Calcium in Human Health

NUTRITION ◊ AND ◊ HEALTH

Adrianne Bendich, Series Editor

Calcium in Human Health, edited by **Connie M. Weaver and Robert P. Heaney,** 2006
Nutritional Health: *Strategies for Disease Prevention, Second Edition,* edited by **Norman J. Temple**, **Ted Wilson, and David R. Jacobs, Jr.,** 2006
Nutrients, Stress, and Medical Disorders, edited by **Shlomo Yehuda and David I. Mostofsky**, 2006
Preventive Nutrition: *The Comprehensive Guide for Health Professionals, Third Edition,* edited by **Adrianne Bendich and Richard J. Deckelbaum,** 2005
The Management of Eating Disorders and Obesity, *Second Edition,* edited by **David J. Goldstein,** 2005
Nutrition and Oral Medicine, edited by **Riva Touger-Decker, David A. Sirois, and Connie C. Mobley,** 2005
IGF and Nutrition in Health and Disease, edited by **M. Sue Houston, Jeffrey M. P. Holly, and Eva L. Feldman,** 2005
Epilepsy and the Ketogenic Diet, edited by **Carl E. Stafstrom and Jong M. Rho,** 2004
Handbook of Drug–Nutrient Interactions, edited by **Joseph I. Boullata and Vincent T. Armenti,** 2004
Nutrition and Bone Health, edited by **Michael F. Holick and Bess Dawson-Hughes,** 2004
Diet and Human Immune Function, edited by **David A. Hughes, L. Gail Darlington, and Adrianne Bendich,** 2004
Beverages in Nutrition and Health, edited by **Ted Wilson and Norman J. Temple,** 2004
Handbook of Clinical Nutrition and Aging, edited by **Connie Watkins Bales and Christine Seel Ritchie,** 2004
Fatty Acids: *Physiological and Behavioral Functions,* edited by **David I. Mostofsky, Shlomo Yehuda, and Norman Salem, Jr.,** 2001
Nutrition and Health in Developing Countries, edited by **Richard D. Semba and Martin W. Bloem,** 2001
Preventive Nutrition: *The Comprehensive Guide for Health Professionals, Second Edition,* edited by **Adrianne Bendich and Richard J. Deckelbaum,** 2001
Nutritional Health: *Strategies for Disease Prevention,* edited by **Ted Wilson and Norman J. Temple**, 2001
Clinical Nutrition of the Essential Trace Elements and Minerals: *The Guide for Health Professionals,* edited by **John D. Bogden and Leslie M. Klevey**, 2000
Primary and Secondary Preventive Nutrition, edited by **Adrianne Bendich and Richard J. Deckelbaum**, 2000
The Management of Eating Disorders and Obesity, edited by **David J. Goldstein,** 1999
Vitamin D: *Physiology, Molecular Biology, and Clinical Applications,* edited by **Michael F. Holick,** 1999
Preventive Nutrition: *The Comprehensive Guide for Health Professionals,* edited by **Adrianne Bendich and Richard J. Deckelbaum**, 1997

Calcium in Human Health

Edited by

Connie M. Weaver, PhD

Department of Foods and Nutrition, Purdue University, West Lafayette, IN

and

Robert P. Heaney, MD

John A. Creighton University Professor, Creighton University, Omaha, NE

Foreword by

Lawrence G. Raisz, MD

Division of Endocrinology and Metabolism, University of Connecticut Health Center, Farmington, CT

Humana Press
Totowa, New Jersey

999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

www.humanapress.com

Cover design by Patricia F. Cleary
Production Editor: Robin B. Weisberg

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel.: 973-256-1699; Fax: 973-256-8341; E-mail: orders@humanapr.com; or visit our Website: www.humanapress.com

This publication is printed on acid-free paper. ∞
ANSI Z39.48-1984 (American National Standards Institute) Permanence of Paper for Printed Library Materials.

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

eISBN 1-59259-961-3

Library of Congress Cataloging-in-Publication Data

Calcium in human health / [edited by] Connie M. Weaver and Robert P. Heaney
; foreword by Lawrence G. Raisz.
p. ; cm. -- (Nutrition and health)
Includes bibliographical references and index.
ISBN 1-58829-452-8 (alk. paper)
1. Calcium in the body. 2. Calcium in human nutrition.
[DNLM: 1. Calcium--metabolism. 2. Calcium--pharmacology. 3. Nutritional Requirements. QV 276 C14363 2005] I. Weaver, Connie, 1950- II. Heaney, Robert Proulx, 1927- III. Series: Nutrition and health (Totowa, N.J.)
QP535.C2C26355 2005
612.3'924--dc22

2005006564

Dedication

Calcium in Human Health incorporates many of the main findings of our research careers. It also has chapters written by many of our favorite colleagues and collaborators. Our interest in calcium spans nearly 80 years of work between us. We had more than a decade of collaboration as co-investigators on our long-running bioavailability project. We continue as colleagues and friends, learning from one another still. The wisdom and rich experience that the other brings to our collaborative efforts have shaped much of the basic framework with which we approach research and nutritional policy. We dedicate this book to our wonderful laboratory groups, who work tirelessly, and to the students who continually teach us. We also dedicate this book to our families who have always supported our work (which is more like play to us) with much love, and on occasion even given generously of their time and skills to our research projects.

Connie M. Weaver
Robert P. Heaney

Series Editor's Introduction

The *Nutrition and Health Series* of books have had great success because each volume has the consistent overriding mission of providing health professionals with texts that are essential because each includes (1) a synthesis of the state of the science; (2) timely, in-depth reviews by the leading researchers in their respective fields; (3) extensive, up-to-date fully annotated reference lists; (4) a detailed index; (5) relevant tables and figures; (6) identification of paradigm shifts and the consequences; (7) virtually no overlap of information between chapters, but targeted, interchapter referrals; (8) suggestions of areas for future research; and (9) balanced, data-driven answers to patient/health professionals' questions that are based on the totality of evidence rather than the findings of any single study.

The series volumes are not the outcome of a symposium. Rather, each editor has the potential to examine a chosen area with a broad perspective, both in subject matter as well as in the choice of chapter authors. The international perspective, especially with regard to public health initiatives, is emphasized where appropriate. The editors, whose trainings are both research- and practice-oriented, have the opportunity to develop a primary objective for their book; define the scope and focus, and then invite the leading authorities from around the world to be part of their initiative. The authors are encouraged to provide an overview of the field, discuss their own research, and relate the research findings to potential human health consequences. Because each book is developed *de novo*, the chapters are coordinated so that the resulting volume imparts greater knowledge than the sum of the information contained in the individual chapters.

Calcium in Human Health, edited by Drs. Connie M. Weaver and Robert P. Heaney, is a critical addition to the *Nutrition and Health Series* and fully exemplifies the goals of the series. As an essential mineral that forms the structural components of bones and teeth, calcium is integral to our health and well-being. However, the critical role of calcium in the functioning of nerves and muscles, cellular membrane interactions, the clotting of blood, and even our mood states is less well known. Moreover, there are newer areas of research concerning the importance of calcium in estrogen-related conditions, such as the premenstrual syndrome and the polycystic ovarian syndrome, that may provide clinically relevant options for many women. This volume has been developed to examine the current investigations concerning the importance of calcium in the functioning of the human body and mind, disease prevention, and treatment, and to put these areas of research and medical practice into historic perspective as well as point the way to future research opportunities.

Calcium and Human Health joins three other volumes in the *Nutrition and Health Series* in providing in-depth information about vitamin and mineral nutrients that are essential to bone as well as overall health. Dr. Michael Holick's edited volume, entitled *Vitamin D*, was published in 1999 and is being updated in the Second Edition that is due to be published in 2007. In 2004, both Dr. Holick and Dr. Bess Dawson-Hughes edited

the comprehensive volume, *Nutrition and Bone Health*. The editors of this volume on calcium have contributed valuable chapters to the *Nutrition and Bone Health* volume. Dr. Heaney has informative chapters in *Clinical Nutrition of the Essential Trace Elements and Minerals*, edited by Drs. John D. Bogden and Leslie M. Klevay and in the recently published Third Edition of *Preventive Nutrition*, edited by myself and Dr. Richard J. Deckelbaum. Thus, the editors of this volume, Dr. Connie M. Weaver and Dr. Robert P. Heaney, have added greatly to the series and have provided a key volume on calcium that makes the series a place where researchers can look for the best up-to-date information on calcium and other minerals, vitamin D, and bone health.

Both of the editors are internationally recognized leaders in the field of calcium research. Both are excellent communicators and they have worked tirelessly to develop a book that is destined to be the benchmark in the field because of its extensive, in-depth chapters covering the most important aspects of the complex interactions between diet and its nutrient components, bone formation and function, consequences of calcium deficiency as well as potential adverse effects of calcium excess on major body systems. Moreover, the volume includes insightful chapters that review the role of calcium and related nutrients including, but not limited to, vitamin D, in maintaining mental as well as physical health, and an extensive evaluation of its critical importance in the prevention of major disease states. The introductory chapters provide readers with the basics of calcium's biological functions so that the more clinically related chapters can be easily understood. The editors have contributed several chapters and have also chosen 23 of the most well-recognized and respected authors from around the world to contribute the 28 informative chapters in the volume. Key features of this comprehensive volume include the bulleted Key Points that are at the beginning of each chapter, the more than 115 detailed tables and informative figures, the extensive, detailed index, and the more than 1800 up-to-date references that provide the reader with excellent sources of worthwhile information about calcium and human health. To add further value to this benchmark volume, the editors have included five appendices that make this the "go-to" text for useful referenced materials including the detailed tabulation of the Dietary Reference Intake values for calcium across the age span as well as the criteria used to support the intake values; a table that lists the major food sources of calcium and the clinically derived absorption efficiency of calcium from each food source; a detailed dietary assessment tool for calculating daily calcium intakes; and lists of both relevant books and websites where the reader can find further information about calcium.

The book chapters are logically organized in six sections to provide the reader with a basic understanding as well as an appreciation of the development of the field of calcium research, its relationship to organ system functions and the potential for calcium nutriture to affect these variables. The first two sections review basic scientific information on the cellular and metabolic functions of calcium that is essential to understanding the following sections. In these chapters, the reader is introduced to the leading techniques for determining calcium status through both dietary as well as kinetic studies. For every nutrient, there are concerns about the veracity of dietary recall, the actual daily intake requirement and the bioavailability of the nutrient that is consumed in a mixed diet and/or through supplementation or fortification. Each of these factors is crucial in understanding the complexities of the disease states as well as the development of drugs to treat relevant diseases such as osteoporosis. The third section includes chapters that review

calcium requirements, tabulate recommendations in the United States compared to 33 other nations, and examine the food sources, supplements, and their bioavailability compared with milk, which is used as the standard. The fourth section examines in depth the body's responses to low calcium intake and its regulation at the molecular level. Figures in this section clearly illustrate the relationships between the internal and external compartments in bone and how these affect bone strength. In addition to internal factors, certain lifestyle choices, such as exercise, smoking, and alcohol consumption can impact on one's calcium status. Moreover, there are data that point to a "calcium appetite," which is discussed in a separate, well-referenced chapter in this section. Equally important is the understanding of the potential for calcium nutriture to affect responses to growth, pubertal changes, and pregnancy and lactation. The fifth section reviews the interactions between the bones, nervous, and endocrine systems and also includes detailed information about the differences in responses between males and females as their bodies undergo maturation.

The sixth and final section of the volume includes 10 chapters that address the interactions between calcium and the major clinical diseases that affect both men and women. The editors have included extensive chapters on calcium's role in the development of osteoporosis in the bones of the central and peripheral skeleton as well as in the oral cavity; the newest research on the potential for calcium to affect the development of, as well as the treatment of, obesity and a separate chapter on the effects of calcium on insulin sensitivity and diabetes; the growing clinical findings of calcium's effects in colon and other cancers; calcium's effects on blood pressure; and a related chapter on the importance of calcium balance in renal disease. Two additional chapters examine the consequences of low calcium status on the development and treatment of the premenstrual and polycystic ovarian syndromes.

This important reference text provides practical, data-driven integrated resources based on the totality of the evidence to help the reader evaluate the critical role of calcium, especially in at-risk populations, in optimizing health and preventing calcium-related chronic illnesses. The overarching goal of the editors is to provide fully referenced information to health professionals so they may have a balanced perspective on the value of foods and nutrients that are routinely consumed and how these help to maintain calcium status to assure both mental as well as physical health.

In conclusion, *Calcium in Human Health*, edited by Weaver and Heaney, provides health professionals in many areas of research and practice with the most up-to-date, well referenced, and easy-to-understand volume on the importance of calcium in reducing the risk of developing chronic diseases and optimizing health. This volume will serve the reader as the benchmark in this complex area of interrelationships between diet, calcium, and other relevant specific nutrients, skeletal, muscle, renal, cardiac, and hormonal functions; environmental factors and their effects on calcium status including exercise, smoking, and alcohol consumption; and calcium's role in obesity, diabetes, cancer, cardiovascular, and kidney disease prevention as well as treatment. The editors are applauded for their efforts to develop the most authoritative resource in the field to date and this excellent text is a very welcome addition to the *Nutrition and Health Series*.

Adrianne Bendich, ***PhD, FACN***
Series Editor

Foreword

In *Calcium in Human Health*, 25 authors have accomplished the daunting task of not only demonstrating the importance of calcium in human health, but also defining its many and complex roles. The roles of calcium in biology became much more complex and critical when animals emerged from the sea, although the fundamental regulatory roles of calcium in cells persisted. The first eukaryotes developed systems for excluding calcium from the intracellular fluid so that nanomolar concentrations could be maintained inside the cell in the face of millimolar concentrations outside, and changes in these concentrations could be used to alter cellular function. Perhaps these primordial organisms developed in an environment of about 1.3 m*M* calcium, similar to that of our own extracellular fluid. As organisms evolved in the sea, the calcium concentration rose, and new mechanisms for preventing excessive calcium entry developed, which may now be expressed in the limited intestinal absorption of this critical element in mammals. As organisms moved into fresh water and ultimately onto dry land, a new problem needed to be solved. Calcium was no longer abundant in the environment, but scarce. One solution was the development of a calcium-rich skeleton, but the critical functions of calcium in cell regulation and its equally critical role in maintaining a structural framework for the organism now came into conflict.

Calcium in Human Health begins, in Chapters 2 and 3, by setting out the fundamentals of this conflict, not only by indicating the multiple roles of calcium, but also by summarizing the mechanisms by which some of the conflict can be resolved. To understand the role of calcium, it is important to have methods that can accurately measure its bioavailability, absorption, and kinetics. These are described in detail in Chapters 4–6. The next three chapters cover the complex issue of calcium consumption, requirements, and bioavailability. Despite the extremely wide variation in calcium intakes and differences in Recommended Daily Allowances in different countries, it can be concluded that calcium deficiency is a major problem and calcium excess a rare one.

The complex regulation of calcium absorption, distribution, and excretion, as well as the multiple interactions of diet, lifestyle, and physical activity in calcium homeostasis are outlined in Chapters 10–14. Chapter 15 summarizes the evidence for a "calcium appetite" in humans and experimental animals and points out the interesting possibility that our current high intakes of salt and fat may blunt this appetite. This provides a potential explanation for the inadequacy of calcium intake in societies where ample supplies are available. However, another factor may be the decrease in total food intake that has occurred as humans become less physically active in an industrialized society.

Chapters 16–18 cover the special aspects of calcium economy that occur in infancy, childhood, adolescence, and with pregnancy and lactation. These are particularly important areas of public health concern, as emphasized in the recent Surgeon General's report on Bone Health and Osteoporosis.[1]

A unique and exciting aspect of this book is the discussion of specific roles of calcium in a variety of clinical disorders, in the last 10 chapters. Although much has been written about the role of calcium in maintaining the skeleton and of calcium deficiency as a pathogenetic factor in osteoporosis, other interactions have not received as much attention. The chapters on calcium and oral health, obesity, reproductive disorders, and the metabolic syndrome, provide new insights and raise new questions. Much more needs to be learned about the role of calcium in these disorders. Similarly, there is clear evidence that calcium and vitamin D can play a role in cancer, but here again further definition is needed. With the availability of drugs that can alter the function of the extracellular calcium receptor, the complex changes in calcium and phosphate regulation that occur in renal disease and the potential role of calcium in hypertension and vascular disease, which are summarized in the last two chapters, represent additional areas where new studies are both needed and feasible.

Calcium in Human Health might have the subtitle, "Everything You Wanted to Know About Calcium and Needed to Ask." It contains a vast amount of information, but also indicates many gaps in our knowledge. One major gap is the discrepancy between knowledge and practice in the area of public health. Perhaps a companion volume on what must be done to improve the calcium economy of our population and how this can be accomplished could be a next step. Based on present information, this might be a slim volume indeed, but we do have much of the necessary scientific background needed to define both the problems and the opportunities for doing more about calcium in human health.

Lawrence G. Raisz, *MD*
Division of Endocrinology and Metabolism
University of Connecticut Health Center
Farmington, CT

[1]*Bone Health and Osteoporosis: A Report of the Surgeon General* can be accessed on the web at www.surgeongeneral.gov.

Preface

More research is done on calcium with resultant publications than for any other mineral. This interest in calcium is appropriate with its diverse biological functions, the dietary inadequacies in calcium all over the world, and the relationship of calcium status to so many disorders. Calcium serves as a second messenger for nearly every biological process and stabilizes many proteins. It is an unusual nutrient in that the storage reserve of calcium in the skeleton has a biological function. Bone mass predicts risk of fracture. Aside from bone health, calcium insufficiency has been associated with hypertension, cardiovascular health, stroke, polycystic ovary disease, kidney stones, certain types of cancer, weight loss, diabetes, and insulin resistance syndrome.

The aim of *Calcium in Human Health* is to provide students, scientists, and health professionals including physicians, nutritionists, dentists, pharmacists, dietitians, and health educators with up-to-date research on calcium function and its relationship to health. The amount of new information has been almost explosive linking calcium to health in the last decade with the associations to weight loss, diabetes, and insulin resistance syndrome evolving in the last 5 years. Equally exciting are the discoveries coming from molecular biology and genetics. Our basic understanding of calcium absorption and the influence of gene polymorphisms is evolving. Single book chapters cannot do justice to the amount of new information available.

Calcium in Human Health is divided into six parts. Part I discusses calcium function as the main element in bone, as an intracellular messenger, and as a stabilizer of proteins. This section explains why calcium status is part of the etiology of so many disorders. Part II discusses methods for estimating calcium intakes of various populations as well as how to conduct controlled feeding studies. The ability to determine calcium intake sheds light on interpretation of studies of the relationship of calcium intake to disease. The third section discusses calcium intakes, requirements, and dietary sources of calcium. One chapter illustrates how widespread calcium deficiencies are throughout the world. Circumstances that create calcium excesses and the implication of exceeding upper tolerable levels are reviewed. Another chapter discusses calcium bioavailability and food factors that influence calcium absorption. Part IV reviews calcium homeostasis. Molecular mechanisms of calcium absorption and regulators of calcium homeostasis from genetics to lifestyle choices are reviewed in this section. One chapter suggests an interesting role for regulation of intake driven by calcium appetite. The influence of total diet and lifestyle choices on calcium metabolism is also covered in this section. A fifth section covers calcium through development. Various chapters in this section cover infancy and childhood, adolescence, pregnancy, and lactation. The last section covers many of the diseases now associated with calcium intake. Each chapter begins with an overview of the literature, but the emphasis is on recent findings.

We have devoted most of our careers to the study of calcium and its relationship to health. As editors, we hope *Calcium in Human Health* will serve as a critical resource for

health professionals to enhance their ability to improve health outcomes of individuals; for researchers who study calcium function and application; for students of health science, nutrition, and medicine; and for those setting dietary requirements and developing disease-prevention programs. This comprehensive coverage of calcium in human health is assembled by the leading researchers in the field of calcium. We believe that *Calcium in Human Health* will serve as a useful text and reference. We invite comments from users of this book about its content and use of various chapters in their investigations and in training.

Connie M. Weaver, ***PhD***
Robert P. Heaney, ***MD***

Contents

Contributors

STEVEN A. ABRAMS, MD • *Pediatrics/Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX*

D. LEE ALEKEL, PhD, RD • *Department of Food Science & Human Nutrition, Iowa State University, Ames, IA*

EMMANUEL M. AWUMEY, PhD • *Cardiovascular Disease Research Program, Julius L. Chambers Biomedical/Biotechnology Research Institute, North Carolina Central University, Durham, NC*

CAROL BOUSHEY, PhD, MPH, RD • *Department of Foods and Nutrition, Purdue University, West Lafayette, IN*

RICHARD D. BUKOSKI, PhD • *(deceased) Cardiovascular Disease Research Program, Julius L. Chambers Biomedical/Biotechnology Research Institute, North Carolina Central University, Durham, NC*

BESS DAWSON-HUGHES, MD • *USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA*

JAMES C. FLEET, PhD • *Department of Foods and Nutrition, Purdue University, West Lafayette, IN*

KELI M. HAWTHORNE, RD • *Pediatrics/Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX*

ROBERT P. HEANEY, MD • *John A. Creighton University Professor, Creighton University, Omaha, NE*

PETER R. HOLT, MD • *Strang Cancer Research Laboratory, Rockefeller University, Professor of Medicine (Emeritus), Columbia University, New York, NY*

HEIDI J. KALKWARF, PhD • *General & Community Pediatrics, Children's Hospital Medical Center, Cincinnati, OH*

ELIZABETH A. KRALL, PhD, MPH • *Health Policy & Health Services Research, Boston University, Goldman School of Dental Medicine, Boston, MA*

ANNE C. LOOKER, PhD • *Division of Health and Nutrition Examination Statistics, Centers for Disease Control and Prevention, National Center for Health Statistics, Hyattsville, MD*

DAVID S. LUDWIG, PhD • *Division of Endocrinology, Children's Hospital, Boston, MA*

OKSANA MATVIENKO, PhD • *Physical Education, Leisure Services, School of Health, University of Northern Iowa, Cedar Falls, IA*

DAVID A. MCCARRON, MD • *Academic Network, Portland, Oregon; Department of Nutrition, University California at Davis, Davis, CA*

LAWRENCE G. RAISZ, MD • *Center for Osteoporosis, Department of Medicine, University of Connecticut Health Center, Farmington, CT*

ZAMZAM K. (FARIBA) ROUGHEAD, PhD, RD • *Novartis Medical Nutrition, St. Louis Park, MN*

Bonny L. Specker, PhD • *E.A. Martin Program in Human Nutrition, South Dakota State University, Brookings, SD*
Dorothy Teegarden, PhD • *Department of Foods and Nutrition, Purdue University, West Lafayette, IN*
Michael G. Tordoff, PhD • *Monell Chemical Senses Center, Philadelphia, PA*
Susan Thys-Jacobs, MD • *Department of Medicine, St. Luke's-Roosevelt Hospital Center, Columbia University, New York, NY*
Matthew Vukovich, PhD • *Exercise Physiology Laboratory, South Dakota State University, Brookings, SD*
Meryl E. Wastney, PhD • *Metabolic Modeling Service, Dalesford, Hamilton, New Zealand*
Connie M. Weaver, PhD • *Department of Foods and Nutrition, Purdue University, West Lafayette, IN*
Yongdong Zhao, MD • *Department of Foods and Nutrition, Purdue University, West Lafayette, IN*

1 Introduction

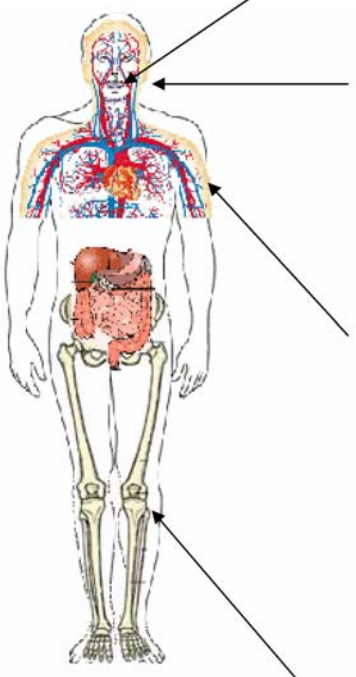
Connie M. Weaver and Robert P. Heaney

Calcium is one of 21 elements known to be essential to humans. It is one of three minerals required in the diet in relatively large quantities and for which a Dietary Reference Intake (DRI) has been established by the Food and Nutrition Board. At this writing, calcium requirements are set as Adequate Intakes (AI) rather than as Recommended Dietary Allowances (RDA). The decision to set an AI rather than an RDA by the 1997 Food and Nutrition Board related more to the use of a new approach for determining optimal calcium intakes than to the stated paucity of data for determining calcium requirements. Calcium is the most studied of the minerals in relationship to human health. In Spring 2004, a Medline search for articles about minerals published between 1994 and 2004 yielded 62,852 articles about calcium. The next most cited minerals were iron (14,963 articles), zinc (10,399 articles), and magnesium (10,097 articles). The most cited common mineral deficiencies in the world are in iron, iodine, and zinc. Yet, more people are further from their recommended intakes for calcium than for any of these minerals. Inadequate calcium intake has such a long latency period before signs of disease are apparent that its association with health is not adequately appreciated. This book covers the functions of calcium, the approaches for determining calcium intakes for optimal health, and the relationship of calcium status to long-studied and newly identified diseases.

Adequate calcium nutrition has such far-reaching impact because of calcium's unique chemistry. Calcium has an intermediate binding affinity. For example, it is not so tightly bound to proteins—as is zinc—that it cannot readily be removed. Thus, it can serve as an on/off switch in cell regulation. It has only one oxidation state so it is not prone to be toxic at high concentrations or to cause tissue damage under various conditions. As part of hydroxyapatite, it forms a material strong enough to support our bodies for many decades, but light enough to allow mobility. Like other minerals, calcium is immutable, and therefore cannot be synthesized or degraded. This is a huge advantage for analysis, even after long-term storage, so long as samples are protected from contamination from extraneous calcium sources.

Calcium is not efficiently absorbed or retained by the body. It can form complexes that are poorly digested. Much of the small fraction that is absorbed is excreted by obligatory losses or is affected by other dietary constituents. Determining bioavailability of calcium and factors that influence the calcium economy is facilitated by the availability of many useful isotopic tracers of calcium.

From: *Calcium in Human Health*
Edited by: C. M. Weaver and R. P. Heaney



Current Status	Knowledge Gaps	Environment	Needed Future Research
Sources Ca:			
Calcium content of food and bioavailability of major sources known	How to improve calcium absorption and retention efficiency	Extensive Ca addition to food supply and supplements	Bioavailability testing of all manufactured sources of Ca Identifying lifestyle choices that optimize Ca economy
Ca Requirements:			
Ca requirements based on optimal retention	Ca Requirements determined primarily only on data for Caucasians	DRIs for Ca given AI not RDA	Ca requirements for various subgroups Relationship to other lifestyle choices – dietary and physical activity. Need better markers for Ca status
Tissue [Ca^{2+}]:			
Serum concentration detected by Calcium - sensing receptor and regulated by vitamin D/PTH homeostatic regulatory mechanism	Soft tissue regulation	Tools available for sampling and measurement are for serum not soft tissue [Ca^{2+}]	Tissue level regulation vit D-Ca interaction Relationship to health and disease Quantitative nutrition studies (controlled diet) to explore Ca interactions and bone health and population specific relationships
Skeleton:			
Relationship of bone mass to bone health well studied	Role of Ca in context of total lifestyle choices for bone health throughout lifecycle	Ca supplements typically advised with insufficient attention to whole diet	Ability of calcium to redistribute to areas of low bone mass

Fig. 1. Status of knowledge of calcium and human health.

The status of knowledge about calcium and human health is briefly summarized in Fig. 1. Some of the most pressing gaps in our knowledge about calcium and needed research are also included. The interplay of calcium with other environmental factors and its regulation and requirements at the soft-tissue level are the least understood areas, both because they are difficult to measure and because complex research design is required to answer these questions.

Calcium as a nutrient is not useful to health in isolation. For example, utilization of calcium depends on adequate vitamin D status. Dietary sodium greatly influences renal calcium reabsorption. Adequate bone mass requires protein, phosphorus, magnesium,

and several trace nutrients as well as nondietary factors including sex steroid hormones and mechanical loading. None of the diseases addressed in this book has as a single etiology calcium deficiency. Nevertheless, it is useful to assemble our knowledge of the broad influence of calcium and its relationship to human health in one book for perspective and convenience.

I Calcium Functions

2 Bone as the Calcium Nutrient Reserve

Robert P. Heaney

KEY POINTS

- Bone is the body's calcium nutrient reserve.
- This reserve, over the course of evolution, acquired a secondary function—mechanical strength and rigidity—serving to support work against gravity.
- The reserve is added to or drawn upon by net addition or removal of microscopic units of bony tissue, not by simple withdrawal or addition of calcium atoms.
- The size of the reserve is determined by a combination of mechanical loading and net dietary calcium availability.
- Calcium is a threshold nutrient, in that bone mass increases as calcium intake increases up to the point where mechanical needs are met; above that level, no further calcium retention occurs and absorbed calcium is simply excreted.

1. INTRODUCTION

In addition to its obvious structural role, the skeleton is an important reservoir of calcium, serving both to maintain plasma calcium concentrations and to make optimal use of ingested calcium. It serves both functions mainly by adjusting the balance between bone formation (which transfers mineral from blood to bone) and bone resorption (which transfers mineral from bone to blood). It is important to stress at the outset that calcium cannot generally be withdrawn from bone *per se;* instead, it is scavenged from the tearing down of structural bony units. Thus, reduction in skeletal calcium reserves is equivalent to reduction in bone mass, and augmentation of the reserve is equivalent to augmentation of bone mass.

These same processes of formation and resorption are what constitute bone structural remodeling, or turnover. Remodeling of bone continues throughout life, and skeletal tissue is replaced every 10 to 12 yr on average. All bone remodeling occurs at anatomical bone surfaces. Bone-resorbing osteoclasts begin the remodeling process by attaching onto a bone surface, sealing it from the rest of the extracellular fluid (ECF); they then extrude packets of citric, lactic, and carbonic acids to dissolve the bone mineral, and proteolytic enzymes to digest the organic matrix. They thereby remove parcels of bone, leaving behind a cavity, or resorption bay. Later, bone-forming osteoblasts synthesize new bone to fill in the cavity and replace the previously resorbed bone.

From: *Calcium in Human Health*
Edited by: C. M. Weaver and R. P. Heaney

Formation and resorption are coupled both systemically and locally, and when resorption is high, formation is generally high as well. But the coupling is neither continuous nor perfect. Resorption normally exceeds formation during fasting, when no calcium is being absorbed from the intestine, and formation normally exceeds resorption during absorption of calcium from ingested food or supplements. This is how the body adjusts to intermittent intestinal absorptive input. Overall, however, the two processes are about equal when averaged over the day. Continuous net imbalances (i.e., changes in the size of the reserve) do occur in several situations. For example, bone formation exceeds resorption during growth, and resorption exceeds formation during lactation, or in the development of osteoporosis, or in the face of ongoing dietary shortage of calcium.

2. A UNIQUE NUTRIENT

Calcium is a unique nutrient in several respects. It is not the only nutrient with a substantial reserve in healthy individuals, but it is the only one for which the reserve has required an important function in its own right. We use the reserve for structural support (i.e., we literally walk on our calcium nutrient reserve). Calcium is unique also in that our bodies cannot store a continuing surplus, unlike, for example, energy or the fat-soluble vitamins. Calcium is stored not as such but as bone tissue, and the quantity of bone tissue is determined by cellular processes, with the responsible bone cellular apparatus controlled through a feedback loop regulated by mechanical forces, not by calcium intake. In brief, given an adequate calcium intake, we have only as much bone as we need for the mechanical loads we currently experience. Once our skeletons have reached their genetically and mechanically determined mass, unless something intervenes such as pregnancy or pharmacotherapy, we cannot accumulate more bone simply by consuming more calcium.

This feature is the basis for the designation of calcium as a "threshold" nutrient with respect to skeletal status, a term that means that calcium retention rises as intake rises, up to some threshold value that provides optimal bone strength (*see* Fig. 1); then, above that level, increased calcium intake produces no further retention and is simply excreted. This threshold intake is the lowest intake at which retention is maximal,that is, it is the minimum daily requirement (MDR) for skeletal health (*see* Chapter 7). The MDR varies with age, and is currently estimated to be approx 20–25 mmol (800–1000 mg/d) during childhood, 30–40 mmol (1200–1600 mg/d) during adolescence, approx 25 mmol (1000 mg/d) during the mature adult years, and 35–40 mmol (1400–1600 mg/d) in the elderly *(2–4)*. As previously noted, the rise in the published requirement in old age reflects an age-related decline in ability to adapt (i.e., to respond to low intakes with improved absorption and retention).

Calcium is unique in another respect related precisely to the reserve function of the skeleton. The best-attested disease manifestation of calcium deficiency (osteoporosis) is due not to impairment of the metabolic functions of calcium (*see* Chapter 3), which would be the case, for example, with the B vitamins, but instead to a decrease in the size of the reserve. For no other nutrient is this the case. Bone strength is a function of bone mass which, in turn, is equivalent to the size of the calcium nutrient reserve. This reserve is vast relative to the demands of calcium for cell signaling and activation, particularly because these metabolic functions do not actually consume calcium. Hence, nutritional calcium deficiency almost never manifests itself as a shortage of calcium ions in critical cellular or physiological processes. With most other nutrients, the reserve must first be exhausted

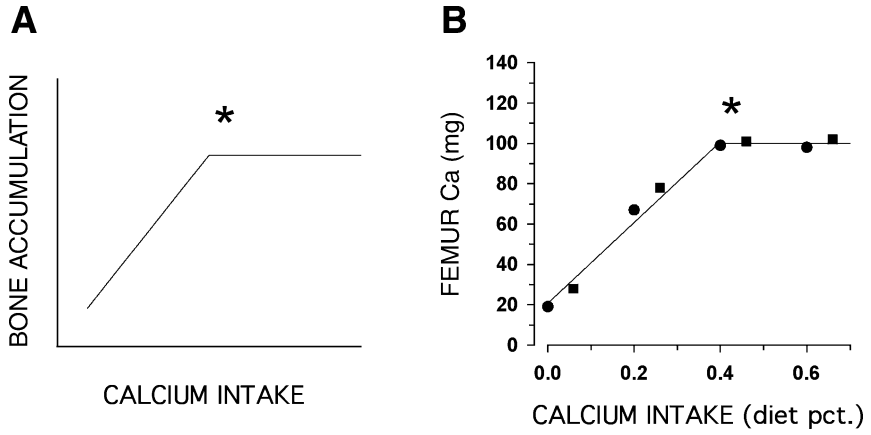


Fig. 1. Threshold behavior of calcium intake. **(A)** Theoretical relationship of bone accumulation to intake. Below a certain value (the threshold, indicated by an asterisk), bone accumulation is a linear function of intake (the ascending line); in other words, the amount of bone that can be accumulated is limited by the amount of calcium ingested. Above the threshold (the horizontal line), bone accumulation is limited by other factors and is no longer related to changes in calcium intake. **(B)** Actual data from two experiments in growing rats showing how bone accumulation does, in fact, exhibit a threshold pattern. (Redrawn from data in Forbes et al. *[1]*. Copyright Robert P. Heaney, 1992. Used with permission.)

before clear manifestations of disease or dysfunction develop. But for calcium, it is the simple reduction in skeletal mass that reduces bone strength and accordingly increases fracture risk. In brief, calcium intake insufficient to offset obligatory losses leads to reduction in bone mass, and is thus one of the causes of osteoporosis.

When excretory and dermal losses exceed absorbed dietary intake, the mechanisms designed to protect ECF [Ca^{2+}] tear down bone to scavenge its calcium. The mechanisms by which the reserves are accessed or augmented are set forth in detail in Chapter 10. Here we note only that parathyroid hormone (PTH) is evoked by a fall in calcium intake. At the same time, PTH is responsible for regulating the prevailing level of bone remodeling. PTH activates remodeling loci, which proceed through an orderly sequence of events consisting of (1) activation, which is manifested morphologically as retraction of lining cells from the bone surface about to undergo remodeling; (2) resorption of bone by osteoclasts; (3) replacement of the osteoclasts by osteoblasts, which lay down new bone to fill the hole created by osteoclastic resorption; and (4) return to the resting state, with the bone surface once again covered by a sheet of lining cells. The destructive, resorptive phase typically takes 3 wk in healthy adults, and the formative, reconstructive phase takes 3–6 mo.

Millions of such remodeling loci, each at different stages of this process, are going through this sequence at any time in the skeleton as a whole, some adding calcium to the blood, and some taking it up into new bone. An acute increase in remodeling activity initially creates an excess of resorption (because the new loci are all in the initial resorptive phase of the cycle). In this way, an increase in remodeling allows bone to contribute calcium to the blood. Conversely, an acute decrease in remodeling initially creates a

temporary excess of formation. These imbalances are how the bone accommodates a relative surplus or shortfall of absorbed calcium, hour by hour and day by day.

In providing the calcium needed to maintain critical body fluid concentrations, the reserve is functioning precisely as it should. But sooner or later there has to be payback, or the reserve becomes depleted, with an inescapable weakening of skeletal structures. During growth, on any but the most severely restricted of intakes, some bony accumulation will usually occur, but the result of an insufficient calcium intake is usually failure to achieve the full genetic potential for bone mass. Later in life, the result is failure to maintain the mass achieved. As also noted in Chapter 24, both low bone mass and osteoporotic fractures have many causes other than low calcium intake. Nevertheless, under prevailing conditions in the industrialized nations, at mid-to-high latitudes, the importance of calcium intake is considerable. Calcium-supplementation trials, even those of short duration, have resulted in reductions in fracture in the elderly amounting to 30% or more *(5,6)*.

3. EVIDENCE LINKING CALCIUM INTAKE TO BONE HEALTH

In addition to a large effect size, the evidence for calcium's role is itself very strong. There have been roughly 80 published reports of investigator-controlled increases in calcium intake with skeletal endpoints, most of them randomized, controlled trials and most of them published since 1990 *(7)*. The vast majority demonstrated either greater bone mass gain during growth, reduced bone loss with age, and/or reduced osteoporotic fractures. The exceptions among these studies were, for example, a supplementation trial in men in which the calcium intake of the control group was itself already high (nearly 1200 mg/d) *(8)*, and a study confined to early postmenopausal women *(9)* in whom bone loss is known to be due predominantly to estrogen deficiency.

Complementing this primary evidence are roughly 130 observational studies testing the association of calcium intake with bone mass, bone loss, or fracture *(7)*. It has been shown elsewhere *(10)* that such observational studies are inherently weak, not only for the generally recognized reason that uncontrolled or unrecognized factors may produce or obscure associations between the variables of interest, but because the principal variable in this case, lifetime calcium intake, cannot be directly measured and must be estimated by dietary recall methods. The errors of such estimates are immense and have been abundantly documented (*11,12; see also* Chapter 4). Their effect is to bias all such investigations toward the null. Nevertheless, more than three-fourths of these observational studies reported a significant calcium benefit. Given the insensitivity of the method, the fact that most of these reports are positive emphasizes the strength of the association; at the same time, it provides reassurance that the effects achievable in the artificial context of a clinical trial can be observed in real-world settings as well.

4. CALCIUM INTAKE, BONE REMODELING, AND SKELETAL FRAGILITY

These observations show clearly that variations in calcium intake in the range commonly encountered in the industrialized nations have substantial influences on the osteoporotic fracture burden (when intakes are low) or protect against fracture (when intakes are high). The most obvious explanation is the effect of calcium intake on opti-

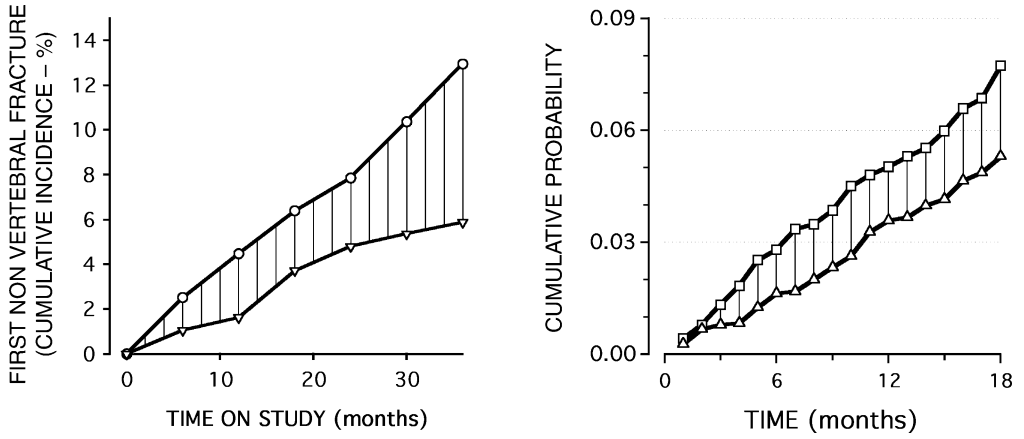


Fig. 2. Plots of the cumulative incidence of fractures, redrawn from the studies of Chapuy et al. *(5)* (right) and Dawson-Hughes et al. *(6)* (left). In both cases, the upper line represents the placebo control subjects, and the lower line represents the calcium and vitamin D-treated subjects. The shaded zones represent the reduction of fracture risk, which, as can be readily seen, starts with the very beginning of treatment. (Copyright Robert P. Heaney, 2004. Used with permission.)

mizing the size of the calcium reserve. But it is likely that there is a second aspect of the reserve involved in bony fragility as well. Examination of the cumulative fracture plots of the calcium intervention trials of Chapuy et al. *(5)* and Dawson-Hughes et al. *(6)* shows that the reduction in fracture risk begins almost immediately after supplementation is started—too soon for there to have been an appreciable effect on bone mass (Fig. 2).

Recent appreciation of the role of bone quality , as distinct from bone quantity , has led to an understanding of the fact that remodeling loci themselves directly contribute to fragility *(13)*, independently of bone mass. Remodeling rate doubles through menopause and continues to rise throughout the remainder of life *(14)*, in part because of inadequate calcium and vitamin D intakes. The immediate effect of calcium and/or vitamin D supplementation in typical postmenopausal women is a reduction of PTH secretion and with it, a corresponding and immediate reduction of bone remodeling. As the data assembled in Fig. 2 show, there is an immediate reduction in bony fragility as well. In brief, not only does low calcium intake contribute to bony fragility by depleting the reserve, but the very process of accessing the reserve itself renders bone fragile. Slowing that process confers an immediate benefit.

Several factors influence the size of the calcium reserve by direct action on bone (rather than by way of the calcium economy). Among these are smoking, alcohol abuse, hormonal status, body weight, exercise, and various medications. Smoking and alcohol abuse exert slow, cumulative effects by uncertain mechanisms that result in reduced bone mass and increased fracture risk. Low estrogen status and hyperthyroidism produce similar net effects, although probably by very different mechanisms. Bone mass rises directly with body weight, again by uncertain mechanisms. Exercise, particularly impact loading, is osteotrophic and is important both for building optimal bone mass during growth and for maintaining it during maturity and senescence.

5. CONCLUSIONS

The body possesses reserve supplies of most nutrients, which it uses to ensure smooth functioning in the face of irregular nutrient intake. Bone is the body's calcium reserve. This reserve is larger than for any other nutrient mainly because it has acquired a secondary, nonnutrient role—internal stiffening and mechanical support of our bodies. The size of the bony reserve is limited at its upper bound by mechanical need, and below that, by net calcium intake. Because the reserve is large, nutritional calcium deficiency virtually never compromises the basic metabolic functions of calcium. Rather, by depleting the reserve, the body weakens bone and jeopardizes its mechanical function. As a consequence and unlike with most other nutrients, reduction in the size of the nutrient reserve has immediate health consequences.

REFERENCES

1. Forbes RM, Weingartner KE, Parker HM, Bell RR, Erdman JW Jr. Bioavailability to rats of zinc, magnesium and calcium in casein-, egg- and soy protein-containing diets. J Nutr 1979;109:1652–1660.
2. NIH Consensus Conference: Optimal Calcium Intake. J Am Med Assoc 1994;272:1942–1948.
3. Dietary Reference Intakes for Calcium, Magnesium, Phosphorus, Vitamin D, and Fluoride. Food and Nutrition Board, Institute of Medicine, National Academy Press, Washington, DC: 1997.
4. Matkovic V, Heaney RP. Calcium balance during human growth. Evidence for threshold behavior. Am J Clin Nutr 1992;55:992–996.
5. Chapuy MC, Arlot ME, Duboeuf F, et al. Vitamin D_3 and calcium to prevent hip fractures in elderly women. N Engl J Med 1992;327:1637–1642.
6. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med 1997;337:670–676.
7. Heaney RP. Calcium, dairy products, and osteoporosis. J Am Coll Nutr 1999;19(2):83S–99S.
8. Orwoll ES, Oviatt SK, McClung MR, Deftos LJ, Sexton G. The rate of bone mineral loss in normal men and the effects of calcium and cholecalciferol supplementation. Ann Int Med 1990;112:29–34.
9. Nilas L, Christiansen C, Rødbro P. Calcium supplementation and postmenopausal bone loss. BMJ 1984;289:1103–1106.
10. Heaney RP. Nutrient effects: Discrepancy between data from controlled trials and observational studies. Bone 1997;21:469-471.
11. Barrett-Connor E. Diet assessment and analysis for epidemiologic studies of osteoporosis, In: Burckhardt P, Heaney RP, eds. Nutritional Aspects of Osteoporosis. Raven, New York, NY: 1991;91–98.
12. Beaton GH, Milner J, Corey P, McGuire V, Cousins M, Stewart E, et al. Sources of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. Am J Clin Nutr 1979;32:2446–2459
13. Heaney RP. Is the paradigm shifting? Bone 2003;33:457–465.
14. Recker RR, Lappe JM, Davies KM, Heaney RP. Bone remodeling increases substantially in the years after menopause and remains increased in older osteoporosis patients. J Bone Miner Res 2004;19:1628–1633.

3 Cellular Functions and Fluxes of Calcium

*Emmanuel M. Awumey and Richard D. Bukoski**

KEY POINTS

- Ionized calcium is an important signaling ion, and its cellular concentration is regulated by the intestine, kidney, bone, and the placenta (during pregnancy).
- The concentrations of Ca^{2+} in extracellular spaces and intracellular compartments are regulated by hormones and through membrane proteins that facilitate transient changes in cellular Ca^{2+} that are vital to cell function.
- Voltage-dependent channels, receptor-operated channels (many coupled to G proteins), and a myriad of transport proteins, all operating by different influx/efflux mechanisms, regulate intracellular Ca^{2+} levels.
- Perturbations in these Ca^{2+} influx/efflux mechanisms lead to various disease states.

1. INTRODUCTION

The divalent cation, or ionized, calcium—Ca^{2+}—is a mineral that is critical to normal human health, playing vital roles in fertilization, metabolism, blood clotting, nerve impulse conduction, muscle contraction, structure of the bony skeleton, and cellular communication. As covered in detail in Chapter 9, the primary dietary sources of calcium in contemporary diets are dairy products and to a lesser extent, leafy green vegetables. Dietary recommendations for calcium vary with age and pregnancy, as discussed in Chapter 8. When considering dietary sources, it is important to recognize the fact that ionized calcium is the biologically active form of the mineral and that bioavailability of calcium varies among different food groups.

Ionized calcium translates external signals into internal signals in the cell, a function facilitated by its small size and its affinity for protein molecules. The Ca^{2+} signal is translated by Ca^{2+}–protein interaction within the secondary and tertiary structure of the peptide. Ca^{2+} is much more suitable as a signaling ion than other prevailing ionic species because of the size of its ionic radius, which is smaller than that of potassium ions (K^+)

*Deceased

From: *Calcium in Human Health*
Edited by: C. M. Weaver and R. P. Heaney

and chloride ions (Cl^-) but larger than that of magnesium ions (Mg^{2+}) and small enough to fit into intracellular pores, whereas that of sodium ions (Na^+) is too small. In addition to this property, the two positive charges on the Ca^{2+} ion and a coordination number of 6–8 make Ca more flexible in interacting with the polypeptide structure, without constraint, to effect conformational changes necessary for signal transduction.

Cell activity is coordinated and controlled by a variety of signaling mechanisms, many or all of which involve the release of Ca^{2+} from critical intracellular compartments into the cytoplasm.

Furthermore, because the mean path length of Ca^{2+} entering through the plasma membrane is only a fraction of the cell diameter, it has been necessary for cells to evolve an elaborate intracellular calcium storage mechanism, which is activated to release Ca^{2+} into the cytosol in response to appropriate signals. For example, during striated muscle contraction, the initial trigger Ca^{2+} enters the cell from the extracellular space as a result of membrane depolarization. This activates intracellular Ca^{2+} release from internal storage sites into the myoplasm and its subsequent binding to regulatory sites to initiate cross-bridge formation. Relaxation follows when Ca^{2+} is removed from the myoplasm.

In view of the critical role that Ca^{2+} plays in the normal health and function of all cells, it is therefore not surprising that elaborate regulatory mechanisms for the transport and storage of Ca^{2+} have evolved at the whole-body and cellular levels. Failure of some or all of these regulatory mechanisms can lead to significant changes in the level of circulating Ca^{2+} that, in some instances, will not be compatible with life.

From this overview, it should be apparent that Ca^{2+} is a critical ion for the maintenance of life. Not surprisingly, elaborate and highly complex mechanisms are involved in maintaining its level within narrow limits in the cell (Fig.1). Calcium homeostasis is complex because it involves the gastrointestinal (GI) tract, kidney, and bones. It is our goal to review these systems with primary emphasis on cellular Ca^{2+} regulation. Where possible, we provide examples of syndromes that are associated with disturbances in Ca^{2+} fluxes.

2. FUNCTIONS OF Ca^{2+} IN CELLS

Activation of excitable cells results in Ca^{2+} influx from extracellular space through voltage-dependent and/or receptor-operated Ca^{2+} channels in the plasma membrane and release from intracellular storage sites to raise the cytosolic Ca^{2+} concentration from n*M* to μ*M* levels. To return the Ca^{2+} concentration to resting levels, ATP-driven Ca^{2+} transport to the extracellular space and into intracellular stores occurs *(1)*. Ca^{2+} is the main point of intersection for many distinct molecular signaling pathways and in living organisms plays a dual role, both as an ion required for cell survival and as an inducer of cell death. The presence of excess Ca^{2+} in the cytosol or perturbation of intracellular Ca^{2+} compartmentalization leads to Ca^{2+} overload, which triggers apoptotic or necrotic cell death *(2)*. Changes in intracellular Ca^{2+} concentrations are accomplished through modulation of Ca^{2+} influx channels, Ca^{2+} exchange proteins, and various Ca^{2+}-dependent enzymes *(3)*. The loss of regulatory ability of any of these Ca^{2+} influx/efflux mechanisms and the consequent increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) leads to a wide variety of pathological events such as brain trauma, stroke, and heart failure.

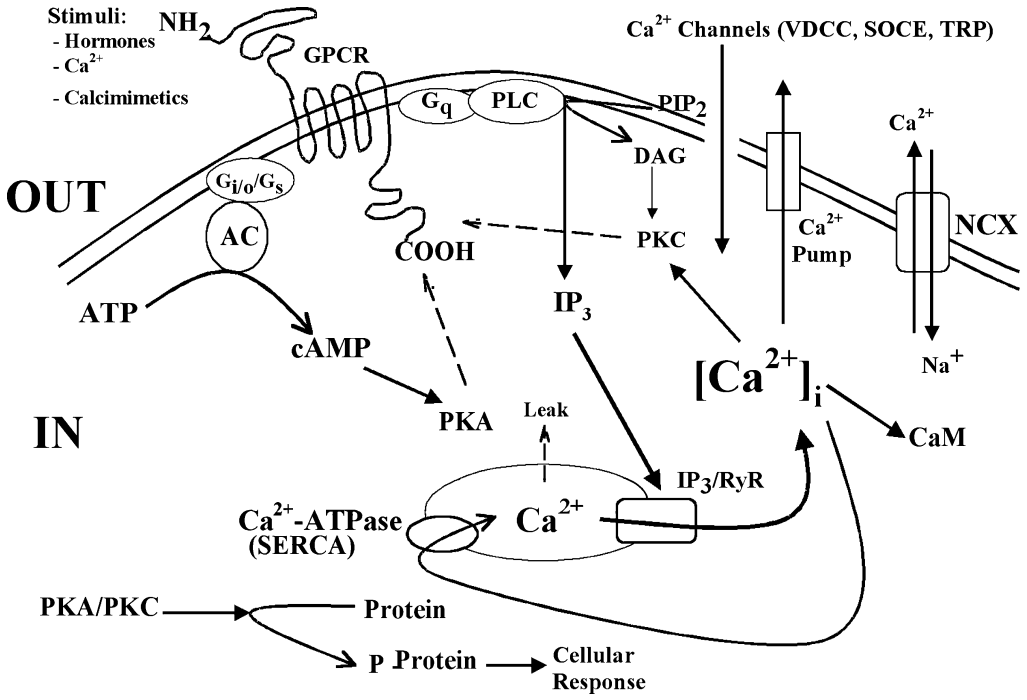


Fig. 1. Cellular Ca^{2+} signal transduction. AC, adenylyl cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; Ca^{2+} pump, plasma membrane Ca^{2+}-ATPase; DAG, diacyl glycerol; GPCR, G protein-coupled receptor; $G_{i/o}$, $G\alpha_{i/o}$ G protein subunit; G_q, $G\alpha_q$ G protein subunit; G_s, $G\alpha_s$ G protein subunit; IP_3, inositol 1,4,5-trisphosphate; IP_3R, inositol 1,4,5-trisphosphate receptor; NCX, sodium-calcium exchanger; PIP_2, phosphotidyl-inositol 4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; P-protein, phosphorylated protein; RyR, ryanodine receptor; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; SOCE, store-operated Ca^{2+} entry; TRP, transient receptor potential; VDCC, voltage-dependent Ca^{2+} channel.

In nonexcitable cells, changes in $[Ca^{2+}]_i$ are initiated by cellular responses to hormones and growth factors that act through the hydrolysis of membrane-bound inositol phospholipid and that are mediated by at least two second messengers, namely diacyl glycerol (DAG), which activates protein kinase C (PKC), and inositol 1,4,5-trisphosphate (IP_3), which binds to the inositol 1,4,5-trisphosphate receptor (IP_3R) in the endoplasmic reticulum (ER) membrane to release Ca^{2+} into the cytosol *(4)*. The interaction of cells with their environment occurs through interdependent signals that are mediated by receptors in the plasma membrane, and activation of these receptors by their ligands leads to conformational changes and the transmission of signals across the membrane to trigger a cascade of events in the cell that result in alteration of its function. An increase in the concentration of intracellular Ca^{2+} initiates diffusion, waves, or oscillations of Ca^{2+} that propagate in the nucleus to affect gene transcription or are sequested by the ER or mitochondria *(5–8)*. These events are regulated by the interplay of multiple counteracting processes in the cell.

3. REGULATION OF CELLULAR Ca^{2+}

Normal $[Ca^{2+}]_i$ is maintained between 20 and 100 n*M*, relative to the extracellular space calcium concentration ($[Ca^{2+}]_e$) of approx 1.3 m*M*. In addition to free cytosolic Ca^{2+}, there are storage sites in the cell that can hold Ca^{2+} at a concentration between 10 and 20 m*M* Ca^{2+} *(9)*. Thus, there are steep Ca^{2+} gradients across the plasma membrane from the interstitial space to the cytoplasm, and across intracellular membranes from storage sites. The main cellular storage sites for Ca^{2+} are the sarcoplasmic reticulum (SR), ER, and mitochondria. As a result of these separate compartments and the fact that $[Ca^{2+}]_i$ can rise to μ*M* levels, systems are in place to regulate it within narrow limits so as to protect the cell from Ca^{2+} overload and subsequent cell death. To achieve this purpose, receptors, transporters, and channels in the cell membrane play important roles. Ca^{2+} movement from the cell to the extracellular space occurs against a Ca^{2+} gradient of 20–100 n*M* (inside) and 1.3 m*M* (outside) and is mediated by a Ca^{2+} pump (Ca^{2+}-ATPase) and a Na^+/Ca^{2+} exchanger (NCX). The Ca^{2+}-ATPase plays a major role, and the NCX a minor role, in regulating cellular Ca^{2+} fluxes. The Ca^{2+}-ATPase uses ATP to pump Ca^{2+} out of the cell or into ER/SR against concentration and electrical gradients. Many of these Ca^{2+} transport proteins are influenced by $1,25(OH)_2D_3$, which regulates the transcription of genes that code for these proteins.

3.1. Calcium Influx Pathways

Calcium enters the cell from the interstitial space mainly via voltage-dependent or receptor-operated Ca^{2+} channels in the plasma membrane. There are several of these Ca^{2+} entry pathways in mammalian cells, and their characteristics and functions may vary from tissue to tissue. In addition, intracellular Ca^{2+} pumps in organelles rapidly sequester Ca^{2+}, thus restricting its diffusion internally unless it is required. The three main types of Ca^{2+} channels that have been extensively described are voltage-dependent calcium channels (VDCC) *(10)*, receptor-operated calcium channels (ROCC) *(11)*, and store-operated calcium entry (SOCE) or capacitative calcium entry (CCE) channels *(12)*. The CCE mechanism is a very important influx pathway in nonexcitable cells; however, its role in the function of neuronal cells has also been reported *(13)* and may be implicated in some neuropathological conditions *(14–16)*. In addition to these channels, the Ca^{2+}-sensing receptor (CaSR) *(17)* and transient receptor protein (TRP) channels *(18)* also constitute significant Ca^{2+} entry pathways, albeit operating to mediate influx by different mechanisms.

3.1.1. Voltage-Dependent Calcium Channels

VDCC are employed largely by excitable cells (muscle and neurons) to move Ca^{2+} from the extracellular space into the cell. They often exist as multiple isoforms, with tissue-specific expression and different gating characteristics, and are activated by the depolarization of the plasma membrane. Different types of VDCCs have been identified in mammalian tissues and have been shown to mediate specialized cellular functions *(19)*. The voltage-dependent Ca^{2+} channels are important therapeutic targets because of their specific characteristics. The two main types, found in the cardiovascular system, are the L and T type channels, which have distinct electrophysiological properties and may have distinct roles in this tissue. In cardiac and smooth muscle cells, VDCC control excitation–contraction coupling. The L-type Ca^{2+} channel is the best known and charac-