Perspective on post-menopausal osteoporosis: establishing an interdisciplinary understanding of the sequence of events from the molecular level to whole bone fractures

L. M. McNamara*

Department of Mechanical and Biomedical Engineering, National University of Ireland Galway, Galway, Ireland

Current drug treatments for post-menopausal osteoporosis cannot eliminate bone fractures, possibly because the mechanisms responsible for bone loss are not fully understood. Although research within various disciplines has significantly advanced the state of knowledge, fundamental findings are not widely understood between different disciplines. For that reason, this paper presents noteworthy experimental findings from discrete disciplines focusing on post-menopausal osteoporosis. These studies have established that, in addition to bone loss, significant changes in bone micro-architecture, tissue composition and micro-damage occur. Cellular processes and molecular signalling pathways governing pathological bone resorption have been identified to a certain extent. Ongoing studies endeavour to determine how such changes are initiated at the onset of oestrogen deficiency. It emerges that, because of the discrete nature of previous research studies, the sequence of events that lead to bone fracture is not fully understood. In this paper, two sequences of multi-scale changes are proposed and the experimental challenges that need to be overcome to fully define this sequence are outlined. Future studies must comprehensively characterize the time sequence of molecular-, cellular- and tissue-level changes to attain a coherent understanding of the events that ultimately lead to bone fracture and inform the future development of treatments for post-menopausal osteoporosis.

Keywords: bone; post-menopausal osteoporosis; tissue composition; biomechanics; cell biology

1. INTRODUCTION

Osteoporosis is a disease which causes bone loss and fractures and leads to severe pain, deformity and in certain cases secondary complications that result in death (Johnell et al. 2004). The disease is classified clinically to be either primary or secondary osteoporosis. Primary osteoporosis refers to both bone loss occurring in post-menopausal women (type I) and bone loss owing to the normal ageing process (type II). Secondary osteoporosis refers to bone loss that ensues as a secondary effect of other diseases or drug treatment. Post-menopausal osteoporosis (type I) is the most common form of the disease and is believed to initiate when oestrogen production is deficient following the menopause (Riggs et al. 2002). During normal physiology, bone can renew itself to maintain bone strength and mineral homeostasis by means of coordinated cellular activity whereby osteoclasts continuously resorb aged or damaged bone and osteoblasts reform new bone tissue in its place (Parfitt 1984). However, during oestrogen deficiency, osteoclasts remove excess bone without adequate formation by osteoblasts. Bone loss ensues when the basic trabecular bone units (trabeculae) become thin and resorb completely or fracture (Eriksen et al. 1990), and ultimately bone fractures occur under minimal trauma in the bones of the hip, wrist and spine.

Approximately 40 per cent of women over 50 will suffer a fracture related to post-menopausal osteoporosis during their lifetime (Melton et al. 2005). Current diagnosis of the disease involves the use of dual energy X-ray absorptiometry (DEXA) to quantify bone mass as a surrogate to predict the likelihood of
fracture. However, these techniques are inefficient as changes in bone mass alone do not fully explain susceptibility to bone fracture (Dempster 2000; Garnero & Delmas 2004; Wainwright et al. 2005). It is now clear that other features of bone quality including tissue properties, composition, micro-architecture (Parfitt 1987; Comptston et al. 1989; Lane et al. 1998) and micro-damage (Burr et al. 1997; Dai et al. 2004) also influence susceptibility to fracture in combination with the changes in bone mass, albeit that ongoing research seeks to determine the precise contribution.

Hormone therapy and anti-resorptive therapy (e.g., bisphosphonates, selective tissue oestrogenic activity regulators, calcitonins) are popular treatments for bone loss, and, while they have different modes of operation, the common primary aim is to reduce fracture incidence by inhibiting osteoclast resorption and maintaining bone mass (Kloosterboer & Ederveen 2002). However, even with continuous use, these drugs reduce the propensity to fracture by only approximately 50 per cent (Randell et al. 2002), suggesting that the quality of the remaining tissue may be compromised (Flora et al. 1981; Hirano et al. 2000). More recently developed drugs, including parathyroid hormone (PTH), alendronate, ciclosporin A and strontium ranelate, have an anabolic effect on bone and thereby promote new bone formation (Liberman et al. 1995; Neer et al. 2001; Reginster et al. 2003; Yeo et al. 2006). Nonetheless, these drugs still reduce fracture risk by only approximately 40 per cent (Liberman et al. 1995; Neer et al. 2001; Reginster et al. 2003). Furthermore, even with drug treatment, the quality of life of a sufferer of osteoporosis cannot be fully restored as 50 per cent experience disability and 75 per cent never regain the same level of health or independence as they had before suffering an osteoporotic fracture (Compston & Rosen 2002).

While abundant research has been undertaken to identify the exact cause of post-menopausal osteoporosis, the disease is not yet fully understood and the associated bone fractures cannot be eliminated. The high prevalence, inaccuracy in diagnostic techniques and insufficiencies of treatments have made post-menopausal osteoporosis the subject of intense scientific investigation for research disciplines such as biomechanical engineering, materials science and cell and molecular biology. For the most part, these studies have been discrete with little interdisciplinary interaction at the interface of these disciplines. As a result, fundamental findings are not widely understood between different disciplines and, even with the wealth of data produced, there is still no coherent understanding of the cause of bone loss, or the sequence of events that ultimately lead to bone fractures. The objective of this review is to identify significant experimental findings from discrete research fields with a view to promoting an interdisciplinary knowledge base regarding the complex changes that occur at the onset of oestrogen deficiency at the molecular, cellular, tissue and organ levels. Furthermore, this review seeks to correlate the findings where possible, and define important topics for future research.

2. BONE STRENGTH DURING OSTEOPOROSIS

The fundamental concern with the disease of osteoporosis is that the mechanical properties of the bone are compromised to such a degree that bone fractures occur under normal loading conditions, which are not associated with fracture in healthy bones. Consequently, much research has sought to determine how bone loss alters bone mechanical strength. Different measures of mechanical strength include yield strength (load required to initiate failure), ultimate strength (maximum load experienced), elastic modulus (tendency to deform) and toughness (energy absorbed before fracture). Experimental testing of whole bones or bone samples from post-menopausal subjects and animal models has been performed under various types of loading, such as compression, tension, bending or shear. At the whole bone level, the ultimate stress and maximum elastic modulus are reduced in the human osteoporotic vertebrae (Hasegawa et al. 1993), and the maximum compressive strength of vertebral bodies and the bending strength of femora are decreased in the ovariectomized animal model of osteoporosis (Kasugai et al. 1998; Yoshitake et al. 1999). Mechanical tests of volumes of trabecular bone (approx. 5 mm³) have shown a reduction in the continuum (apparent) compressive strength and elastic modulus of osteoporotic bone compared with normal bone (Li & Aspden 1997; Sugita et al. 1999; Yoshitake et al. 1999; Ciarelli et al. 2000). The cause of these changes in mechanical behaviour is much debated; the overall mechanical strength of bone is determined by two features, bone mass and bone quality (NIH 2000; Judex et al. 2003). Bone mass is a quantitative measure of the total bone present in a specific volume of bone selected for clinical analysis as is discussed further below. Bone quality encompasses a number of features of the bone tissue including (i) morphology, (ii) micro-architecture, (iii) composition, and (iv) the degree of micro-damage. Each of these features contributes to different degrees to create the material properties that allow bone to bear the loading conditions experienced during life (figure 1).

2.1. Bone mass during osteoporosis

The reduction in bone mass, occurring during oestrogen deficiency, has long been regarded as the critical event leading to bone fracture. Bone mineral density (BMD) is a quantitative measure of bone mass and represents the total mineral in a selected volume of bone in the hip or in the spine. This has become the gold standard for the clinical diagnosis of osteoporosis (Brunner & Shelton 2002; Kanis et al. 2002). The World Health Organization defines that a person is diagnosed with osteoporosis if their BMD falls below 2.5 standard deviations (T-score) of the BMD of a young adult (Kanis et al. 2003). A fracture is commonly classified to be osteoporotic if it arises in a patient over 50 and the BMD of the fracture site is sufficiently low (Kanis et al. 2001). DEXA is the preferred technology for quantifying BMD (Cummings et al. 2002; Leib et al. 2004), but other diagnostic tools, including quantitative

J. R. Soc. Interface (2010)
computed tomography (QCT), absorptiometry, quantitative roentgen micro-densitometry and quantitative ultrasound (QUS), may provide more accurate measures of BMD (Richardson et al. 1985; van Berkum et al. 1989). Nonetheless, BMD is a poor predictor of fracture risk as changes in bone mass alone do not fully explain susceptibility to bone fracture; for example, only 10–53% of bone fractures that occur in female post-menopausal patients over the age of 65 can be attributed to a BMD level low enough (i.e. T-score< −2.5) for clinical diagnosis of osteoporosis (Stone et al. 2003; Garnero & Delmas 2004; Wainwright et al. 2005). Hence, current research methods now seek to understand whether changes in bone quality might account for the fractures that are not detected by BMD changes alone (Dempster 2000; Stauber et al. 2006).

2.2. Bone micro-architecture and morphology during osteoporosis

Micro-architecture is a term which refers to the microscopic morphology and organization of both trabecular and cortical bone. It is characterized using bone histomorphometry and micro-CT scanning (μCT) to obtain two-dimensional/three-dimensional measurements of the architecture at various resolutions. The estimation of the three-dimensional properties of the architecture from two-dimensional histological sections is known as stereology. Stereology is typically used to characterize bone micro-architecture by quantifying cortical porosity, cortical thickness, trabecular number, trabecular thickness and trabecular connectivity. Connectivity is a stereological measurement used to quantify the topology of various structures by quantifying the number of redundant connections between locations (Russ & Dehoff 2000). Using such approaches, it has been demonstrated that, at the onset of post-menopausal osteoporosis, trabecular thinning, deeper resorption cavities, micro-fracture and loss of trabecular connectivity occur (Parfitt 1987; Compston et al. 1989; Lane et al. 1998). The micro-architecture is statistically significantly more anisotropic than normal, with fewer trabeculae transversing to the primary load axis (Ciarelli et al. 2000). Until recently, it was believed that bone loss occurred throughout the trabecular architecture to the same extent, and that this loss was irreversible. However, with the recent generation of high-resolution in vivo micro-CT scanners, new findings have challenged this premise considerably; Waarsing et al. (2004, 2006) found that, following the initial trabecular thinning in OVX rats, the few remaining trabeculae subsequently slowly increase in thickness. Interestingly, this was not merely due to the disappearance of thin trabeculae,
but rather certain trabeculae were preserved and in fact increased in thickness or merged with each other (Waarsing et al. 2006). Computational finite element models have been developed that incorporate the trabecular micro-architecture using data from high-resolution micro-CT scanning (Faulkner et al. 1997; Homminga et al. 2001; Testi et al. 2001). By applying these models, it has been shown that fracture risk prediction is improved by approximately 13 per cent as compared with predictions based on bone mass indicators (BMD) alone (Testi et al. 2001). This improvement is a result of the inclusion of changes in the trabecular micro-architecture such as resorption cavities, which act as stress concentrations (McNamara et al. 2006a), and thinning and loss of trabecular connectivity. There is also evidence that the micro-architecture of cortical bone is altered in hip fracture patients by means of an increase in porosity in the bone tissue of the femoral neck compared with controls (Bell et al. 1999). Localized thinning of the cortical bone of the femoral neck also occurs in hip fracture patients (Crabtree et al. 2001).

While much research has been dedicated to assessing bone micro-architecture, the whole bone morphology (geometry) has received much less attention. It has been reported that the cross-sectional geometry of femora is altered during osteoporosis to compensate for bone loss (Cordey et al. 1992; Jiang et al. 2008). A separate study reported that a profound loss of cortical bone dramatically alters the shape and dimensions of the medullary canal of the femur, whereas little or no changes occur in the metaphysis or external dimensions of the femur (El-Zaïm et al. 2007).

As a result of these studies, osteoporotic fractures are now believed to occur when both the bone mass and the micro-architecture of the bone are degraded to such an extent that bone strength is reduced and they occur under normal physiological loads. It is unknown whether these changes occur as a direct result of oestrogen deficiency or whether alternative mechanisms are responsible. By studying the other aspects of bone quality such as tissue composition, it may be possible to address this question.

2.3. Bone tissue properties and composition during osteoporosis

While bone loss and architecture have been extensively characterized, it is less clear how the remaining bone tissue is altered. Bone is a porous composite material, consisting of a mineral phase (mainly calcium and phosphorus in the form of hydroxyapatite crystals) and an organic phase (collagen, non-collagenous proteins and cells). The mechanical properties of this tissue are determined by the quantity and mechanical integrity of each phase, the structural organization of the different phases and the physical interaction between them. The organic phase of bone comprises approximately 35 per cent of bone mass and provides post-yield behaviour and strength, whereas the mineral phase allows the tissue to resist deformation under applied loading, which is known as the stiffness of the tissue. Recent studies have now established that, while overall bone mass and BMD are reduced during oestrogen deficiency, the yield strength and elastic modulus of the remaining tissue increased by 40–90% of control values (McNamara et al. 2005, 2006b). These tissue-level changes suggest that post-menopausal osteoporosis may alter the primary constituents of bone tissue, namely collagen, mineral and non-collagenous proteins (NCPs) and previous studies have focused on identifying such changes.

2.3.1. Collagen. Collagen is synthesized by osteoblast cells during the initial phase of formation when unmineralized bone (osteoid) is formed. The collagen molecules are assembled extracellularly and immature ketomine and aldime cross-links are formed, which contribute to the formation of mature pyridinium or pyrrole cross-links. Together, these cross-links result in fibril formation and these fibrils act as a scaffold for bone minerals and provide strength (Eyre et al. 1988; Viguet-Carrin et al. 2006). The collagen in bone is primarily type I collagen, but types III, IV and VI are also present (Miller 1969; Miller 1984). Various changes in the compositional properties of the collagen have been reported at the onset of post-menopausal osteoporosis; a recent study suggests that type I collagen synthesis increases during osteoporosis, whereas earlier studies reported a reduction in the amount of type VI collagen and type III collagen (Bailey et al. 1993). Increased hydroxylation of lysine residues occurs (Batge et al. 1992; Kowitz et al. 1997) and this modulates the nature of the collagen cross-links that are formed extracellularly. Indeed, decreases in the quantity of ketomine, pyridinium (Mansell & Bailey 2003), aldime (Bailey et al. 1993; Oxlund et al. 1996) and pyrrole cross-links have been reported for osteoporotic bone (Knott et al. 1995). Interestingly, these changes were inversely correlated to bone volume and were more prevalent in the femoral neck, a site which is highly susceptible to fracture (Bailey et al. 1993). The details of these experimental studies are summarized in table 1. It has been proposed that such changes increase fracture susceptibility by altering the strength of the collagen network or the normal mineralization of the matrix (Bailey et al. 1993; Bailey & Knott 1999). Increased collagen metabolism (Mansell & Bailey 2003) or molecular-level defects (polymorphisms) in the type I procollagen genes (COLIA1, COLIA2) (Spotila et al. 1991; Grant et al. 1996; Harris et al. 2000; Efstatiadou et al. 2001) may be responsible for changes in collagen content and organization. Further studies are required to fully understand these changes and determine whether altered collagen plays a role in bone loss and fracture. Given that collagen acts as a scaffold for bone minerals, alterations in the collagen framework are likely to be accompanied by altered tissue mineralization.

2.3.2. Non-collagenous proteins. The NCPs in bone, in particular osteopontin, osteocalcin, alkaline phosphatase and bone sialoprotein, are essential for matrix organization by acting as mineral crystal nucleation sites on the organic matrix (Hunter & Goldberg 1993; Tye et al. 2003; Qiu et al. 2004), binding of mineral crystals to the collagen matrix (Tye et al. 2005) and
facilitating cell attachment (Fantner et al. 2005). Therefore, these proteins indirectly regulate the mechanical properties of the collagen–mineral interface. In addition, recent studies now suggest that NCPs influence bone matrix material properties in a direct fashion by forming tough bonds with molecular self-healing abilities (Fantner et al. 2007; Thurner et al. 2009). Interestingly, in vitro studies have shown that these bonds may have both energy dissipation (Fantner et al. 2005) and energy storage capabilities (Fantner et al. 2005; Zappone et al. 2008). These findings are relatively new and further studies are required to determine how such bonds could facilitate energy storage. However, if it can be confirmed that these bonds play a mechanical role in vivo, then this will dramatically alter the current understanding of bone fracture mechanics.

There is limited evidence that NCPs are altered during post-menopausal osteoporosis. While the overall levels of NCPs have been reported to be considerably reduced (Grynpas et al. 1994), other studies report that the levels of circulating alkaline phosphatase, osteocalcin and bone sialoprotein are increased following oestrogen deficiency (Jilka et al. 1998; Faassbender et al. 2000; Zhu et al. 2008). Furthermore, the number of osteopontin-expressing osteocytes increases in bones that tend to resorb after OVX (Ikeca et al. 1996).

The details of these experimental studies are summarized in table 1. Future studies are required to determine whether such changes relate to the overall reduction in bone, or whether tissue-level changes in NCP content also occur. Furthermore, the link between mechanical properties, in particular fracture susceptibility, and NCPs must be delineated.

2.3.3. Mineral. Bone mineralization occurs when mineral crystals are deposited by osteoblasts on the organic matrix, bind in the presence of NCPs and over time the size of the mineral particles increases slowly (secondary mineralization). It is widely accepted that bone strength and stiffness are correlated to the degree of tissue mineralization (Currey 1984). As such, micro-structural changes in tissue properties (McNamara et al. 2006b) suggest that a corresponding change in tissue mineralization should occur. However, conflicting data exist from previous studies; some studies report a decrease in tissue mineral content (Gadeleeta et al. 2000), and others reveal an increase in the mineral content (Dickenson et al. 1981; Boyd et al. 1998; Zioupos & Aspden 2000; McNamara et al. 2006b). These findings have been shown to differ for trabecular and cortical bone; while iliac cortical tissue has a lower mineral : matrix ratio than control, this was not seen for trabecular tissue (McCreadie et al. 2006).

There have also been varying reports that the mineral crystal size is increased (Paschalis et al. 1997; Gadeleeta et al. 2000), decreased or not altered (Baud et al. 1988). Variations in experimental methods, choice of animal model or anatomical location (table 1) might explain this large variability. Indeed, a recent study observed that mineral distribution is more heterogeneous in bone tissue from female fracture patients, with both higher and lower mineralization values than non-fracture control subjects (Ciarelli et al. 2003). Further studies are required to delineate the exact nature of such changes, the mechanisms by which such changes initiate and whether such changes are limited to specific anatomical locations or occur in human osteoporotic bone.

2.4. Osteoporosis and bone micro-damage

Bone micro-damage has long been considered to be a key determinant of bone strength (Burr et al. 1998) and it is also believed that, during normal bone remodelling, osteoclasts maintain bone strength by resorbing mature bone thereby preventing damage accumulation (Frost 1960b; Burr et al. 1985). There is some evidence that the extent of micro-damage is increased during oestrogen deficiency (Dai et al. 2004). This damage may have accumulated as a result of the increased tissue mineralization (McNamara et al. 2006b). Such changes might reduce the fracture toughness of bone tissue and thereby render the tissue more brittle and susceptible to damage, as has been well explored (Mashiba et al. 2001; Burr 2003; Schaffler 2003).

Alternatively, bone tissue may be more susceptible to damage following bone loss when the remaining tissue bears higher loads than normal. It is not known whether such damage is sufficient to deteriorate the mechanical behaviour of the tissue to such an extent as to predispose it to failure. Nonetheless, increased remodelling may be attributable to an accelerated removal of damaged tissue (Burr et al. 1997) and as such this increased damage may indeed play a significant role in bone loss and subsequently fracture. Future studies are required to definitively prove that micro-damage repair is increased during post-menopausal osteoporosis. Such understanding might be gained through a greater understanding of the physiological activity of bone cells throughout the disease state.

3. BONE PHYSIOLOGY DURING OSTEOPOROSIS

The normal bone remodelling process is regulated by bone cells, osteoblasts and osteoclasts, which operate simultaneously in a unit known as the basic multi-cellular unit (BMU). The process initiates when bone-lining cells degrade unmineralized osteoid and increase the expression of growth factors to recruit osteoclast progenitors to differentiate from the haematopoietic progenitors in the bone marrow. These osteoclasts differentiate into mature multi-nucleated cells that attach to bone surfaces, via binding of the integrin αvβ3 and vitronectin, and secrete acids and enzymes to digest bone matrix. Following resorption, additional growth factors are released which recruit osteoblast precursors to differentiate and produce new bone matrix (Parfitt 2005) in the place of resorbed tissue. Osteoblasts also produce growth factors that regulate osteoclast activity. Osteoblasts are derived from osteo-progenitors that reside in the bone marrow and periosteal lining of bone surfaces. These progenitors are induced to differentiate into mature osteoblasts in the presence of various growth factors. Certain osteoblasts become encased in mineralized bone matrix and
Table 1. Changes in bone tissue constituents during oestrogen deficiency (−− indicates a reduction in the amount of constituent; ++ increase in the amount of constituent; +/− the variable changes in tissue constituent).

<table>
<thead>
<tr>
<th>tissue constituent</th>
<th>origin</th>
<th>function/role</th>
<th>study design</th>
<th>key findings</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>collagen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>type I collagen</td>
<td>collagen molecule produced by osteoblasts</td>
<td>provides mechanical strength and acts as a scaffold for mineralization</td>
<td>enzyme-linked immunosorbent assay (ELISA) of bone tissue from normal and osteoporotic human bone</td>
<td>++</td>
<td>Mansell &amp; Bailey (2003)</td>
</tr>
<tr>
<td>type III collagen</td>
<td>collagen molecule produced by osteoblasts</td>
<td>provides mechanical strength and acts as a scaffold for mineralization</td>
<td>SDS-PAGE electrophoresis of femoral bone tissue from normal and osteoporotic human bone</td>
<td>−−</td>
<td>Bailey et al. (1993)</td>
</tr>
<tr>
<td>type VI collagen</td>
<td>collagen molecule produced by osteoblasts</td>
<td>regulates collagen fibre diameter and orientation of the fibres in the tissue</td>
<td>SDS-PAGE electrophoresis and western blotting of femoral bone tissue from normal and osteoporotic human bone</td>
<td>−−</td>
<td>Bailey et al. (1993)</td>
</tr>
<tr>
<td>hydroxylation</td>
<td>intracellular (osteoblast) post-translational modification of collagen molecules</td>
<td>governs the nature of cross-links formed and regulates collagen fibre diameter</td>
<td>amino acid analysis (AAA) of femoral bone tissue from normal and osteoporotic human bone and avian bone</td>
<td>++</td>
<td>Batge et al. (1992), Knott et al. (1995), Kowitz et al. (1997), Mansell &amp; Bailey (2003)</td>
</tr>
<tr>
<td>of lysine residues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hydroxylation</td>
<td>intracellular (osteoblast) post-translational modification of collagen molecules</td>
<td>governs the nature of cross-links formed and regulates collagen fibre diameter</td>
<td>amino acid analysis (AAA) of femoral bone tissue from normal and osteoporotic human bone and avian bone</td>
<td>++</td>
<td>Batge et al. (1992), Knott et al. (1995), Kowitz et al. (1997), Mansell &amp; Bailey (2003)</td>
</tr>
<tr>
<td>ketoimine collagen</td>
<td>immature collagen cross-link formed extracellularly</td>
<td>provides mechanical strength between collagen fibrils</td>
<td>AAA of femoral bone tissue from normal and osteoporotic human bone</td>
<td>−−</td>
<td>Mansell &amp; Bailey (2003)</td>
</tr>
<tr>
<td>collagen cross-links</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aldime collagen</td>
<td>immature collagen cross-link formed extracellularly</td>
<td>provides mechanical strength between collagen fibrils</td>
<td>AAA of femoral and vertebral bone tissue from normal and osteoporotic human bone</td>
<td>−−</td>
<td>Bailey et al. (1993), Oxlund et al. (1996)</td>
</tr>
<tr>
<td>pyridinium collagen cross-links</td>
<td>immature collagen cross-link formed extracellularly</td>
<td>provides mechanical strength between collagen fibrils</td>
<td>AAA of femoral bone tissue from normal and osteoporotic human bone</td>
<td>−−</td>
<td>Mansell &amp; Bailey (2003)</td>
</tr>
<tr>
<td>pyrrole collagen cross-link</td>
<td>mature collagen cross-link derived from the immature ketoimine cross-links</td>
<td>provides mechanical strength between collagen fibrils</td>
<td>biochemical analysis of bone tissue from normal and osteoporotic hens by reaction with ρ-dimethylaminobenzaldehyde</td>
<td>−−</td>
<td>Knott et al. (1995)</td>
</tr>
<tr>
<td>non-collagenous proteins</td>
<td>non-collagenous protein produced by osteoblasts</td>
<td>act as sites for mineral crystal nucleation</td>
<td>biochemical analysis (autoanalyser with ρ-nitrophenyl phosphate) of bone tissue from normal and osteoporotic human bone and enzyme immune assay (EIA) of bone tissue from normal and ovariectomized rats</td>
<td>++</td>
<td>Mansell &amp; Bailey (2003)</td>
</tr>
<tr>
<td>alkaline phosphatase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued.)
differentiate to become osteocytes, which are essential for secondary mineralization (Frost 1960a; Inoue et al. 2006; Gluhak-Heinrich et al. 2007) and can transduce mechanical signals into biochemical signals to osteoclasts and osteoblasts to alter bone mass (Cowin et al. 1991; Lanyon 1993). During normal physiology osteoclasts, osteoblasts and osteocytes work in concert to maintain bone mass.

### 3.1. Osteoclasts and haematopoietic progenitors

Osteoclasts possess oestrogen receptors (ERα, ERβ), which are normally activated to permit protein synthesis when they bind to oestrogen (Braidman et al. 2001). Oestrogen has an inhibitory effect on osteoclasts as the proteins produced following oestrogen binding inhibit bone resorption by decreasing the formation of mature osteoclasts (Oursler et al. 1991b) and increasing osteoclast apoptosis (Hughes et al. 1996). When oestrogen levels are deficient, the number of haematopoietic progenitors increases (Jilka et al. 1992, 1995; Brockstedt et al. 2000b). In addition, osteoclast apoptosis

---

**Table 1. (Continued.)**

<table>
<thead>
<tr>
<th>tissue constituent</th>
<th>origin</th>
<th>function/role</th>
<th>study design</th>
<th>key findings</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>osteopontin</td>
<td>non-collagenous protein produced by osteoblasts</td>
<td>act as sites for mineral crystal nucleation, provide mechanical bonding between collagen fibrils</td>
<td>in situ hybridization of bone tissue of normal and ovariectomized rats</td>
<td>++</td>
<td>Ikeda et al. (1996)</td>
</tr>
<tr>
<td>osteocalcin</td>
<td>non-collagenous protein produced by osteoblasts</td>
<td>act as sites for mineral crystal nucleation, provide mechanical bonding between collagen fibrils</td>
<td>radioimmunoassay of serum obtained from normal and ovariectomized mice</td>
<td>++</td>
<td>Jilka et al. (1998)</td>
</tr>
<tr>
<td>bone sialoprotein</td>
<td>non-collagenous protein produced by osteoblasts</td>
<td>act as sites for mineral crystal nucleation and regulate crystal growth, provide mechanical bonding between collagen fibrils</td>
<td>radioimmunoassay of serum obtained from human osteoporotic patients</td>
<td>++</td>
<td>Fassbender et al. (2000)</td>
</tr>
</tbody>
</table>

**mineral tissue mineral content**

| calcium phosphate produced by osteoblasts and binds to collagen in the presence of non-collagenous proteins | regulates the mechanical stiffness of bone tissue | fourier transform infrared micro-spectroscopy (FTIRM) of femoral bone tissue from normal and osteoporotic human bone | -- | Boyde et al. (1998) |
| calcium phosphate produced by osteoblasts and binds to collagen in the presence of non-collagenous proteins | regulates the mechanical stiffness of bone tissue | quantitative backscattered electron imaging (qBEI) of bone tissue from ovariectomized monkeys | -- | Gadeleta et al. (2000) |
| calcium phosphate crystals mature and grow over time | regulates the mechanical stiffness of bone tissue | FTIRM of iliac and vertebral bone tissue from normal and osteoporotic human and monkey bone | ++ | Paschalis et al. (1997), Gadeleta et al. (2000) |
| distribution of mineral crystal deposition within the bone matrix | regulates the mechanical stiffness of bone tissue | backscattered electron microscopy of bone tissue from normal and osteoporotic human bone | ++ | Ciarelli et al. (2003) |
is inhibited and these events together lead to prolonged resorption, deeper resorption cavities and trabecular perforation (Bell et al. 1996). The perforated trabeculae are removed by further remodelling and this loss of bone mass increases the fragility of bones, resulting in a greater propensity for fracture. These findings and details of the experimental studies are summarized in table 2.

Although osteoclasts are the primary target for conventional drug treatments, inhibiting osteoclast activity alone does not completely prevent osteoporotic fractures from occurring. Interestingly, a recent study
found that oestrogen inhibited osteoclast formation in vitro when osteoclast precursors were cocultured with osteoblastic cells, but that the hormone did not directly affect osteoclast precursors when they were cultured alone (Michael et al. 2005). This study strongly suggests that oestrogen's regulation of osteoclast activity is governed by osteoblasts (Michael et al. 2005) and hence the role of osteoblasts during post-menopausal osteoporosis merits further research.

3.2. Osteoblasts, osteocytes and osteoprogenitors

Osteoblasts and osteocytes also possess receptors for oestrogen (Braidman et al. 2001), hence their function may be affected when oestrogen production is deficient during post-menopausal osteoporosis. Evidence suggests that oestrogen allows the normal response of osteoblasts and osteocytes to loading (Lanyon 1996) and osteoblastic cells deprived of oestrogen display deficient osteogenic responses to mechanical stimuli in vitro (Sterck et al. 1998; Jessop et al. 2004). Oestrogen deficiency induces osteocyte apoptosis (Tomkinson et al. 1997; Kousteni et al. 2001), which might result in hypermineralization of the surrounding tissue (Frost 1960a; Kingsmill & Boyde 1998; Boyde 2003).

In addition, the organization of the osteocyte network is altered (Knothe Tate et al. 2004) and osteocyte density is increased in post-menopausal bone, which might indicate that osteoblasts produce less bone per cell during osteoporosis (Mulleder et al. 1996). Together, these changes might alter the mechanoresponsiveness of the tissue (Tatsumi et al. 2007).

Changes in osteoblastic activity during oestrogen deficiency might initiate when the cells are in the immature osteoprogenitor state. Indeed, it has been reported that the number of mesenchymal progenitors capable of committing to the osteoblast lineage (osteoblastogenesis) increases in the murine bone marrow in an ovariectomized animal model of osteoporosis (Jilka et al. 1998; Rosen 2000b). In contrast to these findings, no difference has been found in the proliferation of osteoprogenitors from normal in human osteoporotic bone (Stenderup et al. 2001). In vitro cell culture experiments confirm that mesenchymal stem cells from osteoporotic patients maintain their ability to differentiate along the osteogenic lineage (Justesen et al. 2002). Further studies are required to fully understand the differences in these experimental findings (table 2) and to comprehensively characterize the physiology of osteoprogenitors, osteoblasts and osteocytes during post-menopausal osteoporosis. Given the tightly coupled nature of bone cell activity, such studies may provide further insight into osteoclast activity and bone loss.

4. MOLECULAR SIGNALLING AND GENE EXPRESSION DURING OSTEOPOROSIS

A number of cytokines and growth factors govern the activity of bone cells during normal bone remodelling. In particular, cytokines and growth factors, namely the receptor activator of nuclear factor kappa B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) and osteoprotegrin (OPG) secreted by osteoblasts and bone-lining cells, are the primary regulators of the process. Normal osteoclast activity is governed by RANKL and M-CSF, which bind to the paracrine receptor RANK on osteoclast precursors and stimulate proliferation and differentiation into mature osteoclasts (Sarma & Flanagan 1996; Lerner 2006).

OPG acts to inhibit osteoclast differentiation at the end of the resorption process by binding to RANKL and blocking RANK (Lerner 2006). Many studies have sought to understand post-menopausal bone loss by studying the expression of these factors. RANKL expression is upregulated in osteoporotic bone (Ikeda et al. 2001; Eghbali-Fatourechi et al. 2003) and M-CSF expression is increased in primary cells harvested from the animal models of post-menopausal osteoporosis (Srivastava et al. 1998). Decreased serum levels of OPG have been reported in osteoporotic patients (Yano et al. 1999), albeit that the overexpression of OPG in an animal model leads to a severe osteoporotic phenotype (Bucay et al. 1998; Mizuno et al. 1998).

Other growth factors and cytokines also regulate osteoclast activity: tumour necrosis factor (TNF-α), the interleukin cytokines (interleukin-1 (IL-1), IL-6, IL-7), transforming growth factor (TGFB), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) (Lam et al. 2000; Lerner 2006; Teitelbaum 2006). Therefore, an alternative view is that bone loss initiates through the altered expression of such factors, instead of changes in RANK, M-CSF or OPG expression. Of these, TNF-α production is increased in post-menopausal osteoporosis (Zheng et al. 1997; Cenci et al. 2000; Lam et al. 2000), TGFB-1 and TGFB-3 exhibit a marked decrease in expression during oestrogen deficiency (Orlic et al. 2007), IL-1 and IL-6 expression is upregulated in human osteoporotic bone (Ralston 1994; Zheng et al. 1997), while IL-7 and the IL-7 receptor are increased in ovariectomized animal models (Weitzmann et al. 2002; Orlic et al. 2007; Sato et al. 2007). Therefore, osteoclast recruitment may occur as a secondary response to these changes. The origin of these factors, observed changes and implication of such changes are summarized in table 3.

There is also evidence that the expression of molecules that modulate osteoblast activity may be altered during post-menopausal osteoporosis. During normal bone resorption, growth factors are released from the extracellular matrix, including insulin growth factor-I (IGF-I) and TGF-β, which recruit and activate osteoblasts to begin collagen synthesis. In vitro studies have shown that the proliferation of osteoblasts from osteoporotic donors is altered as a result of impaired IGF-I receptor signalling (Perrini et al. 2008) and also that IGF-I expression is decreased during oestrogen deficiency (Lerner 2006). However, it is still unclear whether the expression is indeed altered during post-menopausal osteoporosis (Rosen 2000a). Deposition of TGFB-1 is also reduced during oestrogen deficiency in vitro and in vivo (Oursler et al. 1991a; Finkelman et al. 1992). These findings are summarized in table 3.
Table 3. Differentially expressed molecules and genes during oestrogen deficiency (−− indicates a reduction in expression; ++ is an increase in expression; +/- the variable changes in expression).

<table>
<thead>
<tr>
<th>molecule modulating osteoclast function</th>
<th>origin</th>
<th>function in bone</th>
<th>expression during oestrogen deficiency</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANKL</td>
<td>cytokine produced by osteoblasts and bone lining cells</td>
<td>binds to RANK on the osteoclasts to stimulate osteoclast proliferation and differentiation</td>
<td>++</td>
<td>Ikeda et al. (2001), Eghbali-Fatourechi et al. (2003)</td>
</tr>
<tr>
<td>M-CSF</td>
<td>cytokine produced by osteoblasts and bone lining cells</td>
<td>stimulates osteoclast proliferation and differentiation, prevents apoptosis</td>
<td>++</td>
<td>Srivastava et al. (1998)</td>
</tr>
<tr>
<td>OPG</td>
<td>cytokine produced by T cells and osteoblasts</td>
<td>binds to RANKL on osteoblasts to inhibit the differentiation of osteoclast precursors into osteoclasts</td>
<td>−−</td>
<td>Yano et al. (1999)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>cytokine produced by T cells, macrophages and fibroblasts</td>
<td>stimulates differentiation of osteoclast progenitors and increases the activity of mature osteoclasts</td>
<td>++</td>
<td>Zheng et al. (1997), Cenci et al. (2000), Lam et al. (2000)</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>protein produced by osteoblasts and released from extracellular matrix during resorption</td>
<td>stimulates the differentiation of monocytes into osteoclast precursors and enables osteoclastogenesis</td>
<td>−−</td>
<td>Ikeda et al. (1993), Oursler et al. (1991a), Finkelman et al. (1992)</td>
</tr>
<tr>
<td>TGF-β3</td>
<td>protein produced by osteoblasts and released from extracellular matrix during resorption</td>
<td>stimulates the differentiation of monocytes into osteoclast precursors and enables osteoclastogenesis</td>
<td>−−</td>
<td>Orlic et al. (2007)</td>
</tr>
<tr>
<td>IL-1</td>
<td>cytokine produced by monocytes in the bone marrow</td>
<td>stimulates the differentiation of osteoclast precursors and bone resorption</td>
<td>++</td>
<td>Ralston (1994), Zheng et al. (1997)</td>
</tr>
<tr>
<td>IL-6</td>
<td>cytokine produced by T cells in the bone marrow</td>
<td>promotes osteoclastogenesis</td>
<td>++</td>
<td>Ralston (1994), Zheng et al. (1997)</td>
</tr>
<tr>
<td>IL-7</td>
<td>growth factor secreted by narrow stromal cells and osteoblasts</td>
<td>stimulates osteoclastogenesis by upregulating T-cell-derived cytokines, including RANKL</td>
<td>++</td>
<td>Weitzmann et al. (2002), Orlic et al. (2007), Sato et al. (2007)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>molecules modulating osteoblast function</th>
<th>origin</th>
<th>function in bone</th>
<th>expression during oestrogen deficiency</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>hormone produced by osteoblasts and released from extracellular matrix during resorption</td>
<td>recruits and activates osteoblasts to begin collagen synthesis</td>
<td>−−</td>
<td>Lerner (2006)</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>protein produced by osteoblasts and released from extracellular matrix during resorption</td>
<td>promotes osteoprogenitor proliferation and osteogenesis, inhibits mineralization</td>
<td>−−</td>
<td>Oursler et al. (1991a), Finkelman et al. (1992)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>genes modulating osteoclast function</th>
<th>origin</th>
<th>function in bone</th>
<th>expression during oestrogen deficiency</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREM-2</td>
<td>gene encoding cell surface receptors expressed on osteoclast precursors</td>
<td>regulates the differentiation of osteoclast precursors into mature multi-nucleated osteoclasts</td>
<td>++</td>
<td>Hopwood et al. (2009)</td>
</tr>
<tr>
<td>CCL2</td>
<td>gene expressed by mature osteoclasts that encodes the chemokine (C–C motif) ligand 2</td>
<td>regulates the recruitment and fusion of osteoclast precursors</td>
<td>++</td>
<td>Hopwood et al. (2009)</td>
</tr>
<tr>
<td>RANK</td>
<td>gene encoding the protein RANK expressed by osteoclasts</td>
<td>regulates osteoclastogenesis by binding to RANKL on osteoblasts</td>
<td>++</td>
<td>Hopwood et al. (2009)</td>
</tr>
</tbody>
</table>
Table 3. (Continued.)

<table>
<thead>
<tr>
<th>molecule</th>
<th>origin</th>
<th>function in bone</th>
<th>expression during oestrogen deficiency</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>gene encoding the cytokine IL-6 secreted by osteoblasts</td>
<td>promotes osteoclastogenesis</td>
<td>++</td>
<td>Hopwood et al. (2009)</td>
</tr>
<tr>
<td>CD14</td>
<td>gene encoding the surface protein CD14 expressed by monocytes in the marrow</td>
<td>cell surface protein that may regulate bone mass</td>
<td>++</td>
<td>Hopwood et al. (2009)</td>
</tr>
<tr>
<td>CTSB</td>
<td>gene encoding the production of cathepsin B produced by osteoblastic cells</td>
<td>activates enzymes involved in the osteoclast bone resorption process</td>
<td>++</td>
<td>Hopwood et al. (2009)</td>
</tr>
<tr>
<td>genes modulating osteoblast function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGFB1</td>
<td>gene encoding the production of TGF-β1</td>
<td>recruits and activates osteoblasts to begin collagen synthesis</td>
<td>−−</td>
<td>Langdahl et al. (1997), Yamada et al. (1998), Hopwood et al. (2009)</td>
</tr>
<tr>
<td>TGFB3</td>
<td>gene encoding the production of TGF-β3</td>
<td>promotes osteoprogenitor proliferation, osteogenesis, inhibits mineralization</td>
<td>−−</td>
<td>Orlic et al. (2007)</td>
</tr>
<tr>
<td>SPP1</td>
<td>gene encoding the production of the protein osteopontin</td>
<td>facilitates the attachment of osteoclasts to mineralized matrix</td>
<td>++</td>
<td>Hopwood et al. (2009)</td>
</tr>
<tr>
<td>CTGF</td>
<td>gene encoding the connective tissue growth factor</td>
<td>promotes proliferation of osteoblast progenitors</td>
<td>++</td>
<td>Hopwood et al. (2009)</td>
</tr>
<tr>
<td>CTSB</td>
<td>gene encoding the production of cathepsin B produced by osteoblastic cells</td>
<td>may regulate the osteoblast mineralization process</td>
<td>++</td>
<td>Hopwood et al. (2009)</td>
</tr>
<tr>
<td>RUNX2(Cbfa1)</td>
<td>transcription factor produced by mesenchymal cells and osteoblasts</td>
<td>inhibits mesenchymal cell proliferation but promotes differentiation towards the osteoblast lineage and matrix production</td>
<td>alleles identified which are correlated to BMD</td>
<td>Geoffroy et al. (2002), Vaughan et al. (2002)</td>
</tr>
</tbody>
</table>

A vast number of genes regulate the normal bone turnover process (Garnero et al. 1994; Lerner 2006) and various genetic mutations have been identified in subjects with lower bone mass (Baldock & Elsman 2004) or who have experienced an osteoporotic fracture (Tsangari et al. 2004; Hopwood et al. 2009). In particular, polymorphisms in the ER promoter have been identified that are correlated to bone loss during osteoporosis (Weel et al. 1999). Putative candidate genes for the development of osteoporosis are RUNX2 (also known as Cbfa1) (Vaughan et al. 2002); Chemokine receptor 3 (CCR3), histidine decarboxylase (HDC) and glucocorticoid receptor (GCR) (Liu et al. 2005); matrix metalloproteinase 8 (MMP8), procollagen types I and VI, PTH receptor 1 and WD repeat domain 5 (Orlic et al. 2007), all of which are involved in skeletal development or have regulatory effects on bone cells. While these studies provide much insight, each of the studies focused on a particular subset of genes and could not provide a complete picture of alterations that occur.

It must be noted that only one study (Hopwood et al. 2009) has comprehensively characterized differential gene expression in post-menopausal osteoporotic human bone. This study confirmed alterations in several genes that regulate osteoclastogenesis and bone resorption, in particular TREM-2, CCL2, RANK and IL-6 identified in previous studies (Tsangari et al. 2004). Furthermore, this study confirmed that genes responsible for osteoblast maturation are differentially expressed in human osteoporotic tissue, in particular CTSB, TGFB1 which had previously been identified (Langdahl et al. 1997; Yamada et al. 1998), but also SPP1 and CTGF (Hopwood et al. 2009). As these genes are known to regulate mineralization and NCP production in vitro, this study provides further evidence that tissue-level changes in bone composition occur. The details of the studies which identified these changes are summarized in table 3. It is crucial that these findings are widely understood across multiple disciplines and further investigated.

Several molecular signalling candidates and genes that regulate osteoclast behaviour have been identified as putative candidates for the development of post-menopausal osteoporosis through in vitro and in vivo studies. More recent studies indicate that molecules that govern osteoblast activity are also altered. Future studies are required to delineate the sequence with which these molecules are expressed, specifically
whether expression is altered as a primary or secondary effect of oestrogen deficiency.

5. UNDERSTANDING THE SEQUENCE OF EVENTS IN THE BONE LOSS CASCADE

A vast amount of research has been undertaken to understand the pathogenesis and biomechanical consequences of post-menopausal osteoporosis and has provided strong evidence of complex changes in molecular and cellular biology, tissue composition, tissue integrity and architecture. However, significant findings from distinct disciplines are not widely reported or understood in an interdisciplinary fashion. As a result, the sequence with which such changes occur in the bone loss cascade is unknown.

It is intriguing to speculate on this sequence based on the existing knowledge. It has long been presumed that osteoclasts are primarily affected by oestrogen deficiency and there is sufficient evidence to support a subsequent sequence of events within this context. Firstly, molecules that promote osteoclast activity (MCS-F, RANKL, TNF-α, interleukins) are upregulated during oestrogen deficiency, whereas those that inhibit osteoclasts (OPG, TGF-β) are downregulated (figure 2a). Accordingly, osteoclastogenesis increases and osteoclast apoptosis decreases leading to deeper resorption for a prolonged duration (figure 2a).

Together, these changes accelerate bone resorption and thereby reduce bone mass. The complex changes in osteoblast behaviour, tissue composition and micro-damage may occur as a secondary response to bone loss (figure 2b) because the remaining tissue and mechanosensitive osteocytes will be subject to elevated loading. This might initiate an adaptive response whereby osteoblasts and osteocytes alter local tissue composition (NCPs and mineral) and mechanical properties of this remaining tissue to

Figure 2. (a) Primary sequence of events; molecules governing osteoclast activity are upregulated leading to increased osteoclastogenesis, while molecules inhibiting osteoclasts are downregulated. These events reduce apoptosis and result in excess bone resorption. (b) Secondary responses to bone loss; mechanical loading on the remaining tissue is increased and tissue mineralization is altered to compensate for bone loss. Increased mineralization may dissociate collagen or non-collagenous protein bonds and lead to damage accumulation and ultimately bone fracture. Feedback mechanisms are indicated by dashed lines. Unfilled rectangle, in vitro; filled rectangle, osteoporosis/OVX.
compensate for bone loss (figure 2b). Concurrently, larger or more numerous mineral crystals might dissociate collagen cross-links or prevent the formation of mature cross-links (Otsubo et al. 1992). Damage may accumulate as a result of the increasingly brittle nature of the tissue and/or the increased loading. These events combined will increase the fracture susceptibility of the tissue. Although substantial evidence exists to support such a sequence of events, it has not yet been established whether changes in tissue composition are indeed a secondary response to bone loss. Furthermore, it remains that suppressing osteoclast resorption does not prevent osteoprotic fractures, which suggests that osteoclastic activity may not be primarily responsible for the bone loss cascade.

An alternative premise proposed here is that post-menopausal bone loss is not a straightforward case of accelerated osteoclast bone resorption, but rather that the bone loss cascade initiates through oestrogen’s effects on osteoblasts. Osteoblasts maintain bone strength by producing, organizing and regulating the tissue matrix, but also possess oestrogen receptors suggesting that these functions may be altered when oestrogen levels are deficient. An alternative sequence is therefore proposed; firstly, differential expression of genes known to regulate osteoblast activity may occur (CTSB, TGFB1, SPP1, and CTGF) followed by the decreased production of IGF-I and TGF-β (figure 3a). As these proteins are the regulators of collagen matrix and NCP production (table 3) it would be expected that the changes in both tissue constituents occur (figure 3a). It is known that the NCP osteocalcin promotes tissue mineralization (Roy et al. 2001) so increased secondary mineralization may occur as a result of the increased levels of this protein (figure 3a). This would thereby increase the elastic modulus of the tissue (McNamara et al. 2006b). Within this context, bone resorption is proposed to be...
a secondary response to altered tissue stiffness (figure 3b); firstly, the deformation of the tissue matrix would be reduced under normal loading and this may in turn initiate an osteoclastic resorptive response to unloading, similar to stress shielding. Increased crystal size may dissociate collagen cross-links and lead to increased micro-damage accumulation, and together these events would ultimately result in bone fractures. Experimental evidence to support such a sequence is limited, but recent computational studies applied mechanoregulation models and predicted that (i) osteocytes residing in stiffer tissue are subject to reduced strain levels and initiate resorption leading to trabecular perforation (Mulvihill et al. 2008) and (ii) if bone tissue stiffness increases, the structure adopts to achieve a lower mass and increased anisotropy similar to osteoporotic bone (van der Linden et al. 2004). Future studies must provide the experimental evidence to delineate whether such a sequence occurs during post-menopausal osteoporosis.

6. KEY CHALLENGES TO UNDERSTANDING THE DISEASE OF OSTEOPOROSIS

While two putative sequences have been considered here, further studies are required to definitively describe the sequence of events in the bone loss cascade. A fundamental challenge to understanding the sequence of events lies in the fact that bone adaptation is tightly coupled and any alteration in one of the features of bone quality has resultant effects on each of the others. Therefore, it is imperative to determine whether the observed changes in gene expression, molecular signalling, cell physiology and tissue composition occur immediately at the onset of oestrogen deficiency, and whether they may be causative, or occur later and are thereby secondary responses to bone loss.

To answer such questions, and advance current understanding of the disease, the key challenges are to perform comprehensive multi-disciplinary experiments using both animal models and human subjects. In particular, these studies will require

(i) Comprehensive monitoring of gene and molecular expression immediately post-ovariectomy or in the early stages of oestrogen deficiency (<1 week) to determine the sequence with which regulatory molecules are altered. Such studies would define the initial event postulated in figures 2 and 3 and thereby distinguish whether osteoclast or osteoblast activity is primarily altered in post-menopausal osteoporosis.

(ii) A combination of in vivo and in vitro studies to monitor osteoblastic activity, e.g., molecular expression, matrix production and mineralization, in the early stages of oestrogen deficiency (>1 week) and at multiple time points as oestrogen deficiency continues. These studies would provide the essential knowledge to understand how bone tissue composition is altered and whether this is a primary (figure 2) or a secondary (figure 3) effect of oestrogen deficiency. In vitro experiments are confounded by the difficulties associated with extrapolating in vivo results to understand in vitro physiology and pathology. Therefore, a greater understanding will be gained by combining in vivo histology and in vitro culture models to focus on the effects of oestrogen deficiency on osteoblastic cells.

(iii) Quantification of complex changes in tissue composition and micro-architecture in the early-stage oestrogen deficiency and at multiple later time points. Such studies would delineate the exact nature of tissue-level changes and when these arise thereby identifying whether these are altered as a primary or a secondary response to oestrogen deficiency.

(iv) Mechanical characterization at the tissue level and whole bone level at multiple time points from the early-stage oestrogen deficiency. Correlation between mechanical properties, bone mass and tissue composition will delineate how these properties are interrelated and contribute to fracture risk.

If these quantities were better understood, then it might be possible to understand the origins of post-menopausal osteoporosis and inform the development of effective treatments to inhibit the disease. For example, if osteoblastic activity is found to alter tissue composition prior to bone loss, then such findings could pave the way for developing a drug treatment that targets osteoblastic activity rather than osteoclast resorption. Alternatively, if tissue composition is altered to compensate for bone loss, then treatments may be developed to capitalize on this phenomenon. Given the inadequacies of the current treatments, advancements are undoubtedly required and further interdisciplinary research at the interface of biomechanical engineering, materials science and cell and molecular biology is required to derive the necessary information to assist in such developments.

7. CONCLUSION

It remains the case that bone fracture during post-menopausal osteoporosis has not yet been eliminated. Future research studies should include multi-disciplinary analyses at multiple time points to comprehensively characterize the sequence of changes in molecular signalling, cell physiology, tissue composition, micro-architecture, damage and bone mass. Such research will be fundamental to deriving a coherent understanding of the disease and defining the sequence of events that ultimately lead to bone fractures.

REFERENCES


Boyde, A. 2003 The real response of bone to exercise.


J. R. Soc. Interface (2010)


Langlalh, B. L., Knudsen, J. Y., Jensen, H. K., Gregersen, N. & Eriksen, E. F. 1997 A sequence variation: 713–8delC in the transforming growth factor-beta 1 gene has higher prevalence in osteoporotic women than in normal women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women. Bone 20, 289–294. (doi:10.1016/S8756-3282(96)00360-8)


J. R. Soc. Interface (2010)


