bones. Thereafter, the craze was for bits of remarkable people.

When the French philosopher Voltaire died, his admirers took his heart and brain, a heel bone and a few teeth. The Marquis de Vilette kept the heart in a small mausoleum inscribed with the words: “His heart is here, but his spirit is everywhere.” A century later, the heart in question was the subject of a sordid quarrel. Skeletons were desecrated and bones dispersed, but many vandals also took their pick of gruesome souvenirs. First come first served: a leg here, an arm there, a few teeth, wisps of hair, whiskers. The Sun King, Louis XIV (who built the Château de Versailles), lost his last few teeth during these days of pillaging in the summer of 1793. Meanwhile, in the south of France, the sans-culottes (meaning “without knee-breeches,” in reference to the poorer members of society) opened the tomb of the famous 16th-century apothecary and reputed seer Nostradamus, and drank out of his skull. The beverage is not recorded, but vin ordinaire is probably a safe bet.

While no one was overly bothered that royal bones should be strewn across byways and highways, could not the bones of illustrious men and women beloved of the people serve...
the cause of the Revolution? As goblets, perhaps, so worthy citizens could drink to the health of the nation? With this idea in mind, the bones of the playwright Molière and of the poet La Fontaine were taken to the Paris Hôtel des Monnaies (which struck coins and medals). Fortunately, this egregious plan was stymied by political events which, in those troubled times, were moving at a frightening pace. The chemist at the Hôtel des Monnaies did, however, send a piece of Molière’s jaw to the Comédie-Française, the great Parisian theater, where it remains to this day, not far, it may be added, from a statue whose base contains Voltaire’s brain, swapped by its owners in 1924 for two free seats in the stalls for twenty years.

Descartes fared no better. A few years after his death in Stockholm in 1650, his body was dug up for return to France. The French ambassador overseeing the exhumation pocketed part of the philosopher’s index finger, considering it “the instrument of immortal writing.” Meanwhile, one of the Swedish guards on duty pilfered the skull and flogged it to pay a few debts. Years later, when entrusted with transferring the philosopher’s remains, the archaeologist Alexandre Lenoir—who devoted his life to saving historical monuments, tombs, and other treasures from the destructive fury of the French Revolution—filched a pelvic bone and sculpted rings for a few friends. As for the skull, it resurfaced in 1821 when it was put up for sale at 37 francs, along with the possessions of a certain Sparman, the manager of a gambling den in Stockholm. It subsequently came into the hands of the Swedish chemist Berzelius. Knowing that the “rest” of Descartes was in France, Berzelius packed the cranium in a pretty hatbox and sent it to his French colleague Georges Cuvier, one of the pioneers of a whole new discipline known variously as phrenology, craniology, craniometry, or physiognomy. Cuvier and the like-minded Franz Joseph Gall, Paul Broca, and George Combe claimed that personality traits could be divined by examining the shape of a person’s braincase.

As luck would have it, there was at the time a steady supply of skulls thanks to the zealous use that had recently been made of “Madame Guillotine” (also dubbed “The National Razor”) in the Place de la Révolution (now Place de la Concorde) in Paris. One such came from Charlotte Corday. A noted figure in the history of France, Charlotte had in July 1793 assassinated Jean-Paul Marat, scourge of the “enemies of the Revolution” and one of the most influential politicians during The Terror, the 14-month period when revolutionary fervor was
at its height and perhaps as many as forty thousand victims had been guillotined. That’s something like one every 8 minutes for a 12-hour working day (no vacation or days off, la Révolution oblige). Charlotte’s skull later found its way into the possession of a fervent enthusiast of craniology, while the rest of her was lost to history.

A little over a century later, in 1889, the skull turned up at the Exposition Universelle organized on the occasion of the centenary of the Storming of the Bastille, the flashpoint of the revolution. A small label informed the curious that its owner was Prince Roland Bonaparte, a nephew of Napoleon. At the Prince’s request, five experts examined the skull. Two declared that in no way was it that of a criminal; the other three had rarely seen a more villainous-looking specimen. So much for experts. Fingering the contours of the heads of the famous or infamous was all the rage in the 19th century. The heads of criminals naturally excited much interest. Gall studied the skulls of Cartouche (17th-century highwayman) and Lacenaire (19th-century murderer and would-be wordsmith), and of the Marquis de Sade, not to mention that of Descartes who, wherever he (or rather the rest of him) was, must have thought things had come to a sorry pass to be lumped together with these scoundrels. “I think, therefore I am (not like them).”

**THE FABULOUS RELIQUARY OF VIVANT DENON**

“The Emperor’s eye” was how Goethe nicknamed Dominique Vivant Denon (1747-1825), creator of the Louvre Museum, Director General of Fines Arts for more than fifteen years during the First Empire (1804-1814) and the Restoration (1814-1830). Instructed by Napoleon to gather for the Louvre the most exceptional collection of works of art in Europe, Denon went about his task with brio, while quietly amassing on the side his own personal collection. Among his treasures was a strange reliquary from the Renaissance: bone fragments from El Cid and his wife Doña Jimena Díaz, pieces of bone from the 12th-century lovers Abélard and Héloïse, hair from Agnès Sorel (mistress of Charles VII of France) and of Inês de Castro (lover of Peter I of Portugal), part of the mustache of Henri IV of France, a piece of the shroud of Turenne, Louis XIV’s brilliant Field Marshall, bone fragments from Molière and La Fontaine, one of Voltaire’s teeth, Napoleon’s hair, his autograph, a piece of the bloodied shirt he was wearing on the day of his death, a leaf from a willow that weeps over his tomb on the island of Saint Helena. For Denon it was enough that there was a certain inspiration, a glimpse of the sublime, something Homeric in the life of the person thus remembered. Was he not himself an engraver, watercolorist, diplomat, writer, traveler, archeologist? Had he not rubbed shoulders with Louis XV, Madame de Pompadour, Catherine the Great in Saint Petersburg, Frederick the Great at Potsdam, and in Naples with the nefarious Count Alessandro di Cagliostro (once held for nine months in the Bastille on suspicion of involvement in the Affair of the Diamond Necklace, before the charges were dropped for lack of evidence)? Had he not followed Napoleon into Egypt where he had many brushes with death?

Denon’s remarkable reliquary is today displayed in the Musée Bertrand at Châteauroux, in Touraine, its contents a moving homage to famous figures in the history of France.

**Vivant Denon’s reliquary, in Renaissance style (44 cm), now at the Musée-Hôtel Bertrand de Châteauroux.** © Photo by Claude-Olivier Daré/Musées de Châteauroux, Indre, France. With kind permission.

**Detail of the reliquary, showing one its four sides which contains bone chips belonging to Molière and La Fontaine; a piece of cloth from a garment belonging to Marshal Turenne, one of Voltaire’s teeth, a few whiskers from Henri IV and a strand of hair from General Desaix.**
Even Napoleon couldn’t escape the craze. When he died on 5 May 1821, after six years of captivity on the island of Saint Helena, he was autopsied by Dr Antommarchi, who also applied “Gall’s method.” And the startling conclusions of this scrupulous examination of Napoleon’s cranium? Well, that the Emperor had the bump corresponding to—yes, you guessed—conquest. Shrewd indeed. Vive la craniologie! Now, as we are, so to speak, on Saint Helena along with Napoleon’s mortal remains, let us tarry awhile. It goes without saying that the greater a person’s notoriety, the greater the likelihood he will be sliced up post mortem and become part of a collection (or two). And this is exactly what happened to Napoleon. The story of the imperial hair is doubtless the best known.

Napoleon’s brother was in the habit of saying that there was enough supposed imperial hair around to be woven into a huge carpet. In passing, it is worth noting that Napoleon had generously given locks of his hair to his nearest and dearest, as well as to a few admirers. As it turned out this was most prescient since scientists were later able to assay arsenic in the hair and to infer that there had been suspicious amounts in the imperial body. But that’s another (forensic) story.

And what of the other imperial bits and bobs? Well, during the autopsy, Dr Antommarchi secreted in the pocket of his large white apron two rib fragments and a tendon, not to mention a piece of the “imperial penis,” which he gave to the priest who had administered the last rites to the Emperor, the Abbé Vignali. Quite what was going through the good doctor’s mind when offering a piece of imperial genitalia to a man of God is anyone’s guess. The medically preserved penile relic remained in the Abbé’s family until 1969, when it was auctioned at Christie’s for the trifling sum of thirty-eight thousand euros to an American urologist by the name of John K. Lattimer, who kept it in a safety-deposit box at the Columbia Presbyterian Hospital in New York. And what would it fetch today? No need perhaps for wild speculation since Dr Lattimer died two years...
ago and it may only be a matter of time before this curio of Napoleonic masculinity once more finds its way into an auction house.

Judging by the success of auctions of Napoleonic body parts (one of his teeth went for twenty thousand euros in 2005), these relics and bones of the good and the great are not merely memento mori (literally, “remember that you must die”), curios of dubious taste suspended in time (and perhaps in formaldehyde) for lovers of the macabre. Strange though it may seem, illustrious remains are much in the news. Not too long ago Mozart’s skull made the headlines. As for its authenticity, the owner pointed to a decayed tooth and reminded one and all that Mozart was complaining of toothache shortly before he died. No doubts there then.

Only last year, the French government seriously envisaged organizing (yet another) transfer of Descartes’ skull, this time from the Musée de l’Homme in Paris, where it has been held for thirty years, to the school in Touraine where the philosopher had studied as a boy. Historians were outraged that the authorities were more concerned with displaying the skull than with reuniting it with his skeleton, held in the Parisian church of Saint-Germain-des-Prés. What’s more, no comparison of DNAs from the two had been planned. In the end the plan was shelved.

◆ Fetishistic forefathers?
Of course, to our eyes, all these relics have something repugnant about them and we find it hard to understand what prompted our forebears to horde bits of skin and bone and teeth. Yet little by little, as science has advanced, our view of these organic relics has changed. They now seem precious, because they may contain the DNA, the unique and virtually indestructible identity card of the individual from whom they came. In a way, these relics are proof of the existence of a form of everlasting life.

Studies on the DNA of organic relics has many a time resolved historical mysteries. If someone hadn’t kept a polyp from Anna Anderson, who spent her whole life claiming to be the Grand Duchess Anastasia, one of the daughters of Nicholas II, the Tsar of Russia murdered with his whole family at Yekaterinburg on 17 July 1918, it wouldn’t have been possible to prove that she was, in fact, unrelated to the Romanovs.

Perf the heart of a boy (the heir to the French throne, Louis XVII) who died in the Temple Prison during the French Revolution had not been kept, together with hairs from the head of Marie-Antoinette, it would have been impossible to prove that the child was indeed the queen’s son. And had not fervent admirers of Beethoven got hold of some threads of the composer’s hair, science would never have been able to discover that he was probably afflicted by chronic lead poisoning, which may explain his notorious mood swings.

Then there is the story of Neil Armstrong, commander of the 1969 Apollo 11 mission and the first man to walk on the moon. A while ago he realized that his barber was cleaning up at every trim. Marx Sizemore, the owner of Marx’s Barber Shop in Lebanon, Ohio, was keeping Armstrong’s hair to sell to collectors, in one case for three thousand dollars. Incensed, Armstrong took legal action against the barber, who claimed to have sold the hair to an agent of John Reznikoff, an American listed by Guinness World Records as the owner of the world’s largest collection of hair from famous people. Insured for a million dollars, the collection includes 115 locks from illustrious figures including Charles Dickens, Abraham Lincoln, Marilyn Monroe, John Kennedy, Albert Einstein, Elvis Presley, and, of course, Napoleon.

Perhaps then we are a tad hasty in expressing disgust at our ancestors’ penchant for “tidbits” of the famous. Readers would do well to remember that there are companies in America, Russia, Switzerland, and the United Kingdom that create diamonds from the ashes of their clients’ dearly departed. Ash contains carbon; diamond is nothing but. So, under conditions that “recreate the forces of nature”—months at extreme...
pressure ($6 \times 10^9$ Pascals, i.e., 60 million times atmospheric pressure) and high temperature (1600-2000°C)—carbon from the cremated remains (shortened to “cremains”) of a loved one or a pet can be converted to diamond. Ashes to ashes, dust to diamond… that is the new fad in terms of relics. Which puts a whole new slant on the Marilyn Monroe song: diamonds really could be “a girl’s best friend.” And not just cremated remains. Hair too. In 2006, one of these companies created three diamonds from ten strands of Beethoven’s hair (plus a pinch or two of exogenous carbon) from the collection of—yes, that’s right—John Reznikoff. Is turning loved ones into brooches, pendants, or perhaps body piercing jewelry really that different from making a ring from Descartes’ bones?

Who now dares smile superciliously at the foibles of those 18th-century eccentrics who sought to preserve their great men in the form of drinking vessels with which to toast the nation’s health? Far from being laughable, these practices bore witness to a profound truth: from time immemorial men and women have shared a craving for immortality.

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**LES OSSEMENTS, PARCELLES D’ÉTERNITÉ : MORCEAUX CHOISIS DE L’HISTOIRE DE FRANCE À TRAVERS LES RELIQUES D’ILLUSTRES PERSONNAGES**

En 2006, le pharaon Ramsès II, ou plutôt ses cheveux, ont été à deux doigts de provoquer un incident diplomatique entre la France et l’Égypte, et ce plus de 3 200 ans après sa mort. En effet, la police française a retrouvé quelques mèches (châtain aux reflets cuivres ma foi très seyant, même après tant d’années) lors d’une perquisition au domicile d’un facteur grenoblois dénommé Jean-Michel Diebolt. Il s’est avéré que Diebolt père avait fait des recherches sur la momie dans les années 70 et légué la kératine pharaonique à Diebolt fils, qui voulut la vendre sur Internet pour 2 000 €. Un tel trafic de reliques, ici pour s’enrichir financièrement, mais ailleurs aussi de façon spirituelle ou profane, procède d’une longue et pittoresque histoire. L’engouement aurait débuté avec quelques marchands vénitiens qui, en 828, dérobèrent les os de saint Marc dans une église d’Alexandrie pour les rapporter à Venise. Depuis lors, la demande pour les restes humains ne s’est jamais tarie, qu’il s’agisse des dents de Bouddha, des jambes de saint Matthieu (il y en a au moins 11 !), du cœur de Voltaire, des cheveux de Napoléon… mais avant tout des ossements : ces derniers se conservent indéfiniment, ne tachent pas et peuvent être transformés en colifichets. Mais après des années de « prééminence »… enfin, prééminence… osseuse, le marché s’est en quelque sorte « consolidé » en acquérant une dimension plus personnelle. Pourquoi s’enticher de restes d’humains qu’on a jamais rencontrés, même s’ils sont illustres, alors que nos chers et tendres sont sous la main ? Prenez un proche ou un animal de compagnie passé à trépas, incinérez-le, extrayez-en le carbone, chauffez-le et compactez-le pendant des mois… et voilà un diamant ! Tu es poussière et tu retourneras en diamant…
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