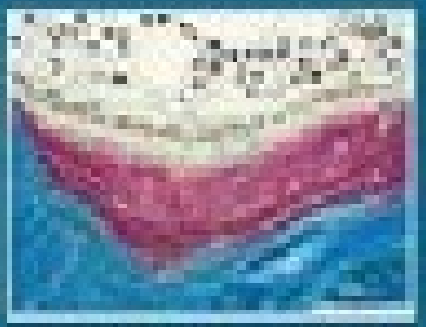
SECOND EDITION

OSTEOPOROSIS IN MEN

THE EFFECT OF GENDER ON SKELETAL HEALTH



EDITED BY
ERIC ORWOLL
JOHN P. BILEZIKIAN
BIRK VANDERSCHUEREN

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Contributors

ROBERT A. ADLER, Hunter Holmes McGuire VA Medical Center and Virginia Commonwealth University School of Medicine, Richmond, VA, USA

MATTHEW R. ALLEN, Departments of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA

SHREYASEE AMIN, Division of Rheumatology, College of Medicine, Mayo Clinic, Rochester, MN, USA

DIANA M. ANTONIUCCI, University of California, San Francisco; Physicians Foundation of California Pacific Medical Center, Division of Endocrinology, Diabetes and Osteoporosis, San Francisco, CA, USA

ANDRE B. ARAUJO, New England Research Institutes, Inc., Watertown, MA, USA

LAURA A.G. ARMAS, Creighton University Osteoporosis Research Center, Omaha, NE, USA

GIAMPIERO I. BARONCELLI, Department of Obstetrics, Gynecology and Pediatrics, 2nd Pediatric Unit, 'S. Chiara' Hospital, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy

SILVANO BERTELLONI, Department of Obstetrics, Gynecology and Pediatrics, 2nd Pediatric Unit, 'S. Chiara' Hospital, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy

SHALENDER BHASIN, Section of Endocrinology, Diabetes and Nutrition, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA

JOHN P. BILEZIKIAN, Department of Medicine, Division of Endocrinology, Metabolic Bone Diseases Unit, College of Physicians and Surgeons, Columbia University, New York, NY, USA

NEIL C. BINKLEY, University of Wisconsin, School of Medicine and Public Health, Madison, WI, USA

STEVEN BOONEN, Center for Musculoskeletal Research, Department of Experimental Medicine, Katholieke Division of Geriatric Medicine, Leuven University Hospital, Department of Internal Medicine, Katholieke Universiteit Leuven, Leuven, Belgium

ADELE L. BOSKEY, Starr Chair in Mineralized Tissue Research and Director, Musculoskeletal Integrity Program, Hospital for Special Surgery, New York; Professor of Biochemistry, Weill Medical College of Cornell University; Professor, Field of Physiology, Biophysics and Systems Biology, Graduate School of Medical Sciences of Weill Medical College of Cornell University; Professor, Field of Biomedical Engineering, Sibley School, Cornell Ithaca; Adjunct Professor, School of Engineering, City College of New York, NY, USA

ROGER BOUILLON, Laboratory of Experimental Medicine and Endocrinology (LEGENDO), Katholieke Univeriteit Leuven (KUL), Leuven, Belgium

DAVID B. BURR, Departments of Anatomy and Cell Biology and Orthopaedic Surgery, Indiana University School of Medicine; Department of Biomedical Engineering, IUPUI, Indianapolis, IN, USA

MELONIE BURROWS, Department of Orthopaedics, University of British Columbia; Centre for Hip Health and Mobility, Vancouver, Canada

FILIP CALLEWAERT, Center for Musculoskeletal Research, Leuven University Department of Experimental Medicine, Katholieke Universiteit Leuven, Leuven, Belgium

GEERT CARMELIET, Laboratory of Experimental Medicine and Endocrinology (LEGENDO), Katholieke Univeriteit Leuven (KUL), Leuven, Belgium

LUISELLA CIANFEROTTI, Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy

JULIET COMPSTON, University of Cambridge School of Clinical Medicine, Cambridge, UK

FELICIA COSMAN, Regional Bone Center Helen Hayes Hospital, West Haverstraw, New York; Department of Medicine, Division of Endocrinology, Metabolic Bone Diseases Unit, College of Physicians and Surgeons, Columbia University, New York, NY, USA

SERGE CREMERS, Division of Endocrinology, Department of Medicine, Columbia University, New York, NY, USA

K. SHAWN DAVISON, Laval University, Quebec City, PQ, Canada

DAVID W. DEMPSTER, Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY, USA

JOHN A. EISMAN, Bone and Mineral Research Program, Garvan Institute of Medical Research; University of New South Wales; St Vincent's Hospital, Sydney, NSW, Australia

GHADA EL-HAJJ FULEIHAN, Calcium Metabolism and Osteoporosis Program, American University of Beirut Medical Center, Beirut, Lebanon

ERIK FINK ERIKSEN, Department of Endocrinology and Internal Medicine, Aker University Hospital, Oslo; Spesialistsenteret Pilestredet Park, Oslo, Norway

MURRAY J. FAVUS, Section of Endocrinology, Diabetes, and Metabolism, University of Chicago, Chicago, IL, USA

DIETER FELSENBERG, Zentrum Muskel- & Knochenforschung, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Freie Universität & Humboldt-Universität Berlin, Berlin, Germany

SERGE FERRARI, Service of Bone Diseases, Department of Rehabilitation and Geriatrics, WHO Collaborating Center for Osteoporosis Prevention, Geneva University Hospital, Geneva, Switzerland

DAVID P. FYHRIE, David Linn Chair of Orthopaedic Surgery, Lawrence J. Ellison Musculoskeletal Research Center, Department of Orthopaedic Surgery, The University of California, Davis; The Orthopaedic Research Laboratories, Sacramento, CA, USA

PATRICK GARNERO, INSERM Research unit 664 and Synarc, Lyon, France

LUIGI GENNARI, Deparment of Internal Medicine, Endocrine, Metabolic Sciences, and Biochemistry, University of Siena, Italy

PIET GEUSENS, Department of Internal Medicine, Subdivision of Rheumatology, Maastricht University Medical Center, Maastricht, The Netherlands; Biomedical Research Institute, University Hasselt, Belgium

VICENTE GILSANZ, Director, Childrens Imaging Research Program, Childrens Hospital Los Angeles, Professor of Radiology and Pediatrics, University of Southern California, Los Angeles, CA, USA

MONICA GIROTRA, Memorial Sloan-Kettering Cancer Center; Joan and Sanford I. Weill Medical College of Cornell University, New York, NY, USA

ANDREA GIUSTI, Department of Gerontology & Musculo-Skeletal Sciences, Galliera Hospital, Genoa, Italy

ANDREA GIUSTINA, Department of Endocrinology & Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands

STEFAN GOEMAERE, Ghent University Hospital, Department of Endocrinology and Unit for Osteoporosis and Metabolic Bone Diseases, Gent, Belgium

DEBORAH T. GOLD, Duke University Medical Center, Durham, NC, USA

X. EDWARD GUO, Department of Biomedical Engineering, Columbia University, New York, NY, USA

PATRICK HAENTJENS, Center for Outcomes Research, University Hospital Brussels, Vrije Universiteit Brussel, Brussels, Belgium

JOHAN HALSE, Department of Endocrinology and Internal Medicine, Aker University Hospital, Oslo; Spesialistsenteret Pilestredet Park, Oslo, Norway

DAVID J. HANDELSMAN, Department of Andrology, ANZAC Research Institute, Concord Hospital, University of Sydney, Sydney, NSW, Australia

ELIZABETH M. HANEY, Oregon Health and Science University, Portland, OR, USA

DAVID A. HANLEY, University of Calgary, Calgary, AB, Canada

ROBERT P. HEANEY, Creighton University Osteoporosis Research Center, Omaha, NE, USA

RAVI JASUJA, Section of Endocrinology, Diabetes and Nutrition, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA

HELENA JOHANSSON, WHO Collaborating Centre for Metabolic Bone Diseases, University of Sheffield Medical School, Sheffield, UK

JOHN A. KANIS, WHO Collaborating Centre for Metabolic Bone Diseases, University of Sheffield Medical School, Sheffield, UK

JEAN-MARC KAUFMAN, Ghent University Hospital, Department of Endocrinology and Unit for Osteoporosis and Metabolic Bone Diseases, Gent, Belgium

ROBERT KLEIN, Bone and Mineral Unit, Oregon Health & Science University and Portland VA Medical Center, Portland, OR, USA

STAVROULA KOUSTENI, Division of Endocrinology, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY, USA

DIANE KRUEGER, University of Wisconsin, Madison, WI, USA

KISHORE M. LAKSHMAN, Section of Endocrinology, Diabetes, and Nutrition, Division of Endocrinology & Metabolism, Boston University School of Medicine, Boston Medical Center, Boston, MA, USA

THOMAS F. LANG, Professor in Residence, Department of Radiology and Biomedical Imaging, and Joint Bioengineering Graduate Group, University of California, San Francisco, San Francisco, CA, USA

BRUNO LAPAUW, Ghent University Hospital, Department of Endocrinology and Unit for Osteoporosis and Metabolic Bone Diseases, Gent, Belgium

JOAN M. LAPPE, Creighton University Osteoporosis Research Center, Omaha, NE, USA

BENJAMIN Z. LEDER, Endocrine Unit, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

WILLEM LEMS, Department of Rheumatology, Vrije Universiteit Amsterdam; VU Medisch Centrum, Amsterdam, The Netherlands

X. SHERRY LIU, Departments of Medicine and Biomedical Engineering, College of Physicians and Surgeons, Columbia University, New York, NY, USA

SHI S. LU, Regional Bone Center, Helen Hayes Hospital, West Haverstraw, New York, NY, USA

HEATHER M. MACDONALD, Schulich School of Engineering, University of Calgary, Calgary, Canada

CHRISTA MAES, Laboratory of Experimental Medicine and Endocrinology (LEGENDO), Katholieke Universiteit Leuven (KUL), Leuven, Belgium

ANN E MALONEY, Maine Medical Center Research Institute, Scarborough, ME, USA

PEGGY MANNEN CAWTHON, San Francisco Coordinating Center, California Pacific Medical Center Research Institute, San Francisco, CA, USA

CLAUDIO MARCOCCI, Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy

LYNN MARSHALL, Department of Medicine, Bone and Mineral Unit, Department of Public Health and Preventive Medicine, Oregon Health & Science University, Portland, OR, USA

GHERARDO MAZZIOTTI, Department of Medical and Surgical Sciences, University of Brescia, Italy

EUGENE V. McCLOSKEY, WHO Collaborating Centre for Metabolic Bone Diseases, University of Sheffield Medical School, Sheffield, UK

HEATHER A. MCKAY, Department of Orthopaedics, University of British Columbia; Centre for Hip Health and Mobility; Department of Family Practice, University of British Columbia, Vancouver, Canada

CHRISTIAN MEIER, Division of Endocrinology, Diabetes and Clinical Nutrition, University Hospital Basel, Basel, Switzerland

PAUL D. MILLER, University of Colorado Health Sciences Center, Medical Director, Colorado Center for Bone Research, Lakewood, CO, USA

BISMRUTA MISRA, College of Physicians and Surgeons, Columbia University, New York, NY, USA

STEFANO MORA, Departments of Radiology and Pediatrics, Childrens Hospital Los Angeles, Los Angeles, California, USA; Laboratory of Pediatric Endocrinology, BoNetwork, San Raffaele Scientific Institute, Milan, Italy

TUAN V. NGUYEN, Bone and Mineral Research Program, Garvan Institute of Medical Research; University of New South Wales; St Vincent's Hospital, Sydney, NSW, Australia

ANDERS ODEN, WHO Collaborating Centre for Metabolic Bone Diseases, University of Sheffield Medical School, Sheffield, UK

CLAES OHLSSON, Center for Bone Research, Department of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

TERENCE W. O'NEILL, Epidemiology arc Unit, University of Manchester, Manchester, UK

ERIC S. ORWOLL, Bone and Mineral Unit, Oregon Health & Science University, Portland, OR, USA

SOCRATES E. PAPAPOULOS, Department of Endocrinology & Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands

RENÉ RIZZOLI, Division of Bone Diseases [WHO Collaborating Center for Osteoporosis Prevention] Department of Rehabilitation and Geriatrics, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland

CLIFFORD J. ROSEN, Maine Medical Center Research Institute, Scarborough, ME, USA

MARTIN RUNGE, Aerpah Clinic Esslingen, Esslingen, Germany

JOHN T. SCHOUSBOE, Park Nicollet Health Services, Minneapolis; Division of Health Policy & Management, School of Public Health, University of Minnesota, MN, USA

EGO SEEMAN, Endocrine Centre, Heidelberg Repatriation Hospital/Austin Health, Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia

MARKUS J. SEIBEL, Bone Research Program, ANZAC Research Institute, The University of Sydney, Sydney, NSW, Australia

DEBORAH E. SELLMEYER, Metabolic Bone Center, The Johns Hopkins Bayview Medical Center, Baltimore, MD, USA

ELIZABETH SHANE, Columbia University College of Physicians & Surgeons, New York, NY, USA

JAY R. SHAPIRO, Bone and Osteogenesis Imperfecta Programs, Kennedy Krieger Institute; Department of Physical Medicine and Rehabilitation, Johns Hopkins University, Baltimore, MD, USA

SHONNI J. SILVERBERG, Division of Endocrinology, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY, USA

STUART L. SILVERMAN, Cedars-Sinai/UCLA and the OMC Clinical Research Center, Los Angeles, CA, USA

RAJAN SINGH, Section of Endocrinology, Diabetes and Nutrition, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA

EMILY M. STEIN, Columbia University College of Physicians & Surgeons, New York, NY, USA

THOMAS W. STORER, Section of Endocrinology, Diabetes and Nutrition, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA

PAWEL SZULC, INSERM Research Unit 831, Hôspital Edouard Heriot, Lyon, France

MAHMOUD TABBAL, Calcium Metabolism and Osteoporosis Program, American University of Beirut Medical Center, Beirut, Lebanon

YOURI TAES, Ghent University Hospital, Department of Endocrinology and Unit for Osteoporosis and Metabolic Bone Diseases, Gent, Belgium

CHARLES H. TURNER, Department of Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis; Department of Biomedical Engineering, IUPUI, IN, USA

LIESBETH VANDENPUT, Center for Bone Research, Department of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

DIRK VANDERSCHUEREN, Center for Musculoskeletal Research, Leuven University Department of Experimental Medicine, Katholieke Universiteit Leuven, Leuven, Belgium

KATRIEN VENKEN, Center for Musculoskeletal Research, Leuven University Department of Experimental Medicine, Katholieke Universiteit Leuven, Leuven, Belgium

LIEVE VERLINDEN, Laboratory of Experimental Medicine and Endocrinology (LEGENDO), Katholieke Universiteit Leuven (KUL), Leuven, Belgium

ANNEMIEKE VERSTUYF, Laboratory of Experimental Medicine and Endocrinology (LEGENDO), Katholieke Universiteit Leuven (KUL), Leuven, Belgium

QINGJU WANG, Endocrine Centre, Heidelberg Repatriation Hospital/Austin Health, Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia

CONNIE M. WEAVER, Department of Foods and Nutrition, Purdue University, West Lafayette, IN, USA

FELIX W. WEHRLI, Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA

SUNIL J. WIMALAWANSA, Professor of Medicine, Endocrinology & Metabolism; Director, Regional Osteoporosis Center, Department of Medicine, Robert Wood Johnson Medical School, New Brunswick, NJ, USA

KRISTINE M. WIREN, Bone and Mineral Unit, Oregon Health & Science University; Portland VA Medical Center, Portland, OR, USA

ROGER ZEBAZE, Department of Endocrinology and Medicine, Austin Health, University of Melbourne, Melbourne, Victoria, Australia

HUA ZHOU, Regional Bone Center, Helen Hayes Hospital, West Haverstraw, New York, NY, USA

Foreword

The field of osteoporosis has grown enormously over the last 4 decades, with a focus upon the issues that relate to skeletal health in women. It was only about 15 years ago that the scientific community began to acknowledge that osteoporosis in men is also important. The first edition of *Osteoporosis in Men,* published in 2001*,* was a seminal event in that it called attention to the problem in an organized series of articles on male skeletal health and bone loss. Now, with this second edition of *Osteoporosis in Men,* further progress in this area is emphasized with particular emphasis on new knowledge that has appeared during the last decade.

Osteoporosis in men is heterogeneous with many etiologies to consider besides the well known roles of aging (Sections 1-4) and sex steroids (Sections 6-8). The roots of the problem in some individuals can be back dated to the pre-pubertal and pubertal growth periods that determine the acquisition of peak bone mass.

In addition, *Osteoporosis in Men, second edition,* deals exhaustively with important clinical issues. Nutritional considerations, the clinical and economic burden of fragility fractures, and diagnostic approaches are particularly strong aspects of the text (Sections 5, 7, 9). These chapters transcend, in part, the specific focus of the volume, making it a useful resource and a valuable reference for an audience not necessarily well-informed in bone and mineral disorders.

The last section of *Osteoporosis in Men, second edition*, highlights therapeutic approaches. Treatment options are less well defined in men than in women because virtually all of the clinical trials involving men have been much smaller and shorter in duration with surrogate, instead of fracture, endpoints. With this smaller database, it nevertheless appears that men respond to available pharmacological approaches to osteoporosis in a similar manner to women (Section 10). Available clinical data support the efficacy of these therapies in men with both primary and secondary osteoporosis.

Finally, *Osteoporosis in Men, second edition* provides a view of the future, underscoring a number of unresolved issues to be included in the agenda for future research in this area. These include discussions related to an appropriate BMD-based definition for male osteoporosis, a further understanding of the factors implicated in age-related bone loss and idiopathic osteoporosis in men, and randomized-controlled studies directly assessing fracture risk reduction, particularly for non vertebral fracture. In all these areas, more definitive information is needed.

This thorough and comprehensive book integrates new, accessible and informative material in the field. It will help investigators, as well as practitioners and students, to improve their understanding of male skeletal health and bone loss. The additional knowledge, assembled in such a readable manner, should help us achieve one of our ultimate goals-better care of men with osteoporosis.

Gerolamo Bianchi, MD
Department of Locomotor System
Division of Rheumatology
Azienda Sanitaria Genovese
Genova, Italy

Preface to the Second Edition

The first edition of *Osteoporosis in Men* was published in 1999, about 15 years after the earliest publications on the subject. Over the past decade, we have witnessed a surge of further interest in the subject of male osteoporosis. This second edition of *Osteoporosis in Men* is, thus, timely. In the second edition, we have made major additions to reflect increased areas of new knowledge, including genetics and inherited disorders. Previous topics are updated and extended to make them timely also. New topics include:

- Important basic processes including bone biochemistry and remodeling
- Mechanical properties and structure
- Genetics and inherited disorders
- Growth and puberty
- Nutrition, including calcium, vitamin D, protein and other factors
- Sex steroids in muscle and bone
- Assessment of bone using DXA, CT, ultrasound, biochemical markers
- Sarcopenia and frailty
- Diagnostic approaches
- Treatment approaches including bisphosphonates, parathyroid hormone, androgens and SARMS and newer agents.

A key element of the book continues to be sex differences in bone biology and pathophysiology that can inform our understanding of osteoporosis in both men and women.

The increased scope of the book is the result of contributions from prominent experts in the field, including many who contributed chapters to the first edition. New authors also have provided novel insights for the second edition. Editorial responsibilities were shared by the three of us.

As was the goal before, *Osteoporosis in Men, Second Edition,* is meant to be useful to a broad audience, including students of the field as well as those already knowledgeable. We have sought to summarize a compendium of information intersecting general and specific areas of interest. This volume will make apparent that information available concerning osteoporosis in men still lags behind what we know about osteoporosis in women. On the other hand, major advances in our understanding of the male skeleton in health and in disease are being translated into practical approaches to their clinical management. We hope this second edition provides a valuable reference source for you and that it also will serve to stimulate further advances in the field.

Eric Orwoll
Portland, Oregon

John Bilezikian
New York, New York

Dirk Vanderschueren
Leuven, Belgium

CHAPTER 1

The Biochemistry of Bone: Composition and Organization

ADELE L BOSKEY

Starr Chair in Mineralized Tissue Research and Director, Musculoskeletal Integrity Program, Hospital for Special Surgery, New York; Professor of Biochemistry, Weill Medical College of Cornell University; Professor, Field of Physiology, Biophysics and Systems Biology, Graduate School of Medical Sciences of Weill Medical College of Cornell University; Professor, Field of Biomedical Engineering, Sibley School, Cornell Ithaca; Adjunct Professor, School of Engineering, City College of New York, USA

INTRODUCTION

As detailed throughout this book, osteoporosis is characterized by increased risk of fracture due to changes in the 'quality' of bone [1]. To appreciate why bone becomes weaker or less resilient to fracture with age in both men and women and in individuals of different races, a general knowledge of bone development and age-dependent changes is necessary. In line with the theme of this book, it is noted that there are both age- and sex-dependent differences in bone properties and composition, some related to the rate at which bones develop in boys and girls, some related to the impact of genes on the X-chromosome which produce proteins important for bone development and/or metabolism and some due to the direct effect of sex steroids on bone cells [2]. To appreciate the discrete differences between bone structure and composition in men and women this chapter reviews the basics of bone composition and organization and the mineralization process from the point of view of sexual dimorphism, where such differences between men and women are recognized. Emphasis is placed on those factors that contribute to bone strength; geometry, architecture, mineralization, the nature of the organic matrix and tissue heterogeneity.

BONE ORGANIZATION

Bone Heterogeneity

The structure of bone appears different depending on the scale at which it is examined. At the centimeter level, whole bone can be viewed as an organ, for example, the tubular (long and short) bones such as the femur and digits, respectively, and the flat bones, such as the calvaria in the skull. Slightly better resolved, at the millimeter level, are the components of the bones, the cortices that surround the marrow cavity, the cancellous bone within the marrow cavity, the marrow cavity itself, the cartilaginous ends, etc. At the micrometer to millimeter level are the individual interconnecting struts of the trabeculae, the lamellae and the osteons that surround the vascular canals. The cells and the composite matrices also can be visualized as part of this microstructure. Finally, at the nanometer level, bone consists of an organic matrix made mainly from collagen fibrils and noncollagenous proteins, lipids, nanometer size mineral crystals (discussed below) and water. There is also heterogeneity in both the size of the collagen fibrils and the composition and sizes of the crystals deposited on this matrix [3, 4]. This heterogeneity is important for the mechanical competence of the tissue [5]. To understand the process of mineralization, knowledge of the cells and the extracellular matrices of bone is required.

Bone Cells

Within the bone matrix are the cells that are responsible for bone formation and bone turnover. Three key cells are of mesenchymal origin – chondrocytes, osteoblasts and osteocytes. The chondrocytes that form cartilage within the epiphysial growth plates produce a matrix that can be mineralized, regulate the flux of ions that facilitate the mineralization of that matrix and orchestrate the remodeling of that matrix and its replacement by bone [6]. The other mesenchymal derived bone cells are the osteoblasts and osteocytes [7]. As seen in the electron micrograph in Figure 1.1,

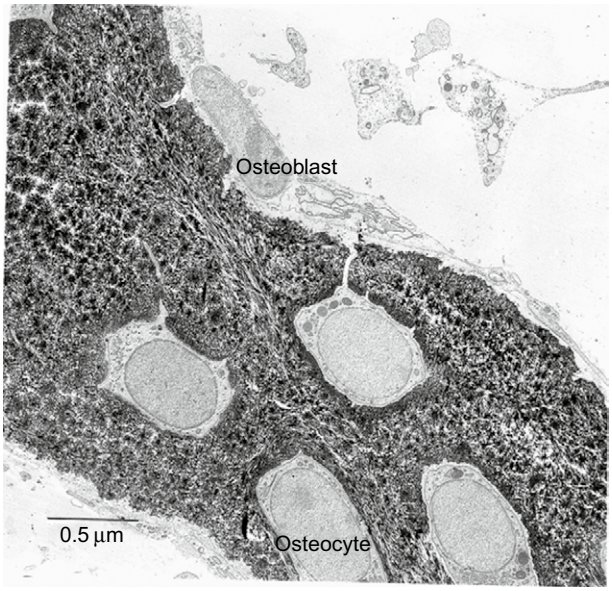

FIGURE 1.1 Transmission electron micrograph showing osteoblasts lining the bone surface in an adult male Sprague-Dawley rat. Inside the bone are the osteocytes, connected to one another by canaliculae. The banded pattern of the collagen is also visible. Magnification is marked on the figure. Courtesy of Dr Stephen B. Doty, Hospital for Special Surgery, New York.

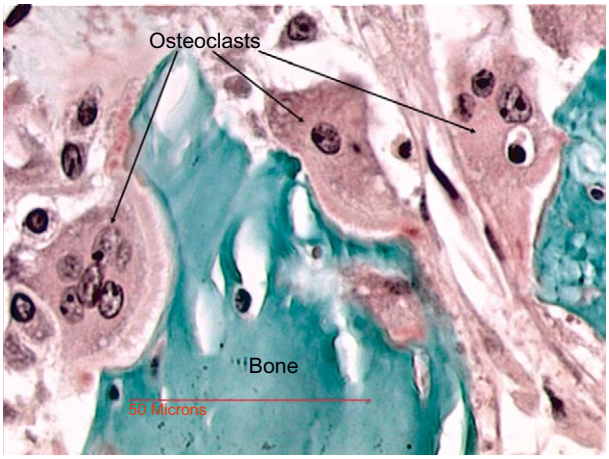

FIGURE 1.2 Transmission electron micrograph of an osteoclast on the bone surface of a 70-year-old woman. The ruffled borders sealing the cell to the mineralized surface are indicated along with the magnification. Courtesy of Dr Stephen B. Doty, Hospital for Special Surgery, New York.

osteoblasts line the surface of the mineralized bone. They synthesize new matrix and regulate the mineralization and turnover of that matrix. Once these osteoblasts become engulfed in mineral they become osteocytes and connect with one another by long processes (canaliculae) (see Figure 1.1). The osteocytes are the cells that sense mechanical signals and then convey them through the matrix. Osteocytes produce many of the same proteins as osteoblasts, but the relative concentrations of these proteins are not the same and the ways in which these cells use regulatory pathways differ. As reviewed in detail elsewhere [8], the osteoblasts use the WNT/beta-catenin pathway [9] to regulate synthesis of new bone; the osteocytes use the WNT/beta-catenin pathway to convey mechanical signals. Osteoblasts synthesize more alkaline phosphatase, more type I collagen and more bone sialoprotein than osteocytes, while osteocytes specifically produce sclerostin, a glycoprotein that is a WNT and BMP antagonist, and produce high levels of dentin matrix protein 1 [8]. Sclerostin, an osteocytes specific protein, inhibits osteoblast differentiation and, based on the significant increase in bone mineral density in the sclerostin knockout mouse [10], is believed to be important in determining the high bone mass phenotype [11]. This increase in bone mass was noted to be comparable for both sexes [10]. There is sexual dimorphism in the density of osteocytes, as females gain osteoclast lacunar density with increasing age, while males show a decrease in this parameter [12]. This may explain why bone loss in women results in a decrease in trabecular number, while in males there is a thinning of trabeculae [13]. Some of the other functions of osteoblasts and osteocyte proteins will be discussed later.

The cells responsible for the turnover of bone, the osteoclasts, are of hematologic and macrophage origin [14]. As seen in the electron micrograph in Figure 1.2, these multinucleated giant cells attach to the surface of the bone via a 'ruffled border'. They receive signals from osteoblasts that control bone remodeling and regulate the turnover of the mineralized matrix. They remove bone by producing acid and couple that with the transport of chloride out of the cell. The acid dissolves the mineral (see below) and, after the mineral is removed, release proteolytic enzymes that degrade the matrix. During the dissolution of the matrix, signaling molecules communicate with the osteoblasts and new bone formation is triggered. Androgens and estrogens inhibit osteoclast activity to different extents [15] explaining some of the sexual dimorphism in osteoclast activity.

There are a number of other cells in bone, marrow stromal cells, pericytes, vascular endothelial cells, fibroblasts, etc that function as stem cells [16] but their properties are beyond the scope of this chapter and will not be discussed here.

Skeletal Development

The shapes of male and female adult bones are different and, for archeologists, form the basis for the identification of sexes in skeletal remains [17]. The early development of the skeleton contributes markedly to these sexual differences. During development, bone structure changes in length and width and there is a concomitant alteration in tissue density, resulting in a bone that is optimally designed to bear the loads imposed on it [18]. In the long and short tubular bones, endochondral

ossification, in which a cartilage model becomes calcified and is replaced by bone, provides the basis for longitudinal growth, while widening of the bones takes place by apposition on already formed bone in the periosteum concurrent with removal of the inner (endosteal) surfaces.

Endochondral ossification starts during embryogenesis and continues throughout childhood and into adolescence, peaking during the 'growth spurt'. The rate at which changes in bone geometry occur depends on genetics, the environment and hormonal signals [19, 20]. With the exception of individuals with rare genetic mutations, the process of endochondral ossification terminates during adolescence with the closing of the growth plate. This generally occurs in girls around age 13 and in boys around age 18 [21]. In contrast, there is a report of a man who had a bone age of 15, based on bone mineral density (BMD), at age 28 and lacked closed epiphyses and had continued linear growth into adulthood due to a mutation in his estrogen-receptor alpha (ER_{alpha}) gene [22]. His testosterone levels were reported as normal. Other related cases with abnormalities in the ability to synthesize estrogen (aromatase deficiency) had a similar phenotype, but longitudinal growth could be modulated with estrogen treatment [23].

During aging, at least in mice [24] and, most likely, in humans [25], there is a decrease of bone formation (osteogenesis) and an increase of fat cell formation (adipogenesis) in bone marrow. There is also a difference between aging patterns in bones of men and women. In general, in both sexes, bone strength is maintained by the process of remodeling, removal of bone by osteoclasts and formation of new bone by osteoblasts. These coupled processes [26] are not equivalent in men and women. Testosterone decreases this pathway in men [27], perhaps contributing to the delayed start of age-dependent bone loss in males relative to females. In women, menopause-related estrogen deficiency leads to increased remodeling [28] and, with age, bone loss is accelerated and bone loss exceeds formation, causing cortices to being thinner and more porous and trabeculae to become disconnected and thinner. In men, the changes in remodeling lead to bone loss occurring later in life [29]. Concurrent bone formation on the periosteal surface during aging occurs to a greater extent in men than in women, thus diminishing some of the bone loss [30]. In a cross-sectional study of older men and women [29], men had significantly larger cross-sectional bone sizes than women which, in turn, was associated with decreased compressive strength indices at the spine, femoral neck and trochanter and bending strength indices at the femoral neck.

BONE COMPOSITION: THE BONE COMPOSITE

Independent of age, state of development, race and sex, bone is a composite material consisting of mineral crystals deposited in an oriented fashion on an organic matrix. The organic matrix is predominately type I collagen, but there are also non-collagenous proteins and lipids present. The non-collagenous proteins account for a small percentage of the bone matrix, yet they are important for regulating cell–matrix interactions, matrix structure, matrix turnover and the biomineralization process. Knowledge about the functions and critical status of these proteins has come from studies of mutant animals (naturally occurring and those made by genetic manipulation), cell culture studies [31] and analyses of the proteins' activity in the absence of cells.

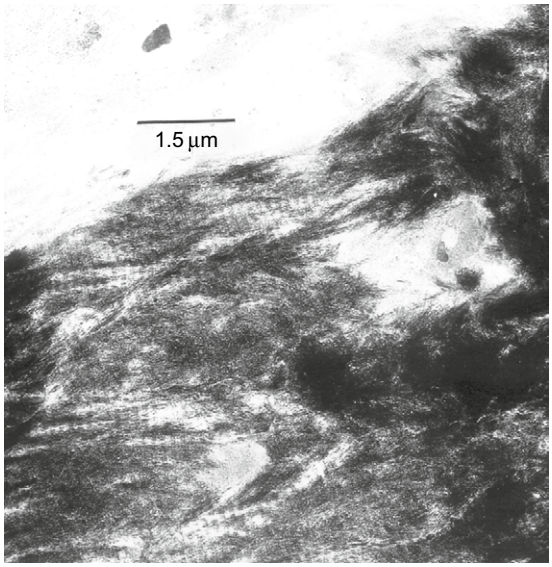

FIGURE 1.3 Transmission electron micrograph of a section of bone from the tibia of an adult male mouse. The electron dense mineral crystals can be seen to lie parallel to the collagen fibril axis. Courtesy of Dr Stephen B Doty, Hospital for Special Surgery, New York.

The Mineral

The mineral component of the bone composite is an analogue of the naturally occurring mineral hydroxyapatite. Bone hydroxyapatite is comprised of nanometer sized crystals [32]. These crystals have the approximate chemical composition $Ca_5(PO_4)_3OH$ but are carbonate-substituted and calcium and hydroxide deficient [33]. The individual crystals have a broad range of sizes, depending on the age of the bone and the health of the subject, but are always oriented parallel to the long fiber axis of the collagenous matrix (Figure 1.3). There is a broad distribution of the amount of mineral in the matrix, again varying with age, environment and disease. The average amount of mineral in the matrix can be measured by burning off the organic matrix (ash weight) or by radiographic measurement of density (bone mineral density or bone mineral content). There is some sexual dimorphism in the ash weight in bones of egg-laying

chicks, with males having, on average, a greater mineral content in any given bone than age matched female bones [34] but, in humans of the same race, the ash content of adult male and female bones is similar [35], perhaps because there is a well defined maximum amount of mineral that can fit into the bone matrix. Only in osteomalacia and related diseases is the mineral content reduced and that occurs in both sexes. Bone mineral density measured by computed tomography, tends to be higher in males than females at each stage of life, but differences are removed when corrected for bone length and cortical thickness [29, 36, 37].

The composition of bone hydroxyapatite varies with age, diet and health due to the substitution of foreign ions and vacancies into the crystal lattice and to the absorption of these ions on the surface of the crystals. The substituted ions also have been reported to differ when male and female mouse bones are compared, although the number of such studies is limited. When attention is paid to the sex of the animal, compositional studies show differences in mineral content and composition [38]. The effects of sex steroids on bone development can explain many of these differences. For example, assessing the effects of sex hormones on bone composition Ornoy et al. [39] compared a variety of compositional parameters in gonadectomized mice treated with male and female sex steroids. While the investigators found that tibial mineral content (ash weight) was comparable in all the groups, Ca and P content increased after ovariectomy. Estradiol treatment increased mineral content and bone Ca and P in ovariectomized and in intact females and orchiectomized mice, while testosterone had smaller effects.

The Extracellular Matrix

Collagen provides the oriented template or scaffold upon which these mineral crystals are deposited. The collagen is predominately type I, a triple helical collagen, with the individual chains having the amino acid sequence (X-Y-Gly)n, where X and Y are any amino acids, often proline and hydroxyproline, and glycine is the only amino acid small enough to fit in the center of the triple helix [40]. The importance of type I collagen for the proper mineralization of the matrix is seen in the different osteogenesis imperfecta diseases, a set of diseases, reviewed elsewhere [41], caused by mutations that lead to altered quantity or quality (composition) of type I collagen and result in brittle bones. There are also other collagen types in bone, including fibrillar type III collagen and non-fibrillar type V collagens [42]. No sex dependent differences in the distribution of collagen types have been reported, however, there are differences in the non-collagenous proteins that are found associated with the collagen matrix. In the next section, these non-collagenous proteins will be presented as families, with emphasis on their roles in mineral formation and turnover and other ways in which they might affect sexual dimorphism in bone strength.

The Non-Collagenous Proteins: GLA Proteins

The most abundant non-collagenous protein in vertebrates is a small protein, osteocalcin, also known as bone gla protein [40]. This small (5.7 kDa) protein has three gamma-carboxy-glutamic acid residues, with a high affinity for hydroxyapatite and calcium as demonstrated by its crystal and nuclear magnetic resonance (NMR) structures [43, 44]. Osteocalcin is frequently used as a biomarker for bone formation [45], although it is also released from bone and hence can reflect remodeling rather than only formation. In studies where bone tissue osteocalcin levels and serum osteocalcin levels were compared as a function of age and sex, the levels in men exceeded those in women at all ages until age 60, when levels in women increased and then decreased, reflecting age-dependent increases in bone remodeling [46, 47]. This most likely is an estrogen-determined effect as, in the rat, estrogen treatment is associated with a decrease in osteocalcin [48].

Knockout mice lacking osteocalcin have thickened bones and, thus, it was initially suggested that osteocalcin was important for bone formation [49]. Further studies led to the suggestion that osteocalcin was important for osteoclast recruitment [50], a suggestion supported by in vitro and in vivo assays [40]. Most recently, Karsenty's group has suggested, from studies in wildtype as well as osteocalcin knockout mice, that the uncarboxylated form of osteocalcin acts as a hormone, regulating glucose levels in cultures of pancreatic cells and in the skeleton [51]. The role of osteocalcin in glucose metabolism is suggested by the observation that osteoblastic bone formation is negatively regulated by the hormone leptin. Leptin, secreted by fat cells (adipocytes), has multiple hormonal functions including, but not limited to: appetite suppression, initiation of puberty in girls and acceleration of longitudinal bone growth in mice, although the data on bone formation have suggested a bimodal pattern [52]. In humans, a recent report showed postmenopausal women with type 2 diabetes had reduced osteocalcin levels [53]. In addition to the identification of osteocalcin as a hormone with a postulated role in metabolic syndrome, readers are reminded that the osteocalcin knockout has a bone phenotype, there is some sex specificity to osteocalcin's action in bone [48] and polymorphisms in the osteocalcin gene have been associated with osteoporosis [54–56].

The second gamma-carboxyglutamic acid containing protein in bone (predominantly in cartilage) and in soft tissues is matrix-gla protein (MGP). MGP is a hydrophobic protein [40] containing five gamma-carboxyglutamate residues that is important for inhibition of soft tissue calcification, as can be seen in the knockout mice where, when MGP is ablated, the animals have excessive cartilage calcification, denser bones and young animals succumb to calcification of the blood vessels and esophagus [57, 58]. Both the full length protein and its component peptides can inhibit hydroxyapatite formation and growth in culture [59]. MGP is more abundant in

soft tissues than in bone, hence it is not surprising that polymorphisms in MGP are not associated with bone density or fracture risk [56].

Non-Collagenous Proteins: Siblings

There is a family of proteins found in bone that have been named the SIBLING proteins (small integrin binding ligand *N*-glycosylated) [60]. These proteins are all located on the same chromosome, all have RGD-cell binding domains, all are anionic and all are subject to multiple post-translational modifications including phosphorylation and dephosphorylation, cleavage and glycosylation [61]. Each is found in multiple tissues in addition to bone and each has signaling functions in addition to interacting with hydroxyapatite and regulating mineralization (Table 1.1). The SIBLING proteins include osteopontin (bone sialoprotein 1), dentin matrix protein 1 (DMP1), bone sialoprotein (BSP2), matrix extracellular phosphoglycoprotein (MEPE) and the products of the dspp gene, dentin sialoprotein (DSP) and dentin phosphoprotein (DPP).

Osteopontin is the most abundant of the SIBLING proteins and has the most widespread distribution. In solution [73, 74], in a variety of cell culture systems [75, 76], in animals in which gene expression has been ablated [71] and in models of pathologic calcifications [77], bone osteopontin is an inhibitor of mineralization. When this glycoprotein is highly phosphorylated it can promote hydroxyapatite formation, most likely due to small conformational changes occurring on binding to the mineral surface [78]. Osteopontin is also involved in the recruitment of osteoclasts and in regulating the immune response [79]. Bone specific conditional knockout of osteopontin results in impaired osteoclast activity at all ages [72], but sexual dimorphism was not noted.

Dentin matrix protein 1 is a synthetic product of growth plate chondrocytes and of osteocytes, although it was first cloned from dentin [40]. DMP1 is not usually found in an intact form but rather it is found as three smaller peptides, an N-terminal peptide, a C-terminal peptide and an N-terminal protein that has a glycosaminoglycan chain attached [65]. It is the only one of the SIBLING proteins to date that has been

TABLE 1.1 Bone non-collagenous matrix proteins* whose modification (deletion (KO) or overexpression (TG)) creates a bone phenotype

Protein or gene	Genotype	Bone phenotype	Proposed function
Biglycan [62]	KO	Decreased mineral content Increased crystal size in young animals Females less affected	Regulation of mineralization
Bone sialoprotein [63]	KO	Variable	Initiation of mineralization Signaling
Decorin [62]	KO	Weaker bones Thinner collagen fibrils	Regulation of collagen fibrillogenesis
Dentin matrix protein-1 [64, 65]	KO	Impaired mineralization Altered osteocyte function	Regulation of mineralization Signaling response to load Phosphate regulation
Dentin sialophosphoprotein gene (dspp) [66]	KO	Increased collagen maturity and crystallinity in young male and female mice	Regulation of initial calcification
Matrix gla protein [57]	KO	Excessive vascular and cartilage calcification	Prevent excessive calcification
Matrix extracellular phosphoglycoprotein [67, 68]	KO	Hypermineralization	Regulation of PHEX activity
	TG	Hypomineralization	Regulation of mineralization
Osteocalcin [49, 50]	KO	Thicker bones, smaller crystals suggest impaired turnover Males/females differ	Regulation of bone turnover Glucose regulation
Osteonectin [69, 70]	KO	Altered collagen maturity	Regulation of collagen fibrillogenesis
	Bone specific KO	Decreased bone density, increased bone fragility	Regulation of bone formation
Osteopontin [71, 72]	KO	Increased bone density, larger crystals, resistant to turnover	Osteoclast recruitment Inhibition of mineralization
	Bone specific KO	Increased bone density	Osteoclast recruitment

*Enzymes, growth factors and cytokines that affect bone are excluded from this table.

associated with a bone disease (autosomal hypophosphatemic rickets) [80]. The intact protein appears to inhibit mineralization, as does the glycosylated N-terminal fragment, but the phosphorylated cleaved fragments can promote mineralization [81, 82]. The knockout mouse has defective mineralization, supporting a role for DMP1 as a nucleator [64], although it appears equally important as a signaling molecule [8].

Bone sialoprotein (BSP) is a specific product of bone forming cells. There are low levels in other mineralized tissues, such as calcified cartilage and dentin. In solution, BSP is a hydroxyapatite nucleator [83, 84], implying a role in in situ mineralization. In culture, BSP facilitates osteoblast differentiation and maturation [85] and thereby stimulates mineralization. The BSP knockout is viable, but has a variable phenotype. In the youngest animals, the bones are shorter, narrower and less mineralized, supporting the in vitro findings. As the animals age, the mineralization normalizes, but the mice have impaired osteoclast activity, as they are resistant to bone loss by hind-limb suspension [63]. These data support the hypothesis that because mineralization is such an important process, it is crucial to have multiple pathways to support mineralization. BSP activity may be different in males and females as knockdown of the estrogen receptor alpha gene in a model of cartilage induced osteoarthritis resulted in decreased expression of BSP, implying some gender specificity to the expression of this protein [86] and studies in chick osteoblasts had previously demonstrated a response of BSP expression to estrogen-like molecules [87].

Matrix extracellular phosphoglycoprotein (MEPE) is made in bone, dentin and also exists in serum as smaller peptides [67]. The MEPE peptides are effective inhibitors of hydroxyapatite formation and growth, while unpublished studies show the intact protein, in phosphorylated form, promotes hydroxyapatite formation. Following gene ablation, the knockout animals have excessive mineralization while the transgenic animal, in which MEPE is overexpressed is hypomineralized [67]. This protein is one of the substrates for PHEX (phosphate regulating hormone with analogy to endopeptidase on the X-chromosome). PHEX is defective in hypophosphatemic rickets, presumably because where normally PHEX binds to MEPE and degrades its inhibitory peptides, in the mutant, this ability to degrade the peptides is absent and the inhibition persists [68]. Thus, MEPE is an important regulator of calcification. Because PHEX is on the X-chromosome, hypophosphatemic rickets is more prevalent and more severe in males than in females, although the female HYP mice have a bone phenotype, but it is less severe than that of the males [88].

Dentin sialophosphoprotein is expressed as a gene, dspp, but an intact protein has not yet been isolated. Its major components, dentin sialoprotein (DSP) and dentin phosphophoryn (DPP) are found mainly in dentin, but the gene is expressed in bone [61], and the dspp gene knockout has a detectable bone phenotype [66]. Both DSP and DPP can regulate mineralization in vitro, thus it is not surprising that the knockout has impaired mineralization both in bone and in dentin.

Non-Collagenous Proteins: SLRPS

Small leucine rich proteoglycans (SLRPS) are the major bone glycoproteins [40]. While small amounts of large aggregating proteoglycans (such as aggrecan and epiphican) are resident in bone as part of residual calcified cartilage, the majority of the bone proteoglycans are smaller. These SLRPS include decorin (the major SLRP produced by osteoblasts), biglycan, osteoadherin, lumican, fibromodulin and mimecan [89]. Each of these proteins binds to collagen and regulates collagen fibrillogenesis, thus they have an important effect on the bone composite and the mechanical strength of bone. In addition, biglycan and decorin are important for regulating cellular activity, perhaps due to the binding of growth factors, and decorin, biglycan and mimecan can regulate hydroxyapatite formation [90]. The properties and functions of these proteins in bone as adapted from these reviews are summarized in Table 1.2, while Table 1.1 includes the properties of the knockouts that had bone phenotypes.

Non-Collagenous Proteins: Matricellular Proteins

Another protein family whose members are found in bone are the so-called ‘matricellular proteins’, named so because they regulate the interactions between the cells and the extracellular matrix. The members of this family found in mineralized bone (as distinct from cartilage) include: osteonectin (SPARC), the matrillins, the thrombospondins, the tenascins, the galectins, periostin and osteopontin and BSP (SIBLINGs). Each of these proteins is expressed in higher amounts during development than in adult life, but they are all upregulated during wound repair (callus formation) in the adult. As noted from studies of mice lacking these proteins, or combinations thereof, matricellular proteins affect postnatal bone structure and turnover when animals are challenged by aging, ovariectomy, mechanical loading and fracture healing regeneration but do not have a visible phenotype during normal development [96].

Non-Collagenous Proteins: Other

In addition to the families of bone matrix proteins noted above, there are other extracellular matrix proteins that are found in glycosylated and phosphorylated form in bone. These include BAG-75 (which is found at the initial sites of mineralization in culture) [97], SPP24 (that regulates the formation of bone via inhibition of BMP-induced osteoblast differentiation) [98] and others proteins that serve as signaling molecules or have other functions that are still being investigated [40].

TABLE 1.2 Small leucine rich proteoglycans (SLRPs) found in bone*

Protein	Structure	Proposed functions
Biglycan	2 GAG chains/protein core	Binds and releases growth factors Cell differentiation Initiates mineralization Expression depressed in patient's with Turner's syndrome
Decorin	Generally 1 GAG chain/protein core	Regulates collagen fibrillogenesis Binds and releases growth factors
Osteoadherin [91]	Keratan sulfate proteoglycan	Facilitates osteoblast differentiation and maturation Regulates HA proliferation
Fibromodulin	4 Keratan sulfate chains in its leucine rich domain	Regulation of collagen fibrillogenesis
Asporin [92]	Possesses a unique stretch of aspartate residues at its N terminus	Negative regulator of osteoblast maturation and mineralization
Osteoglycin/mimecan	Derived from bone tumor Also called osteogenic factor	Induces osteogenesis Regulation of collagen fibrillogenesis Regulation of mineralization
Lumican	Keratan sulfate proteoglycan	Regulation of collagen fibrillogenesis Regulation of mineralization
Osteomodulin [93]	Keratan sulfate proteoglycan	Regulates osteoblast maturation
Periostin (osteoblasts-specific factor 2) [94]	SLRP made in primary osteoblasts	Regulates intramembranous bone formation Regulates collagen fibrillogenesis
Tsukushin [95]	353 amino acid protein upregulated by estrogen – has phosphorylation sites	BMP inhibitor Regulates mineralization

*Adapted from OMIM: On Line Mendelian Inheritance in Man: http://www.ncbi.nlm.nih.gov/sites/entrez/OMIM unless otherwise noted.

Other Matrix Components

Within the extracellular matrix are other proteins including enzymes (Table 1.3), growth factors and other signaling molecules, as well as lipids that are important for regulating cell–cell communication and mineral deposition. The actions of lipids in bone are reviewed in detail elsewhere [40, 103, 104]. The importance of lipid rafts (caveolin) is seen in the caveolin knockout mouse that has increased bone density and matures more rapidly than control mice [105]. There have not yet been reports of sex-dependent differences in these mice, although lipid metabolism is different in men and women.

HOW BONES CHANGE WITH AGE

A key event in the transition from the embryo to the adult is the development of mineralized structures. The cells that deposit the matrix, regulate the flux of ions and control the interaction between the matrix components orchestrate these processes. As shown by Figure 1.3, the mineral in bone is deposited in an oriented fashion on the collagen matrix. It is widely recognized, as reviewed elsewhere [33, 40], that the collagen provides a template for mineral deposition, but the extracellular matrix proteins regulate the sites of initial mineral deposition and control the extent to which the crystals can grow in length and in width. The collagenous matrix is mineralized to a certain extent during development (primary mineralization) and, as the individual ages, the rest of the matrix becomes mineralized (secondary mineralization). A variety of signals, discussed elsewhere in this book, activate the osteoclast to remove bone and this removal exposes stimuli that activate osteoblasts to lay down a new bone matrix, with the matrix proteins mentioned above regulating these processes. With age, the resorption process exceeds the formative one and this occurs earlier in women then in men.

Mouse models in which specific matrix proteins are ablated or inserted provide information both on the sexual dimorphic responses of these proteins, but also on the age-related changes. Mice, in general, achieve their peak bone mass at 16–18 weeks of age, depending on the sex and background. Although the functions of many of these proteins are redundant, because they are so essential for the development of the animal, examining knockout and transgenic animals (see Table 1.1) and the phenotypic appearance of their bones provides clues into the activities of these proteins. The only knockouts that totally lack bone are the osterix [106] and the Runx2 knockouts [107], although the retinoblastoma tumor suppressor gene knockout has severely impaired osteogenesis [108]. The knockout

TABLE 1.3 Some key enzymes* involved in modifying bone structure in health and disease

Enzyme	Substrate/activity	Effect on bone properties
Bone specific alkaline phosphatase [99]	Hydrolyzes phosphate esters	Stimulates new bone formation
Bone morphogenetic protein 1/tolloid [100]	Cleaves matrix proteins including removing pro-peptides form fibrillar collagens	Modulates activity of matrix proteins – turning inhibitors into activators and vice versa preparing matrix for mineral deposition
Cathepsin K [101]	Demineralized matrix	Osteoclast enzyme – when defective results in osteopetrosis
Cl-channel and ATPase [101]	Transports Cl ions out of osteoclasts	When blocked get osteopetrosis
PHEX [67, 68]	Cleaves ASARM peptides	Removes inhibitors of mineralization
Protein kinases [31]	Add phosphate moieties	Activates some proteins/inactivates others
Phosphoprotein phosphatases [31]	Removes phosphate moieties	Activates some proteins/inactivates others
Procollagen peptidases [48]	Removes terminal peptides from collagen	When defective bone fails to cross-link properly resulting in reduced mechanical strength
Tartrate resistant acid phosphatase [102]	Phosphoesters	Marker of osteoclast activity

*Excludes enzymes involved in protein synthesis.

and overexpression of other bone proteins and 'critical' signaling pathways have altered bone properties but none seem to be mandatory, most likely due to the redundancy of the function of these proteins. However, from the analyses of the cell culture and altered phenotype in the animals having too little or too much of these proteins, the following can be identified as important for the formation of the mineralized matrix: type I collagen, bone sialoprotein, dentin matrix protein1, BAG-75, osteopontin, PHEX and alkaline phosphatase. The sequence in which they act is not yet clear.

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CHAPTER 2

Bone Remodeling: Cellular Activities in Bone

HUA ZHOU[1], SHI S LU[1] AND DAVID W. DEMPSTER[2]

[1]*Regional Bone Center, Helen Hayes Hospital, West Haverstraw, New York, NY, USA*
[2]*Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY, USA*

INTRODUCTION

Bone remodeling is a fundamental process by which the mammalian skeleton tissue is continuously renewed to maintain the structural, biochemical and biomechanical integrity of bone and to support its role in mineral homeostasis. The process of bone remodeling is achieved by the cooperative and sequential work of groups of functionally and morphologically distinct cells, termed basic multicellular units (BMUs) or bone remodeling units (BRUs). Changes in the population and/or activities in any component of the BMUs disrupts the harmony of the cellular efforts and leads to changes in bone mass and strength. The cellular activities of bone remodeling units vary within and among the different bones of the skeleton and this variation changes with age, underlying the mechanism of age-related bone loss. This chapter reviews current concepts of bone remodeling with respect to its cellular mechanism, physiological functions and anatomic variation in cellular behavior.

CELLULAR MECHANISM OF BONE REMODELING

Bone remodeling takes place on bone surfaces and is achieved by multicellular units, BMUs [1, 2] or bone remodeling units, BRUs [3], the latter term being used here. The process of remodeling consists of four sequential and distinct phases of cellular events: activation, resorption, reversal and formation [2, 4, 5] (Figure 2.1A–E). The microanatomic basis of BRUs is osteonal units in intracortical bone (Figure 2.1G) and discrete osteonal units or packets in endocortical and cancellous bone (Figure 2.1F), where removal of old bone is coupled in space and in time by replacement by new bone [6, 7].

Activation

Activation is the term used to describe the process of converting a resting bone surface into a remodeling surface. In the human adult skeleton, a new BRU is activated about every ten seconds [3]. Activation involves recruitment of mononuclear osteoclast precursors from hematopoietic origin, penetration by osteoclast precursors through gaps in the bone lining cell layer, fusion of the precursor cells to form multinucleated osteoclasts and functional osteoclasts adhering to mineralized bone matrix [8, 9]. Two cytokines, receptor activator of nuclear factor kappa B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF), are essential and sufficient for osteoclastogenesis [10–12]. RANKL and M-CSF are produced by marrow stromal cells and their derivative osteoblasts in response to pro-resorption stimuli, such as parathyroid hormone (PTH), $1,25(OH)_2D$, interleukin-1 (IL-1) and interleukin-6 (IL-6), and play a crucial role in the formation, activation, activity and life span of osteoclasts (Figure 2.2). The activation of sites on the bone surface is either targeted or random. Selective remodeling targets specific sites where the osteocytes have sensed a change in mechanical strain or matrix damage in the form of microcracks and have conveyed signals to the surface to initiate targeted remodeling. However, most remodeling sites are likely to be random [13, 14].

Resorption

Osteoclasts affix themselves to the bone matrix through integrins such as $\alpha\nu\beta3$ [15, 16]. The adherence to bone induces ruffled membrane formation and creates an annular

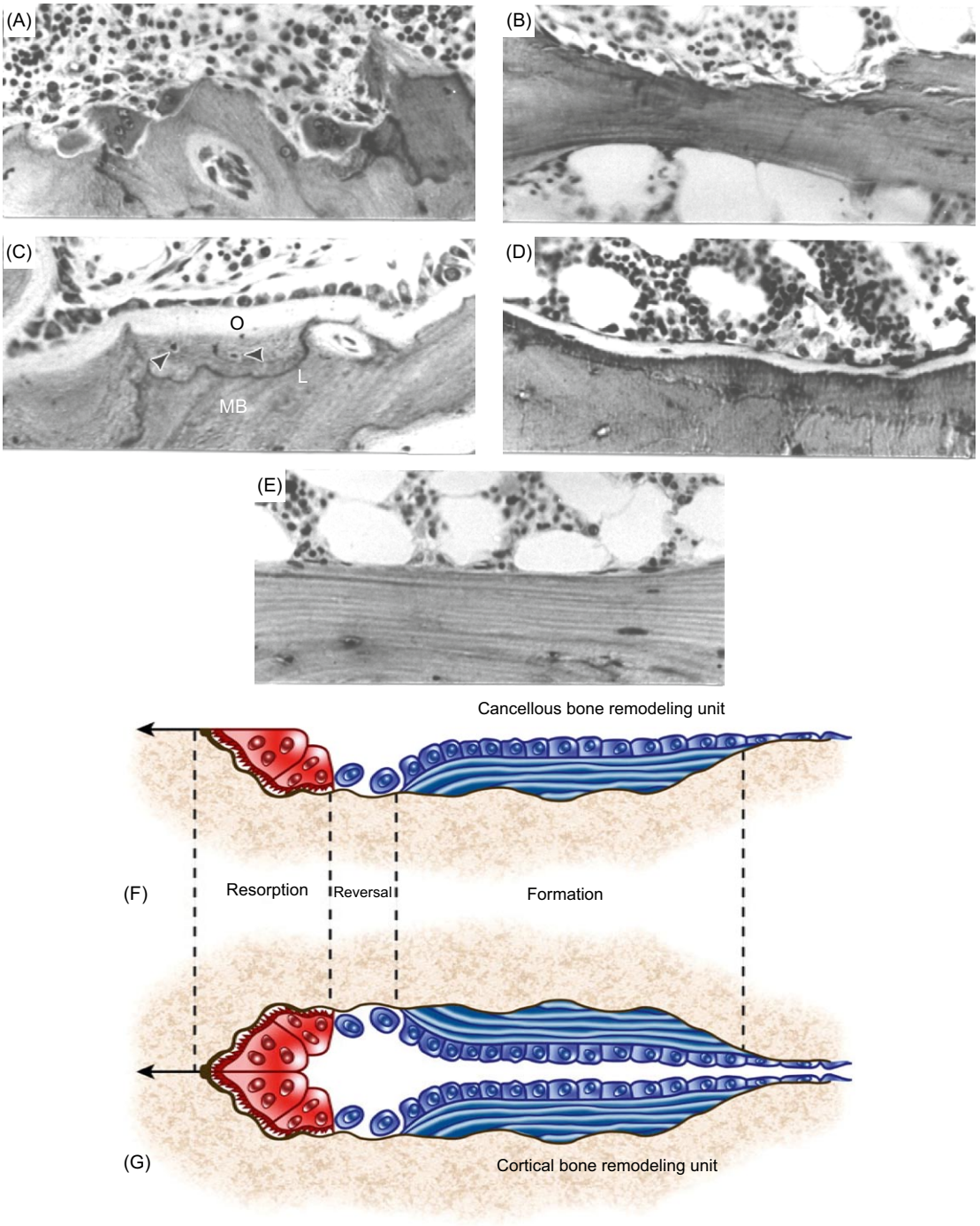


FIGURE 2.1 Light photomicrographs of the principal phases of the remodeling cycle in cancellous bone of human iliac crest biopsy specimens. (A) Resorption. Several multinucleated osteoclasts are seen in excavating a Howship's lacuna. (B) Reversal. The Howship's lacuna contains no osteoclasts but small mononucleated cells in contact with the scalloped surface. (C) Formation. A sheet of plump osteoblasts is seen depositing osteoid (O) on top of mineralized bone (MB). Note the reversal line (L) and osteocyte lacunae (arrowheads) in the mineralized matrix. (D) A later stage of formation where the osteoblasts have become flattened lining cells. Matrix production has ceased, but a thin layer of osteoid still remains to be mineralized. (E) Resting. No remodeling activity is in progress but a layer of attenuated cells lines the surface. Cross-sectional diagrams of BRUs in cancellous bone (F) and cortical bone (G). The arrows indicate the direction of movement through space. Note that the cancellous BRU is essentially one half of the cortical BRU. (A–E, from Dempster DW. Bone remodeling. In Disorders of bone and mineral metabolism. 2nd edn, (eds) Coe F, Favus MJ, pp 315–343, 2002. Lippincott Williams & Wilkins, Philadelphia: with permission. F,G, from Seibel MJ, Robins SP, Bilezikian JP. (eds) Dynamics of bone and cartilage metabolism, 2nd edn, pp 377–389, 2006. Academic Press, New York with permission).

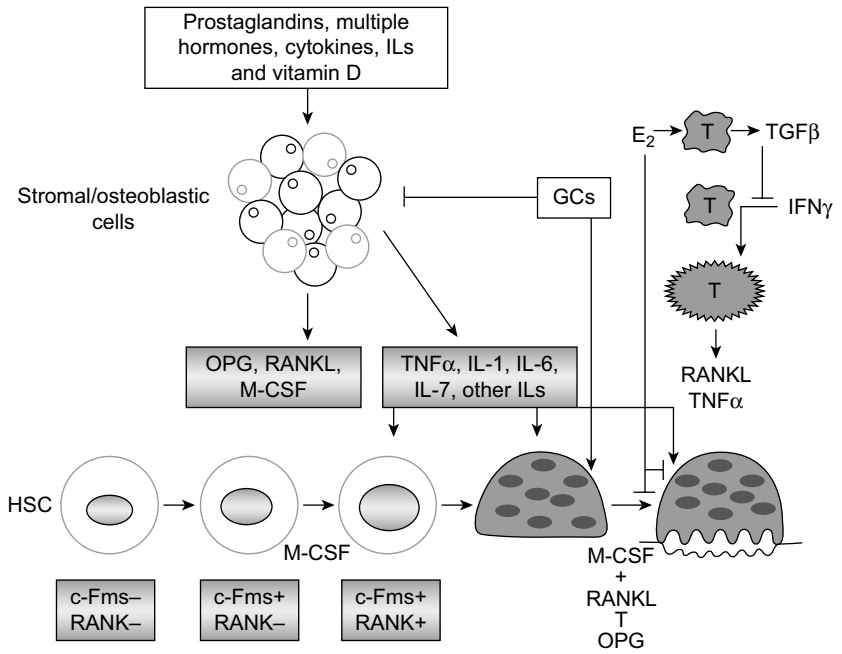


FIGURE 2.2 Role of cytokines, peptide and steroid hormones and prostaglandins in the osteoclast formation and activation. Hematopoietic stem cells (HSCs) express c-Fms (receptor for M-CSF) and RANK (receptor for RANKL) and differentiate to osteoclasts. Marrow mesenchymal cells respond to a range of stimuli by secreting a mixture of pro- and anti-osteoclastogenic factors, the latter consisting primarily of OPG. (From Ross FP. Osteoclast biology and bone resorption. In Primer on the metabolic bone diseases and disorders of mineral metabolism, 6th edn, (ed.) Favus MJ, pp 30–35, 2006. American Society for Bone and Mineral Research, Washington, with permission).

sealing zone, forming a hemivacuole between the osteoclast itself and the bone matrix and isolated from the surrounding extracellular space (Figure 2.3A, B). By means of membrane-bound proton pumps and chloride channels, the osteoclast secretes hydrochloric acid, as well as acidic proteases such as cathepsin K, TRACP, MMP9, MMP13 and gelatinase into the hemivacuole (see Figure 2.3A, B) [17, 18]. The acidified solution in the resorbing compartment mobilizes the mineralized component of the matrix and the proteolytic enzymes, which are most active at low pH, degrade the organic constituents of the matrix. This process creates the crescent-shaped resorption cavities called Howship's lacunae on the cancellous bone surface (see Figure 2.1A and F) and the cutting cones of the evolving Haversian systems within cortical bone (see Figure 2.1G). Generally, the resorption is accomplished by multinucleated osteoclasts, but both in vivo and in vitro evidence suggests that mononucleated cells are also capable of excavating bone and forming resorption cavities and cutting cones [19, 20]. The fate of the osteoclast at the conclusion of the resorption phase is unclear, but at least some undergo apoptosis [21].

Reversal

During this phase, the resorption lacuna is occupied by mononuclear cells, including monocytes, osteocytes that were liberated from bone by osteoclasts and pre-osteoblasts that are being recruited to couple the resorption phase with the formation phase (see Figure 2.1B, F, G) [22]. The mechanism of osteoblast coupling and the exact nature of the coupling signals are currently undefined, but there are a number of interesting hypotheses. One plausible theory is that osteoclastic bone resorption liberates growth factors from the bone matrix and that these factors serve as chemoattractants for osteoblast precursors and then enhance osteoblast proliferation and differentiation. Bone matrix-derived growth factors, such as transforming growth factor-β (TGF-β), insulin-like growth factors I and II (IGF-I and II), bone morphogenetic proteins (BMPs), platelet-derived growth factors (PDGF) and fibroblast growth factor (FGF) are all possible contenders for such coupling factors [23–27]. Another attractive premise is that the coupling of bone formation to resorption is a strain-regulated phenomenon [28]. As bone remodeling units penetrate through cortical bone, strain levels are reduced in front of the osteoclasts, but are increased behind them. Similarly, in cancellous bone, strain is posited to be higher at the base of the Howship's lacunae and lower in the surrounding bone. It is argued that this gradient of strain leads to sequential activation of osteoclasts and osteoblasts, with osteoclasts being activated by reduced strain and osteoblasts, in turn, by increased strain. This hypothesis may account for alignment of osteons along the dominant loading direction of the

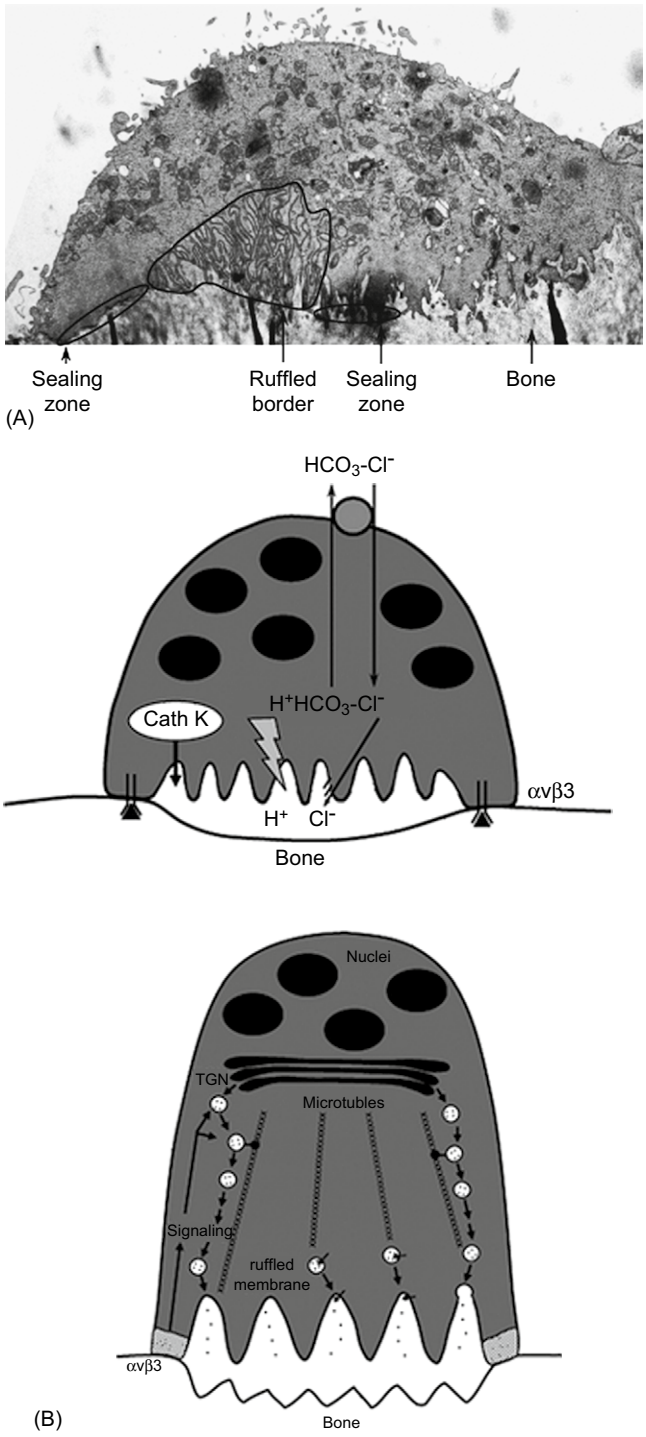


FIGURE 2.3 (A) Transmission electron microphotograph of a multinucleated osteoclast in rat bone. Note the extensive ruffled border, sealing zones and the partially degraded matrix between the sealing zones. (B) Diagram illustrating the primary mechanisms of osteoclastic bone resorption. (From Ross FP. Osteoclast biology and bone resorption. In Primer on the metabolic bone diseases and disorders of mineral metabolism, 6th edn, (ed.) Favus MJ, pp 30–35, 2006. American Society for Bone and Mineral Research, Washington, with permission).

bone [29, 30]. Furthermore, osteoclast to osteoblast forward and reverse signaling has recently been implicated in the coupling mechanism [31, 32].

Formation

Osteoblasts are recruited and differentiate from mesenchymal precursors. There is a gradient of differentiation as the osteoblastic precursors reach the bone surface to refill the resorption cavity and the osteoblast phenotype becomes fully expressed (Figure 2.4A) [33]. Bone matrix formation is a two-stage process in which osteoblasts initially synthesize the organic matrix, called osteoid, and then regulate its mineralization (Figure 2.4B). Osteoid consists of collagenous proteins, predominantly type I collagen, accounting for ≈90% of the organic matrix, with non-collagenous proteins making up the remaining ≈10%, including glycoproteins (i.e. alkaline phosphatase and osteonectin), Gla-containing proteins (i.e. osteocalcin and matrix Gla protein) and others (e.g., proteolipids) [34]. Osteoid is deposited on the bone surface in curved sheets called osteoid lamellae, following the contours of the underlying mineralized bone (see Figure 2.4B). Once the collagenous organic matrix is synthesized, osteoblasts trigger the mineralization process, which occurs after a delay of about 20 days, called the mineralization lag time. This is accomplished by the release of small, membrane-bound matrix vesicles that establish suitable conditions for initial mineral deposition by concentrating calcium and phosphate ions and enzymatically degrading inhibitors of mineralization, such as pyrophosphate and proteoglycans that are present in the extracellular matrix [35]. During this period, the osteoid undergoes a variety of biochemical changes that render it mineralizeable. The mineral content of the matrix increases rapidly to 75% of the final mineral content over the first few days, called primary mineralization, but it may take as long as a year for the matrix to reach its maximum mineral content, called secondary mineralization [36]. The mineral crystals within bone are analogous to the naturally occurring geologic mineral, hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$), including numerous ions which are not found in pure hydroxyapatite, such as HPO_4^{2-}, CO_3^{2-}, Mg^{2+}, Na^+, F^- and citrate, adsorbed to the hydroxyapatite crystals [34].

As bone formation continues, osteoblasts that have reached the end of their synthetic activity embed themselves in the matrix, becoming osteocytes (see Figure 2.4A). Osteocytes are regularly dispersed throughout the mineralized matrix and maintain intimate contact with each other, as well as to the cells on the bone surface, through gap junctions between their slender, cytoplasmic processes or dendrites, which pass through the bone in small canals called canaliculi (Figure 2.5). Osteocytes function as an extensive 3-dimensional network of sensor cells, or 'syncytium', which can detect a change in mechanical strain in bone and respond by transmitting signals to the lining

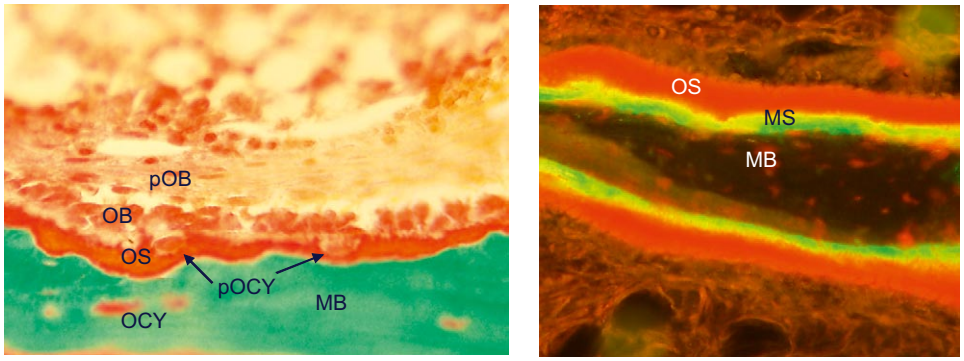


FIGURE 2.4 (A) Light photomicrograph of a human bone biopsy stained with Goldner's trichrome. Osteoblastic lineage in a gradient differentiation: osteoblastic precursors (pOB) reach the bone surface → mature osteoblasts (OB) filling in a resorption cavity → pre-osteocytes (pOCY) become incorporated into osteoid (OS) matrix → osteocytes (OCY) embedded within the mineralized bone (MB). (B) Fluorescent photomicrograph of dog bone. Two steps of bone formation: osteoid matrix forming on bone surface (OS), mineralizing surface (MS) and mineralized bone (MB). (See color plate section).

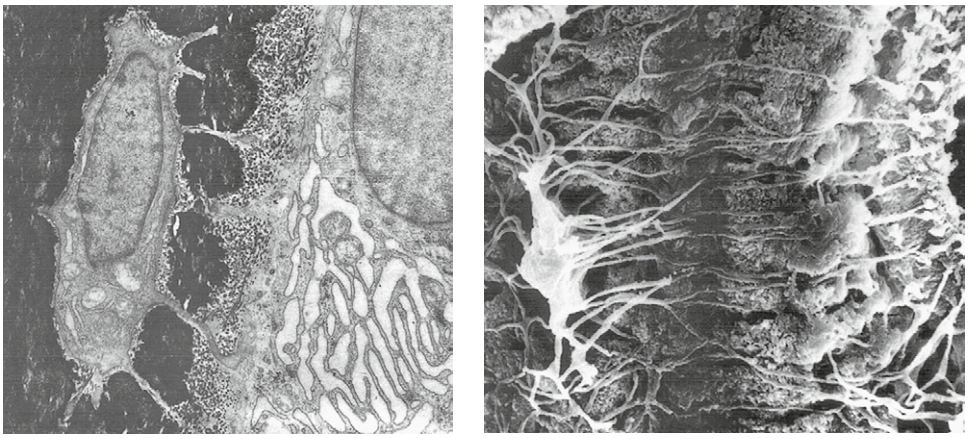


FIGURE 2.5 (A) Transmission and (B) scanning electron micrographs showing osteocyte processes communicating with cells on the bone surface. (From Marotti G. et al. The structure of bone tissues and the cellular control of their deposition. Ital J Anat Embryol 1996;**101**:25-79, with permission).

cells on the bone surface to initiate targeted remodeling or to regulate resorption and formation in the newly initiated bone remodeling cycle [37]. Osteocytes die by apoptosis, which occurs with aging, immobilization, microdamage, lack of estrogen, glucocorticoid excess and in association with pathological conditions, such as osteoporosis and osteoarthritis [38]. Osteocyte apoptosis has also been suggested to play an important role in targeting bone remodeling following the observation that osteocyte apoptosis occurs in association with areas of microdamage and that this is followed by osteoclastic resorption to begin the replacement of the mechanically challenged bone [39].

Osteoblasts suffer one of three fates during and at the end of the bone formation phase of the remodeling cycle: many become incorporated into the matrix they formed and differentiate into osteocytes; some convert into lining cells on the bone surface at the termination of formation; and the remainder die by apoptosis. Bone lining cells were once thought to serve primarily to regulate the flow of ions into and out of the bone extracellular fluid serving as the blood–bone barrier. It has recently been appreciated that, under certain circumstances, for example, stimulation by PTH or mechanical force, bone lining cells can revert back to functional osteoblasts [40, 41]. Another recently discovered important function of the lining cells is to create specialized compartments in cancellous and cortical bone where bone remodeling takes place [42] (Figure 2.6).

The end result of a completed remodeling cycle by a BRU is the production of a new osteon (Figure 2.7A, B). The remodeling process is similar in cancellous and cortical bone with the remodeling unit in cancellous bone being equivalent to half of a cortical remodeling unit [43] (see Figure 2.1F, G).

The difference between the volume of bone removed by osteoclasts and replaced by osteoblasts during BRU remodeling cycle is termed 'bone balance'. As will be discussed later, the bone balance varies with the anatomical location of the bone surface as well as with gender, age and disease.

PHYSIOLOGICAL FUNCTIONS OF BONE REMODELING

The primary functions of bone remodeling are presumed to be maintenance of the mechanical competence of bone by continuously replacing fatigued bone with new, mechanically sound bone and to preserve mineral homeostasis by continuously mobilizing the skeletal stores of calcium and phosphorus to the circulation. It has also been suggested that there must be other, as yet known functions or reasons why the human skeleton undergoes such extensive remodeling [44].

Like all load-bearing structural materials, the skeleton is subjected to fatigue damage as it ages and undergoes repetitive mechanical challenges. Older bone displays increased mineralization density as secondary mineralization continues and the water content diminishes, which causes the matrix to become more brittle [45]. In addition, aging is associated with biochemical changes in the bone matrix constituents, such as accumulation of non-enzymatic glycation end products [46] and increased cross-linking of collagen [47]. These changes render the bone more susceptible to mechanical damage and fracture. It has also been demonstrated that osteocytes that have undergone apoptosis leave empty lacuna that may become occluded by mineralized debris [48] and that fatigue microcracks increase in number with bone age and are spatially associated with missing osteocytes [49]. Moreover, the fact that resorption cavities are frequently located close to bone microcracks [50, 51] provides compelling evidence that targeted remodeling is activated in response to the appearance of such microcracks.

The skeleton is the greatest repository of mineral ions, such as Ca, Mg and P, in the human body and plays an important role in mineral homeostasis by coordinated interplay with the intestine, the site of net ionic absorption, and the kidney, the site of net ionic excretion. Long-term mineral homeostasis is achieved by the BRUs, which mobilize skeletal mineral to blood during bone resorption and return the mineral back to the skeleton during bone formation. However, at least two other mechanisms allow the skeleton to participate in mineral homeostasis: the blood–bone barrier maintained by the bone lining cells and the percolation of bone extracellular fluid through osteocyte lacuno-canalicular network.

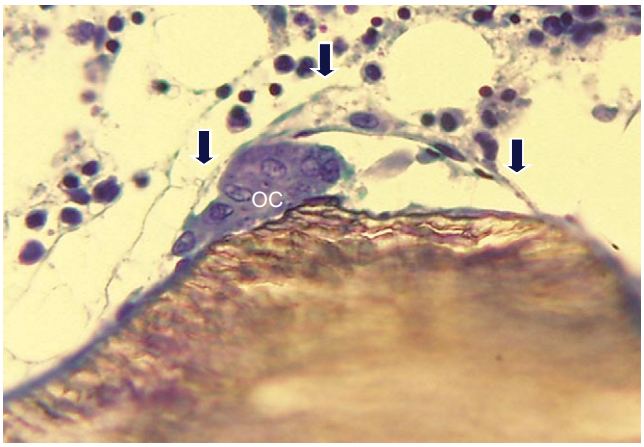
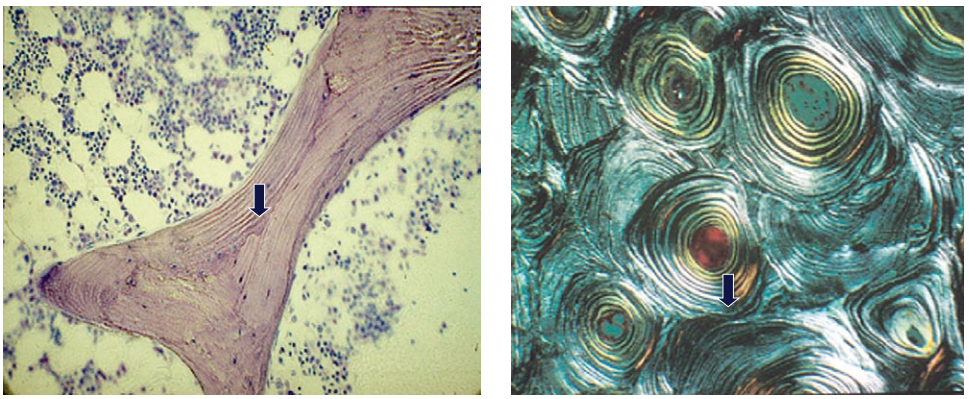


FIGURE 2.6 Light photomicrograph of a human bone biopsy stained with toluidine blue. An osteoclast (OC) is resorbing bone within a specialized compartment formed by a dome-shaped layer of lining cells (arrows). (See color plate section).



(A) (B)

FIGURE 2.7 (A) Completed basic structural units in cancellous bone and (B) cortical bone. The arrowheads delineate reversal lines. (From Dempster DW. Bone remodeling. In Osteoporosis: etiology, diagnosis, and management, 2nd edn, (eds) Riggs BL, Melton LJ, pp 67–91, 1995. Raven Press, New York, with permission.) (See color plate section).