Calcium in Human Health

NUTRITION ◊ AND ◊ HEALTH

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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.

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[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***
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I Calcium Functions

II Techniques for Studying Calcium Metabolism and Its Relationship to Disease

III Calcium Consumption, Requirements, and Bioavailability

IV Calcium Homeostasis

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.

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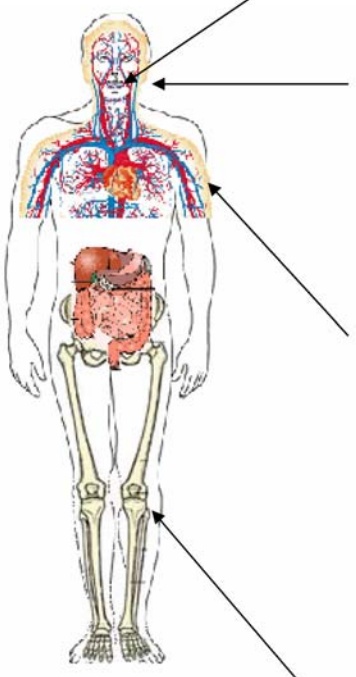

Current Status	Knowledge Gaps	Environment	Needed Future Research
Sources Ca:			
Calcium content of food and bioavailability of major sources known	How to improve calcium absorption and retention efficiency	Extensive Ca addition to food supply and supplements	Bioavailability testing of all manufactured 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.

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.

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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

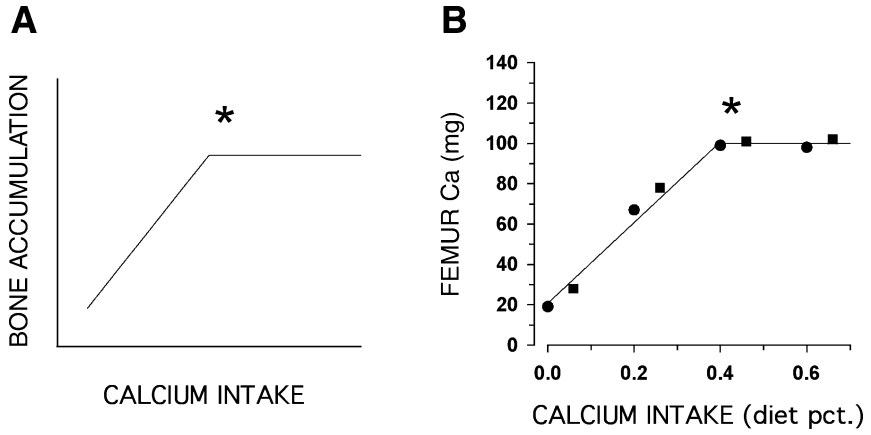

Fig. 1. Threshold behavior of calcium intake. **(A)** Theoretical relationship of bone accumulation to intake. Below a certain value (the threshold, indicated by an asterisk), bone accumulation is a linear function of intake (the ascending line); in other words, the amount of bone that can be accumulated 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-

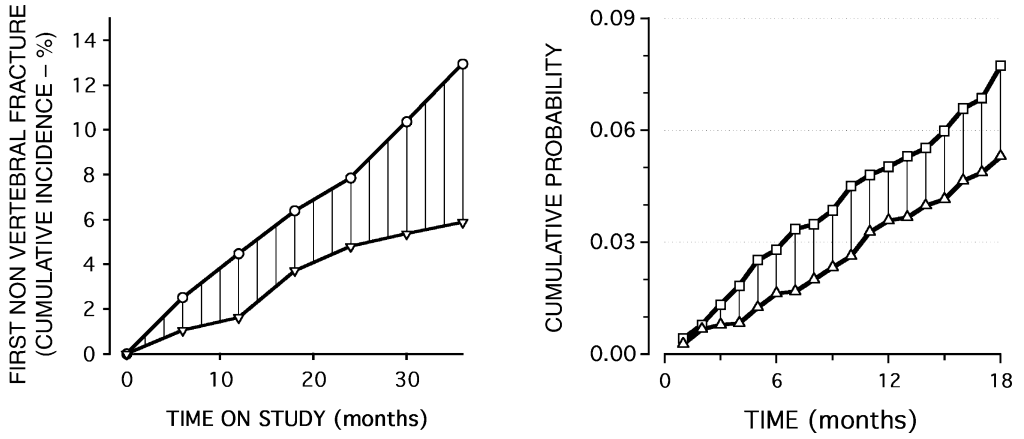

Fig. 2. Plots of the cumulative incidence of fractures, redrawn from the studies of Chapuy et al. *(5)* (right) and Dawson-Hughes et al. *(6)* (left). In both cases, the upper line represents the placebo control subjects, and the lower line represents the calcium and vitamin D-treated subjects. The shaded 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.

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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

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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.

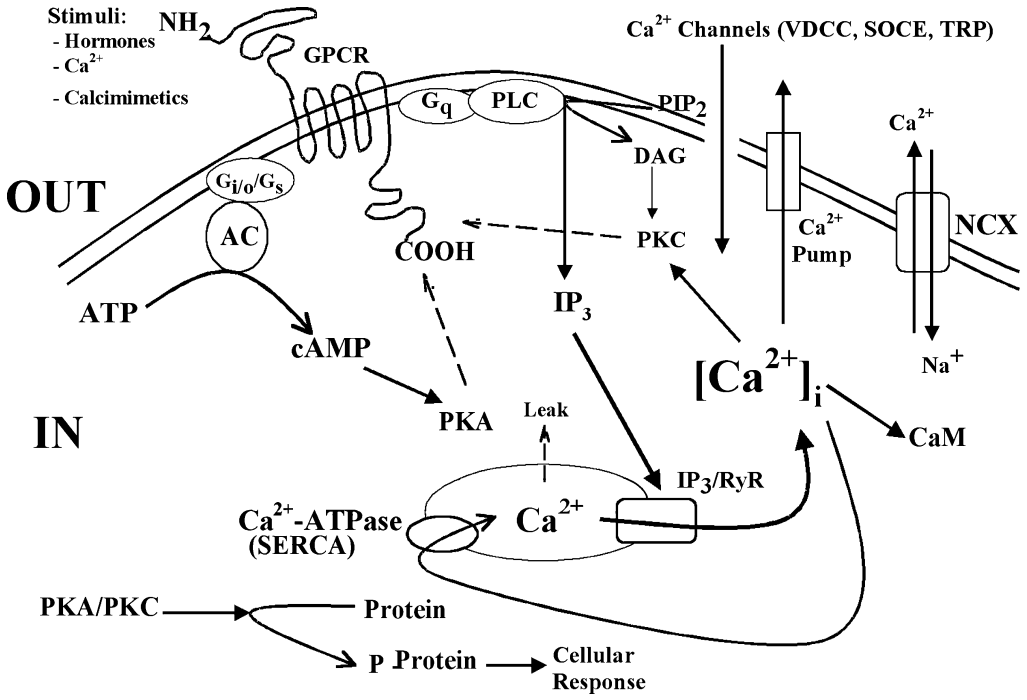

Fig. 1. Cellular Ca^{2+} signal transduction. AC, adenylyl cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; Ca^{2+} pump, plasma membrane Ca^{2+}-ATPase; DAG, diacyl glycerol; GPCR, G protein-coupled receptor; $G_{i/o}$, $G\alpha_{i/o}$ G protein subunit; G_q, $G\alpha_q$ G 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-

terized among the high voltage-activated calcium channels. It is distinguishable from other voltage-dependent Ca^{2+} channels by its sensitivity to 1,4-dihydropyridine compounds such as nifedipine *(20–22)*. The T-type Ca^{2+} channels, which are also expressed in the cardiovascular system, are prominent in conducting and pacemaking cells but are not normally present in adult myocardium. Although very little is known about the T-type Ca^{2+} channels because of the lack of pharmacological tools for their study, the recent discovery of a selective blocker of this channel with important cardiovascular actions indicates that it, too, may become a useful therapeutic target *(23–25)*. These channels are thought to regulate vascular tone, signal conduction, cardiac pacemaking, and the secretion of certain intercellular transmitters and to play an important role in the tissue remodeling that occurs in pathological processes such as cardiac hypertrophy *(26–28)*. Thus, the availability of novel antagonists that selectively block T-type Ca^{2+} channels will facilitate their future characterization. In recent times, neuron-specific calcium channels have been the subject of intense research and a number of agents that are selective for these channels are being investigated for their potential in the therapy of chronic neuropathic pain *(29)*. These channels are large-conductance, Ca^{2+}-activated potassium channels, which play a major role in the regulation of spike waveform and the temporal pattern of repetitive spike discharge that are important in mature neural circuits *(30)*.

Both type 1 and type 2 diabetes mellitus are associated with disturbances in the regulation of $[Ca^{2+}]_i$. Hyperglycemia leads to acute increases in $[Ca^{2+}]_i$ as a result of influx and release from internal storage sites, secondary to activation of dihydropyridine-sensitive Ca^{2+} channels *(31)*. Spontaneous mutations of VDCC in skeletal muscles lead to malignant hyperthermia and familial hypokalemic periodic paralysis.

3.1.2. Receptor-Operated Calcium Channels

Receptor-operated Ca^{2+} channels are structurally and functionally diverse and are prevalent on secretory cells and nerve terminals. The nicotinic acetylcholine and *N*-methyl- D-aspartate (NMDA) receptors are classic examples of receptor-operated Ca^{2+} channels. They are activated by agonist binding to the extracellular domain of the channel. In smooth muscles, Ca^{2+} required for contraction comes from both intracellular release from ER and extracellular entry via several ROCC *(32)*. Several hormones and neurotransmitters activate nonselective Ca^{2+} channels in various tissues *(33,34)*. These entry pathways are dependent on activation of pertussis toxin-sensitive, G protein-coupled receptors (GPCRs) and therefore can be considered ROCCs *(35)*. In a human neuroblastoma cell line, carbachol-stimulated Ca^{2+} entry was shown to be mediated by a receptor-operated Ca^{2+} channel that was dependent on IP_3-induced Ca^{2+} release *(36)*; however, in glomerular mesangial cells, it was mediated by epidermal growth factor-activated, SOCE through an IP_3-independent, phospholipase C (PLC)-dependent pathway *(37)*, indicating tissue-specific mechanism of activation of these receptors. In the myocardium, a number of receptor-mediated signaling pathways are activated through PLC and phospholipase D (PLD) *(38)*.

3.1.3. Store-Operated Calcium Entry Channels

The release of Ca^{2+} from intracellular stores in nonexcitable cells activates Ca^{2+} entry via channels in the plasma membrane, a process known as SOCE or CCE *(12)*. The channels are activated in response to depletion of intracellular Ca^{2+} stores as a result of

physiological Ca^{2+} mobilization or by the action of pharmacological agents such as thapsigargin *(39–41)*. Although the precise mechanism of this entry process is the subject of rigorous research in many laboratories, there is a consensus that it involves conformational coupling between Ca^{2+} entry channels in the plasma membrane and Ca^{2+} release channels in the ER membrane *(42)*. It has been proposed that a diffusible calcium influx factor messenger is synthesized by depleted Ca^{2+} stores and that this activates Ca^{2+} entry channels in the plasma membrane. The store-operated Ca^{2+} channels are ubiquitous, having been demonstrated in many different cell types. The electrophysiological characteristics of these channels differ from cell to cell, giving rise to the demonstration of different types of this channel. Although the molecular identity of the channel has not been established unequivocally, TRP proteins have been implicated *(43)*.

Presenilin-1 is one of the genes implicated in the etiology of early-onset autosomal-dominant or familial-onset Alzheimer's disease *(14)*. Mutant *presenilin-1* deregulates neuronal Ca^{2+} homeostasis by direct attenuation of CCE at the cell surface independent of amyloid precursor protein (APP), and by an indirect increase of ER Ca^{2+} stores via processing of APP and generation of amyloid peptides and C-terminal (C99) fragments of APP.

3.1.4. Ca^{2+}-Sensing Receptor-Mediated Ca^{2+} Entry

The CaSR is a seven-transmembrane GPCR that binds Ca^{2+} at the extracellular domain and transduces the signal through cAMP and phospholipases (PLC, PLD, or PLA) depending on the cell type *(17)*. Activation of PLC leads to IP_3 production and release of stored intracellular Ca^{2+} from ER, thus transiently raising the cytoplasmic Ca^{2+} concentration following activation of IP_3R in the ER membrane. Reduction in $[Ca^{2+}]_i$ is rapidly achieved by Ca^{2+} pumps located in the ER and plasma membranes. However, the released Ca^{2+} also opens plasma membrane SOCE or CCE channels that allow influx of Ca^{2+}, resulting in a sustained plateau *(44–47)* or periodic oscillations of $[Ca^{2+}]_i$ that regulate cytosolic as well as nuclear functions of the cell *(4,48)*. A diverse array of signaling mechanisms is implicated in these events, which are linked to membrane channels; however, the molecular mechanisms of these Ca^{2+} signaling pathways are not clear. Studies have shown that stimulation of the CaSR with $[Ca^{2+}]_e$ produces oscillations in $[Ca^{2+}]_i$, the pattern and frequency of which play a key role in signal transduction; but there are conflicting views on the mechanisms involved.

The generally accepted model is based on the negative feedback effects of PKC on the production of IP_3 or on the regulatory properties of $[Ca^{2+}]_i$ on the IP_3R *(49–51)*. Young et al. *(52)* have suggested that negative feedback by PKC could play a role in the generation of $[Ca^{2+}]_e$-evoked $[Ca^{2+}]_i$ oscillations via the CaSR, contradicting the study by Breitwieser and Gama *(8)*, which concluded that the activity of a variety of protein kinases, including PKC, do not influence the pattern of $[Ca^{2+}]_i$ oscillations elicited by activation of the human parathyroid (hPTH) CaSR by Ca^{2+}. It is therefore clear from these reports that the mechanisms of the CaSR-mediated $[Ca^{2+}]_i$ oscillations are yet to be resolved. The CaSR couples, through the intracellular loops and carboxyl terminal chain, to multiple G proteins that mediate its biological actions, and three modes of coupling have been reported: namely, through $G\alpha_i$ to inhibition of adenylate cyclase (AC) and activation of ERK1/2; through $G\alpha_q$ to stimulation of PLC and PLA_2; and through $G\beta\gamma$ to stimulation of PI3-kinase *(53)*. However, $G\beta\gamma$ is also known to activate PLCβ isoforms,

and the expression profile of these isoforms in cells may dictate the ability of the Gβγ to mediate PI and Ca^{2+} signaling. Phosphorylation of PLCβ by PKA and PKC plays an important role in the regulation of this isoform and provides part of a well-recognized negative feedback loop.

It is clear that many GPCRs can simultaneously initiate multiple second messenger pathways by coupling to more than one Gα subunit and influencing the functional properties of Gβγ *(54–56)*. In studies on the human β_2-adrenergic receptor, which mediates increases in $[Ca^{2+}]_i$ via cAMP, site-specific mutagenesis indicated that low concentrations of agonist induced receptor phosphorylation at PKA sites, whereas higher concentrations induced phosphorylation at PKC and GPCR kinase (GRK) sites *(57–58)*. Evidence from studies on the metabotropic glutamate receptor (mGluR) indicates that the receptor is regulated by agonist-induced, PKC-dependent feedback inhibition of the IP_3 pathway and the agonist-independent, PKA-dependent pathway, which potentiates IP_3 signaling *(59)*. Thus, GPCR activation can lead to functional integration of an intricate network of intracellular signaling pathways as well as stimulation of effectors completely independent of G proteins. Calcium mobilization from intracellular stores triggers events that lead to secretion, contraction, and energy generation in the short term and the regulation of proliferation, differentiation, apoptosis, and gene transcription in the long term *(17)*.

Mutations in the hPTH Ca^{2+}-sensing receptor have been linked to disorders of Ca^{2+} homeostasis due to alterations in the set point of parathyroid hormone (PTH) secretion and control of renal Ca^{2+} excretion. Inactivating mutations in the CaSR gene cause familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT), and activating mutations cause a form of autosomal dominant hypocalcemia *(60–63)*.

3.1.5. Transient Receptor Protein Channels

TRPs were originally named for the Drosophila transient receptor potential mutant *(64)*, and since the identification of mammalian TRPs, a family of homologs and splice variants has been described *(65,66)*. The mammalian TRPs (also known as TRPCs) belong to the short TRP family of which seven (TRPs 1–7) have been described *(67)*. TRP1 and TRP3 are the most widely studied mammalian TRPs and have been implicated in the mediation of store-depleted Ca^{2+} entry in nonexcitable cells *(68,69)*. As previously discussed, activation of PLC leads to IP_3 production and release of stored Ca^{2+} following activation of IP_3R in the ER membrane, and a subsequent influx of Ca^{2+} resulting in a sustained plateau *(45,46)* or periodic oscillations of $[Ca^{2+}]_i$ that regulate cytosolic as well as nuclear functions of the cell *(4,48)*.

The identity of the Ca^{2+} entry channels remains a key question, as does the mechanism by which they are activated. Several studies have indicated that SOCE is associated with TRPs, and various lines of evidence support the hypothesis that TRPs can, in certain circumstances, form part of a store-operated, Ca^{2+}-permeable channel in mammalian cells *(43,68)*; but much confusion still exists as to whether SOCE channels are TRP channels, and vigorous investigation is being carried out in many laboratories to definitively identify store-depletion-activated Ca^{2+} channels as TRP channels. Other studies suggest that expression of the hTRP3 in human embryonic kidney (HEK)293 cells forms a nonselective cation channel that opens after activation of PLC but not after store deple-

tion, indicating that TRP3 may be linked to endogenous proteins to form channels that are sensitive to store depletion *(68)*. There is overlap between store-operated and receptor-operated Ca^{2+} entry because the latter is potentiated by the activation of the former, suggesting that both may belong to the same family of mammalian TRP proteins.

3.2. Calcium Release and Reuptake From Internal Storage Sites

The release of Ca^{2+} from intracellular stores is mediated by distinct messenger-activated channels such as the IP_3R and the ryanodine receptor (RyR). These channels provide most of the signal Ca^{2+} in cells *(70)*. The trigger for release is the binding of ligands, such as hormones and growth factors, to specific receptors in the plasma membrane, resulting in the opening of integral Ca^{2+} channels in the ER membrane. Many of these receptors are coupled to G proteins that are linked to PLC activation to cause the release of Ca^{2+} from ER. Stimulation of one type of GPCR can be influenced by the stimulation of a different type, a phenomenon known as cross-talk *(71)*. These interactions may be important in the control of cell function; however, there is no unifying mechanism to explain the many examples of cross-talk among GPCRs that contribute to the control of intracellular Ca^{2+} release. A number of studies indicate that there is direct modulation of PLC activity via $G\beta\gamma$ *(72–74)*; however, regulation of phosphatidylinositol-4,5-bisphosphate (PIP_2) supply *(75–77)* and sensitization of IP_3Rs *(78,79)*, among other processes, have been suggested to play significant roles in these mechanisms.

Reuptake of Ca^{2+} from the cytosol is facilitated mainly by sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump. Thus, both the IP_3Rs and RyRs are coupled to extracellular signals *(80)*. Studies employing vascular myocytes indicate that activation of IP_3Rs coupled to RyRs releases large quantities of Ca^{2+}, which causes salutatory propagation of $[Ca^{2+}]_i$ waves *(81,82)*. Both the IP_3R and the RyR display considerable amino acid sequence homology and similar channel-opening characteristics *(83–85)*. These systems are controlled by negative feedback mechanisms, which ensure that just enough Ca^{2+} is released to give a meaningful signal, yet avoid Ca^{2+} overload and cytotoxicity.

3.2.1. The IP_3R

There are at least three isoforms of IP_3Rs, which are coded by different genes and have different characteristics, functions, and tissue distribution patterns *(86–90)*. The IP_3R is activated by the second messenger, IP_3 produced from the hydrolysis of membrane PIP_2, following activation of PLC through GPCRs (91,92). The released IP_3 then binds to the IP_3R in the ER membrane to produce conformational change in the receptor, which leads to the opening of the integral channel allowing Ca^{2+}, at high concentration in the store, to move into the cytoplasm. The opening of the channel is regulated by changes in the cytosolic Ca^{2+} concentration, with modest increases (0.5–1.0 μM) enhancing release, and large increases (>1 μM) leading to inhibition and generation of complex patterns such as waves, sparks, and oscillations *(4,93)*.

Oscillations are spontaneous changes in bulk intracellular Ca^{2+} concentrations, resulting from cycles of release and reuptake of stored Ca^{2+}; they are regulated either by protein kinases or phospholipases *(94–96)*. Intracellular Ca^{2+} oscillations play important roles in cellular signaling to the nucleus. In B lymphocytes, the amplitude and duration of Ca^{2+} oscillations have been shown to control differential activation of the pro-inflammatory transcriptional regulators nuclear factor (NF)κB, c-Jun-N-terminal kinase (JNK), and

NF of activated T-cells (NFAT), which are Ca^{2+}-dependent. Downstream effectors in these pathways can decode information contained in the oscillations, revealing a mechanism by which multifunctional second messengers such as Ca^{2+} can achieve specificity in signaling to the nucleus. Multiple sparks summate to form Ca^{2+} waves in cardiac and skeletal muscles, which propagate along the tissue. Spontaneous mutations in the IP_3R in the central nervous system are linked to Alzheimer's disease *(31)*.

3.2.2. The Ryanodine Receptor

The RyR is structurally and functionally analogous to the IP_3R, with twice the conductance and molecular mass of the latter. It has a high affinity for the plant alkaloid ryanodine, from which it derives its name. The Ca^{2+} sensitivity of the receptor is between 1 and 10 μM, and concentrations greater than 10 μM inhibit it. Thus, ryanodine acts both as an agonist and an antagonist at the receptor. The receptor also interacts with multiple exogenous ligands such as toxins, xanthines, and anthroquinones *(97)*. Therefore, the receptor constitutes a rich and important pharmacological target for modulating cellular functions because of its role in regulating intracellular Ca^{2+} concentrations and its ability to bind multiple ligands. The receptors are largely present in excitable cells, such as muscle and neurons, where they play significant roles in impulse transmission and are responsible for Ca^{2+}-induced Ca^{2+} release (CICR) from intracellular stores, which amplifies the signal resulting from membrane depolarization.

There are at least three subtypes (RyR1, RyR2, and RyR3) of the RyR, which are coded for by three different genes and expressed in different tissues *(98)*. RyR1 is predominantly expressed in skeletal muscles and can be gated by direct or indirect coupling to dihydropyiridine-sensitive receptors on the T-tubules. RyR2 is the primary isoform in cardiac muscle, where it is involved in excitation, whereas RyR3 is widely expressed in a variety of tissues, including smooth muscles, where it regulates Ca^{2+}sparks and spontaneous outward currents. The RyR3 may also be co-expressed with RyR1 and RyR2 in some tissues *(99–101)*.

The characteristics and functions of RyRs in cardiac, skeletal, and smooth muscles have been well described. In the brain, RyRs are present in presynaptic entities, where they regulate intracellular Ca^{2+}-concentrations, membrane potential, and the activity of a variety of second messengers *(102)* and play significant physiological roles in modulating local Ca^{2+} levels and neurotransmitter release; these functions are important for an understanding of the cellular mechanisms controlling neuronal function. Cyclic adenosine diphosphoribose (cADPR) is a known intracellular Ca^{2+}mobilizing agent in sea urchins, where it is produced together with IP_3 during fertilization and has been shown to play a role as an endogenous modulator of CICR in longitudinal muscle *(103)* and thought to be a modulator of the RyR Ca^{2+}release channels in bone and pancreatic β-cells *(104–107)*.

In skeletal muscles, mutations of the RyR1 isoform are linked to malignant hyperthermia, and the formation of autoantibodies to the receptor is the cause of myasthenia gravis *(31)*. The reduction in RyR2 expression in the heart is associated with cardiomyopathy.

3.2.3. Sarcoplasmic/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA)

The SERCA pump is structurally and functionally similar to the plasma membrane Ca^{2+}-ATPase. It belongs to a family of highly conserved proteins encoded by three highly homologous genes—*SERCA1*, *SERCA2*, and *SERCA3 (108)*. *SERCA1* gives rise to alternately spliced variants, 1a and 1b, which are expressed in fast-twitch fetal/neonatal and

adult muscle, and *SERCA2* gives rise to the variants 2a and 2b. SERCA2a is the primary isoform found in the heart (where it is the critical determinant of Ca^{2+} handling by SR, which is required for excitation–contraction coupling), whereas SERCA2b is expressed ubiquitously in association with IP_3-gated Ca^{2+} stores. The activity of SERCA is modulated by phospholamban, an integral membrane protein *(109)*. Ca^{2+} transients, as well as the activity and expression patterns of Ca^{2+} handling proteins, especially SERCA2a, are altered in the failing heart; thus, the amount of SR Ca^{2+} that is available for contraction is altered *(110)*.

Spontaneous mutations in *SERCA1* in skeletal muscles are associated with Brody Disease, and reduction in the expression of *SERCA2* in the myocardium leads to hypertrophy and heart failure. Mutations in *SERCA3* in pancreatic β-cells have been linked to diabetes mellitus *(31)*.

3.2.4. Mitochondrial Ca^{2+} Regulation

The mitochondrion functions in the long-term, large-scale regulation of $[Ca^{2+}]_i$ *(111)*. It protects the cell against large fluctuations in $[Ca^{2+}]_i$, a function achieved by the presence of both a low-affinity, high-capacity Ca^{2+} uniporter (which moves large amounts of Ca^{2+} out of the cytosol into storage in the mitochondria) and the NaCX *(112)*. The mitochondria are able to accumulate large amounts of Ca^{2+} in a relatively slow process and store it, for example, under pathological conditions in which the permeability properties of the SR/ER are altered. This ability is related to the existence of a system for the simultaneous uptake of inorganic phosphate, which then precipitates Ca^{2+} in the mitochondrial matrix in the form of insoluble hydroxyapatite. This mechanism allows for storage of excessive amounts of Ca^{2+} without essential changes in the ionic activity of the mitochondrial matrix. Ca^{2+} transport into the mitochondria from the cytosol is mediated by ruthenium red-sensitive Ca^{2+} uniporters and efflux by a Na^+-dependent and –independent mechanisms *(113)* that play important roles, such as the control of metabolic rate for cellular ATP production, modulation of amplitude and shape of cytosolic Ca^{2+} transients, and induction of apoptosis. In addition, other studies have linked the RyR to the dynamic uptake of Ca^{2+} during $[Ca^{2+}]_i$ oscillations *(114)*, suggesting that the RyR may be responsible for the rapid uptake of Ca^{2+}, a process that may be important in the removal of excess Ca^{2+} from the cytosol.

3.2.5. Calcium-Binding Proteins

In addition to the above Ca^{2+} translocation systems, calsequestrin (in SR) and calreticulin (in ER) play significant roles in regulating cellular calcium *(115)*. These hydrophilic, high-capacity and low-affinity proteins are the major Ca^{2+}-binding proteins in muscle and nonmuscle cells, and they achieve this by forming complexes with excess Ca^{2+} in the cytosol. Calsequestrin is an acidic protein which is present in the lumen of the junctional terminal cisternae of the SR and rapidly binds and releases large quantities of Ca^{2+}. It interacts with RyRs, thus ensuring storage of high concentrations of Ca^{2+} near release sites. Calreticulin is present in the ER lumen, where it acts as a chaperone during the synthesis of channel proteins, surface receptors, and transporters and participates in the regulation of intracellular Ca^{2+} homeostasis by modulating ER Ca^{2+} storage and transport *(116)*. Calmodulin is the major Ca^{2+}-binding protein in nonmuscle cells and is the most ubiquitous of intracellular Ca^{2+}binding regulatory proteins. It affects the function of many proteins, enzymes, and ion channels *(117,118)*.

Mutations in cardiac calsequestrin gene (*CSQ2*) are linked to arrhythmias and sudden cardiac death *(119)*.

3.3. Calcium Efflux Pathways

Ca^{2+} extrusion from the cell is carried out mainly by the plasma membrane Ca^{2+}-ATPase and Na^+/Ca^{2+} exchanger.

3.3.1. Plasma Membrane Ca^{2+}-ATPase

The plasma membrane Ca^{2+}-ATPase or Ca^{2+} pump is a high-affinity (K_M:1 μ*M*), low-capacity transport protein expressed in a tissue-specific manner in both excitable and nonexcitable cells *(120)*. The proteins are encoded by four genes, and alternate mRNA splicing gives rise to multiple isoforms with different regulatory properties *(121,122)*. The plasma membrane Ca^{2+}-ATPase is activated directly by calmodulin to increase its affinity for Ca^{2+} *(120)*. Its role is in the fine-tuning of intracellular free Ca^{2+}, which it achieves by using the energy from the hydrolysis of ATP, in the presence of Ca^{2+} and Mg^{2+} to transport two Ca^{2+} ions from the cytoplasm into the extracellular space. This transport is electroneutral in function, in that two protons (H^+) are exchanged for one Ca^{2+}. Studies by Kip and Stoehler *(123)* show that expression of the plasma membrane Ca^{2+}-ATPase in Madin-Darby canine kidney (MDCK) epithelial cells is upregulated by $1,25(OH)_2D_3$, and this increase correlates with increase in transcellular Ca^{2+} influx from the apical toward the basolateral compartment, supporting the relevance of this hormone in kidney tubular Ca^{2+} absorption. Genetic evidence indicates the existence of mammalian Ca^{2+}-ATPase isoforms generated from a multigene family by alternative RNA splicing with different regulatory properties, probably as a consequence of different tissue specificities and physiological requirements *(120)*.

3.3.2. Na^+/Ca^{2+} Exchanger

The NCX is an asymmetric, high-capacity, low-affinity transporter which was initially reported to be abundant in nerve, muscle, and epithelial cells but that has now been identified in a wide range of tissues. Most of the NCXs expressed in other tissues, however, are similar to that found in muscle and neuronal tissue; they are particularly abundant in heart *(124)*. Typically, the NCX moves net Ca^{2+} either out of or into cells depending on the driving electrochemical force, giving rise to "Ca^{2+}-entry" and "Ca^{2+}-exit" modes of exchange. It exchanges two Na^+ ions for every Ca^{2+}. The entry mode is dependent on intracellular Na^+ to drive Ca^{2+} influx and Na^+ efflux, and is insensitive to ouabain *(125)*. The exit mode is dependent on intracellular Ca^{2+} and is sensitive to ouabain but insensitive to tetrodotoxin. Because the affinity of the transporter for intracellular Ca^{2+} is low, under physiological conditions only a small fraction of the exchangers are active at a normal, resting $[Ca^{2+}]_i$ of approx 100 n*M* in most cells. However, at peak activity of excitable and secretory cells, when $[Ca^{2+}]_i$ is in the μ*M* range, the exchanger is fully activated. Thus, this system constitutes a very important mechanism for Ca^{2+} extrusion from excitable and secretory cells, which go through cycles of low- and high-$[Ca^{2+}]_i$. Although cytosolic ATP does not play any role in the extrusion process mediated by the NCX, it substantially alters its kinetics, most likely through phosphorylation at sites in the protein molecule that are important for activity *(126)*. Rosker et al. *(127)* have shown that overexpression of a nonselective TRPC3 cation channel interacts with the NCX in Ca^{2+} signaling, suggesting an association between these transport proteins. A

comprehensive review of the properties of the NCX is provided by Blaustein and Lederer *(125)*.

The common abnormalities of heart failure include hypertrophy, contractile dysfunction, and alteration of physiological properties, which contribute to low cardiac output and sudden death *(128)*. Although prolonged NCX currents are implicated in these events, the involvement of Ca^{2+} currents varies. Alterations in inotropy in dilated human cardiomyopathy are associated with impaired intracellular Ca^{2+} handling as a result of the inability to restore basal Ca^{2+} levels leading to Ca^{2+} overload *(129,130)*. The activity of the NCX is apparently reduced in myocardial ischemia, leading to intracellular Ca^{2+} overload which can result in arrhythmia, myocardial stunning, and necrosis *(131)*. On the other hand, congestive heart failure and myocardial hypertrophy are associated with increased NCX activity and decreased inotropic state.

4. OVERVIEW OF WHOLE-BODY CALCIUM HOMEOSTASIS

Under normal conditions, the maintenance of Ca^{2+} balance in the body is the result of the interplay among the intestines, kidney, and bone; the placenta is also involved in the maintenance of this balance during pregnancy. A complex Ca^{2+} traffic occurs among intestines, kidney, and bone and is controlled mainly by the PTH and $1,25(OH)_2D_3$, which is synthesized from $25(OH)D_3$ in the kidney *(132)*. (*See also* Chapter 10, "The Calcium Economy.")

4.1. Calcium Fluxes in the GI Tract

Adequate absorption of Ca^{2+}, Mg^{2+}, and PO_4^{3-} from the GI tract is necessary for normal mineral homeostasis, which in turn is vital to the control of Ca^{2+} levels in blood, skeletal growth during childhood, and the maintenance of bone mass in adulthood. Ca^{2+} absorption in the GI tract occurs mainly in the intestine by a classical epithelial transport mechanism *(9)*. Ca^{2+} is transported across the intestinal epithelium at the brush border or apical membrane into the cell, translocated by a calcium-binding protein (CaBP), calbindin, to the basolateral membrane on the opposite side, and exported via the Ca^{2+}-ATPase pump into the extracellular space. The amount of Ca^{2+} absorbed is dependent on its availability in the diet and the absorption capacity of the intestine, and the absorption process is regulated at least in part by $1,25(OH)_2D_3$. Approximately 200–300 mg/d of total luminal content of 1000 mg (800 mg daily uptake; 200 mg from pancreatic, biliary, and intestinal secretions) is absorbed, giving a gross GI absorption efficiency of 30%. This absorption is influenced by cellular and paracellular transport, systemic modulators of cell function, and intraluminal factors. In addition to the epithelial transport, Ca^{2+} can be transported directly through the gap junctions in the epithelial layer into the extracellular space when the concentration of Ca^{2+} in the lumen is high *(133–135)*. Thus, GI Ca^{2+} absorption is a sum of two transport processes—namely, a saturable, transcellular uptake that is regulated physiologically, and a nonsaturable, paracellular process that is dependent on the concentration of the mineral in the lumen of the intestine.

The transcellular transport of Ca^{2+} involves specific transport systems, which are dependent on an electrical gradient across the mucosal membrane and on Ca^{2+} binding to calbindin intracellulaly for export via the Ca^{2+}-ATPase in the basolateral membrane *(136)*. Thus this process is active, saturable, and unidirectional. Calbindin rapidly binds

the free cytosolic Ca^{2+} to maintain the basal level around 100 n*M* to prevent cellular Ca^{2+} overload (with its associated effects of necrosis and apoptosis) *(135)*. The mucosal transport, intracellular binding, and basolateral extrusion are influenced by $1,25(OH)_2D_3$ via its effect on the synthesis of transport and binding proteins, with maximum effect being exerted on calbindin and ATPase synthesis.

Paracellular Ca^{2+} transport is a passive, nonsaturable, bi-directional process that occurs when luminal Ca^{2+} concentration is high. Although this process may be independent of $1,25(OH)_2D_3$, this hormone is known to increase the permeability of gap junctions and, therefore, can increase Ca^{2+} transport under such conditions *(137)*.

4.2. Calcium Fluxes in the Kidney

An important role of the kidney is the regulation of inorganic ion balance. The kidney filters 10,000 mg of Ca^{2+} per day; approx 99% of this is re-absorbed in the tubules of the nephron and 1% is excreted in the urine *(132)*. To maintain total body balance, the amount excreted is balanced by absorption in the intestine. Reduction in the plasma concentration of Ca^{2+} is counteracted by intestinal re-absorption, renal tubular resorption, and bone resorption. Generally, Ca^{2+} re-absorption in the kidney proceeds in parallel with Na^+ excretion *(138)*. The bulk of the filtered Ca^{2+} is re-absorbed primarily in the proximal tubule, with some re-absorption occurring in the distal and collecting tubules. The mechanisms of re-absorption of Ca^{2+} in the kidney are similar to those in the intestine, because both involve epithelial transport. In the proximal tubule, absorption occurs by two mechanisms—namely, transcellular (20%) and paracellular (80%) processes. The transcellular transport is an active process in which Ca^{2+} diffuses across the apical membrane down an electrochemical gradient and channels, and leaves the cell across the basolateral membrane against its electrochemical gradient, using Ca^{2+}-ATPase and NCX *(139)*. The kinetics of Ca^{2+} transport in the distal luminal membrane indicate the presence of Ca^{2+} channels, which have been shown to be voltage-dependent *(125)*.

4.3. Calcium Fluxes in Bone

Bone is composed of collagen and crystals of hydroxyapatite, $Ca_{10}(OH)(PO_4)_3$, in a ground substance of glycoproteins and proteoglycans *(141)*. This highly anionic environment allows for high cation binding and is thought to play an important role in the calcification of bone after the collagen fibers and ground substance have been laid down during bone formation by osteoblasts. Dietary calcium plays an important role in the growth and development of bone, and intakes below normal (600–800 mg/d) delay the onset of skeletal maturity and may result in deficits in adults. Calcified bone, therefore, constitutes a large reservoir of Ca^{2+}, which is available through bone remodeling, to buffer rises and falls in extracellular fluid (ECF) $[Ca^{2+}]$ *(142)*. Bone remodeling is the algebraic sum of formation by osteoblasts and resorption by osteclasts, and any imbalance in this system results in the preponderance of one over the other.

In order for Ca^{2+} to be released from bone, osteoclasts in contact with the calcified bone surfaces produce and release proteolytic and lysozomal enzymes, as well as hydrogen ions, into the localized area beneath the apical membrane of the cell, creating an acidic environment to dissolve the crystals and expose the matrix. The extrusion of protons requires the presence of ion exchangers, pumps, and channels in the basolateral membrane of the cell to maintain electrochemical balance. Thus, Ca^{2+} transport proteins

similar to those found in the epithelium of the intestine and kidney are also present in osteoclastic cells, where they regulate Ca^{2+} fluxes during bone resorption. Osteoblastic and odontoblastic cells have been shown to express splice variants of the NCX, and therefore show high Na^{+}-dependent Ca^{2+} extrusion activity *(143)*. Studies also indicate that a Ca^{2+}-sensing receptor present on the surface of osteoclasts senses the changes in the Ca^{2+} concentration in the environment and thereby induces signals that are transmitted into the interior of the cell to alter its function *(144)*. The osteoclast Ca^{2+}-sensing receptor is believed to be linked to a plasma membrane RyR receptor *(145,146)*. An interesting observation, however, is that the signaling by the osteoclast Ca^{2+}-sensing receptor is not dependent on coupling to G proteins, unlike the Ca^{2+}-sensing receptors that have been described previously *(17)*.

4.4. Calcium Fluxes in the Placenta

The placenta is the major organ involved in calcium transport from the mother to the developing fetus, a process regulated by a complex array of hormones *(147–150)*. A prerequisite for the reproductive health of the mother and normal fetal development is proper calcium homeostasis, which is regulated by the placental trophoblast epithelium *(151–153)*. Placental calcium transport is an active process that occurs in the syncytiotrophoblastic epithelium, which separates the maternal and fetal circulations, and is developmentally regulated to handle the increasing Ca^{2+} needs of the growing fetus. Under normal conditions, Ca^{2+} and nutrients are translocated in a maternal-to-fetal direction.

A number of Ca^{2+} transport mechanisms have been reported for the placenta; however, there is no consensus on the exact process except that there is a net influx of Ca^{2+} from the maternal side to the fetal side of the placenta *(154,155)*. Ca^{2+} is transported against a concentration gradient because the concentration in fetal plasma is higher than that in maternal plasma, suggesting an active process requiring ATP. Because the trophoblastic layer is an epithelium, it is assumed that its Ca^{2+}-transport machinery consists of components similar to those found in the GI tract—namely, a channel, a pump, and substrate-binding protein. The main transport proteins involved in Ca^{2+} transport in the placenta are the Ca^{2+}-activated ATPase *(156–159)* and soluble CaBPs such as calbindin-D9K (which is similar to the vitamin D-dependent intestinal protein *[160,161]*), oncomodulin or parvalbumin *(162,163)* (whose role in plancental Ca^{2+} transport is unknown), and a high-molecular-weight CaBP expressed exclusively in the placenta and shown to be functionally involved in placental Ca^{2+} uptake *(164,165)*. The expression of these high-affinity Ca^{2+}-binding proteins in the placenta suggest that they play important roles in regulating or shuttling cytosolic Ca^{2+} in the placenta. The exact roles of these proteins in Ca^{2+} uptake in the placenta, however, have not been established. The Ca^{2+}ATPase may function in similar fashion to the plasma membrane transporter that extrudes Ca^{2+} from the cytosol of cells. The Ca^{2+}-activated ATPase has been identified in human placenta and has been shown to be functionally involved in transmembrane Ca^{2+} uptake *(156–159)*.

4.5. Hormonal Regulation of Ca^{2+} Transport

As described in the previous sections (as well as in Chapter 10), the concentration of Ca^{2+} in the blood can be influenced by input from the intestine and kidney and by rapid mobilization from bone remodeling. The two major hormones involved in the regulation

of serum Ca^{2+} levels are PTH and $1,25(OH)_2D_3$, the former being the primary, acute controller. When the level of Ca^{2+} in the blood falls, PTH is secreted to restore it to normal levels. This is achieved by the CaSR on parathyroid cells sensing the drop in Ca^{2+} level and transducing this signal into the release of the hormone, which then acts on the kidney to increase Ca^{2+} re-absorption, mainly from the proximal tubule of the nephron, and to increase the activity of vitamin D 1α-hydroxylase *(132)*. The increase in 1α-hydroxylase activity in turn increases the synthesis of $1,25(OH)_2D_3$, which then acts on the intestine to increase Ca^{2+} absorption from the lumen by increasing the synthesis of proteins involved in Ca^{2+} absorption. In addition, increased PTH secretion stimulates osteocytolysis and osteoclastic activity in the bone while decreasing osteoblastic activity. These events combine to return plasma Ca^{2+} to normal. On the other hand, when plasma Ca^{2+} levels rise above normal—for example, as in certain disease states—a second hormone, calcitonin, secreted by C cells in the thyroid gland, acts to decrease this level by stimulating the renal excretion of Ca^{2+} and to inhibit 1α-hydroxylase activity and decrease bone remodeling through inhibition of osteoclastic activity. Thus, PTH and calcitonin exert opposite effects on plasma Ca^{2+} levels. The hormonal regulation of plasma Ca^{2+} level is, therefore, an integrated process, with PTH and $1,25(OH)_2D_3$ playing important roles to maintain the level within narrow limits under normal circumstances. From the foregoing, it is obvious that perturbation in any of these systems is bound to have far reaching effects on whole-body Ca^{2+} homeostasis.

The regulation of Ca^{2+} uptake by the placenta is under the primary control of $1,25(OH)_2D_3$. This conclusion is based on the fact that vitamin D receptors *(166,167)*, $1,25(OH)_2D_3$ synthesis *(168)*, and vitamin D hydroxylase activity *(169)* are all present in the placenta and surgical removal of the kidney, the main source of $1,25(OH)_2D_3$ synthesis, reduces transplacental Ca^{2+} gradient, which is restored by the administration of $1,25(OH)_2D_3$. In humans, regulation of calbindin-D9K expression in the placenta is thought to be under the control of estradiol *(161,170)*, suggesting the involvement of estrogens in the regulation of placental Ca^{2+} transport. Furthermore, the possibility that parathyroid hormone-related peptide (PTHrP) is involved in placental Ca^{2+} metabolism *(171–173)* suggests the participation of multiple endocrine pathways in the regulation of Ca^{2+} transport in this tissue.

5. CONCLUSIONS

Ca^{2+} is an important ion with multiple physiological effects that are vital to survival at all levels of organization. Therefore, plasma Ca^{2+} levels must be maintained within narrow limits for internal harmony, and any disturbance in Ca^{2+} homeostasis is bound to have consequences for multiple body systems. The interplay among the intestine, kidney, and bone serves to ensure that the plasma Ca^{2+} is maintained at optimum levels under normal conditions. There is a steep downward Ca^{2+} gradient from the extracellular space and internal storage sites into the cell cytoplasm. Therefore, mechanisms exist to regulate Ca^{2+} fluxes between these compartments to prevent intracellular Ca^{2+} overload and the associated deleterious effects of apoptosis and necrosis. The changes in intracellular Ca^{2+} concentrations that are necessary for normal functioning of the human body are brought about by cells interacting with their environment by means of receptors, channels, and Ca^{2+} transport proteins to initiate critical events within the cell that lead to contraction, secretion, and transcription of genes.

Calcium influx in cells occurs by means of transport systems such as voltage-dependent Ca^{2+} channels, receptor-operated Ca^{2+} channels, store-operated Ca^{2+}+ entry channels, Ca^{2+}-sensing receptor-mediated Ca^{2+} entry, and transient receptor potential channels. Calcium efflux from cells occurs mainly via plasma membrane Ca^{2+}-ATPase and the NCX. In addition to Ca^{2+} influx and efflux from the cytosol, Ca^{2+} release from internal storage sites into the cytosol plays a central role in cellular signal transduction. This release is part of a mechanism by which cells transmit external stimuli to internal signals to initiate events that lead to activation of ion channels, enzymes, contraction, secretion, and gene transcription. These Ca^{2+}-release mechanisms involve IP_3/IP_3R, ryanodine/RyR, and SERCA. Mitochondria and Ca^{2+}-binding proteins such as calsequestrin, calrecticulin, and calmodulin play important roles in maintaining cytosolic Ca^{2+} level within the limits that are necessary for essential cellular functions. Finally, the various Ca^{2+} transport systems play significant roles in regulating Ca^{2+} movement in the main Ca^{2+}-regulating organs of the body—namely, the intestines, kidneys, and bone. Any event that compromises any of these systems, therefore, leads to disruption of the Ca^{2+} homeostasis of the body.

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4 Nutritional Epidemiology

Dietary Assessment Methods

Carol J. Boushey

KEY POINTS

- Estimating dietary exposures is useful for identifying modifiable risk factors for disease.
- Several dietary assessment methods have been developed for use in research.
- Many factors, such as study design and research objectives, must be considered in selecting a dietary assessment method.
- Biomarkers are useful additions to corroborate results from dietary assessment tools.

1. INTRODUCTION

Nutritional epidemiology has developed from an interest in the concept that aspects of diet may influence the occurrence of human diseases. In epidemiology, disease occurrence is measured and related to different characteristics of individuals or their environments. Exposures, or what an individual comes in contact with, may be related to disease risk. The exposure can be a habit such as smoking, which would increase an individual's risk for lung cancer, or the exposure can be an environmental agent such as sun, which may increase an individual's risk for melanoma. In the case of nutritional epidemiology, food and the behaviors surrounding food choices are the exposures. For example, vegetable consumption may reduce an individual's risk for colon cancer, and exposure to television may increase an individual's risk of being overweight secondary to an increased intake of high-energy snack foods.

Measuring dietary intake presents more challenges than other exposures such as smoking. In most cases, the question as to whether an individual smokes can be answered by a simple "never," "yes," or "used to." In addition, smoking is a physiological habit; thus the amount smoked per day is fairly constant. Cigarettes are packaged in uniform amounts, making recall of packs or portions of packs per day fairly straightforward. Most individuals would not be able to tell an interviewer the last time they ate apple pie (unless it was the previous day). On the other hand, an ex-smoker can often tell an interviewer to the month and year, if not the day and hour, when he/she quit smoking.

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Table 1
Suggested Dietary Assessment Methods for Different Study Designs

Dietary assessment methods				
Study design	*Brief methods*	*24-h dietary recall*	*Food frequency questionnaire*	*Food record*
Cross-sectional	X	X	X	X
Surveillance	X			
Case–control	X		X	
Cohort	X	X	X	X
Intervention	X	X	X	X

Despite the difficulties encountered in the collection of food intake data, dietary information provides some of the most valuable insights into the occurrence of disease and subsequent approaches for mounting intervention programs for prevention. Food is a universal language. Fortunately, dietary assessment methods continue to evolve to meet the challenge and there is recognition that further improvements will enhance the consistency and strength of the association of diet with disease risk.

The primary purpose of this chapter is to provide readers with information to insure the selection of an appropriate dietary assessment method for a particular need. As with any assessment tool, choosing the right tool for a dietary project is critical to achieving desired results *(1,2)*. The intent is to focus on dietary assessment methods, and not specific sources of calcium, which is covered in Chapter 9. An overview of the four primarily used dietary assessment methods will be discussed and references to more detailed descriptions will be provided. Then the relationship of dietary assessment methods to study designs as shown in Table 1 is emphasized with examples from the literature.

2. DIETARY ASSESSMENT METHODS

2.1. The 24-Hour Dietary Recall

For the 24-h dietary recall, an interviewee is asked by a trained interviewer to remember foods and beverages consumed in the previous 24 h. To assist the interviewee, food models and pictures are often used as prompts for assistance with portion sizes *(1)*. The interviewer uses structured questions and prompts to help the interviewee remember foods eaten. An interviewer conducts the interview, thus the literacy of the respondent is not an issue as it is with some other dietary assessment methods. Because of the immediacy, respondents are generally able to recall most of their dietary intake. The reduced burden on the respondent allows for a sample of participants that may be more representative than individuals completing a more intensive method, such as the keeping of food records. In addition, an unannounced interview takes place after food is consumed; thus, alteration of usual eating habits is unlikely to occur. However, there are circumstances that may prompt the interviewee to alter his/her usual eating pattern, and therefore the amount of food consumed the previous 24 h may be reduced. This could occur when a 24-h dietary recall is prescheduled or occurs after an overnight fast (a common occurrence when recall is scheduled prior to a fasting phlebotomy session).

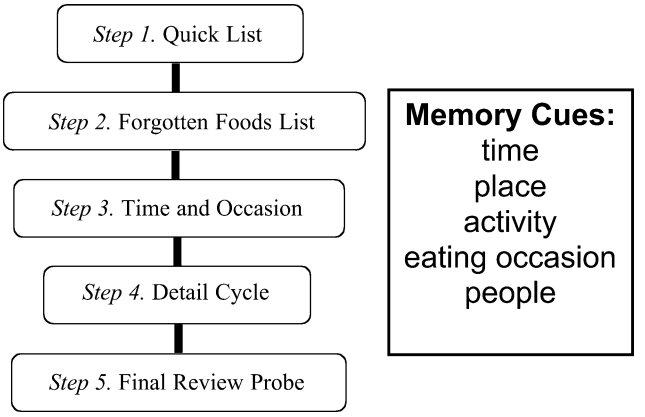


Fig. 1. The sequential steps for the five-step multiple-pass method for conducting a 24-h recall with the topic probes for the memory cues.

The Food Surveys Research Group (FSRG) of the US Department of Agriculture has devoted considerable effort to improving the accuracy of the 24-h recall through development and refinement of the multiple-pass method. The multiple-pass method provides a structured interview format with specific probes. Campbell and Dodds *(3)* found decades ago that interviewees receiving probing while being interviewed reported 25% higher dietary intakes than interviewees without probing. The latest variation of the multiple-pass approach involves five structured sets of probing (*see* Fig. 1) compared with its predecessor, which outlined three passes *(4)*. With this five-step multiple-pass method, the average number of foods reported per day increased by two from the previous triple-pass method, and energy intake increased 17%, suggesting a more complete recall of dietary intake *(5)*. A 24-h recall administered in this style can take 30–60 min.

For the National Health and Nutrition Examination Survey (NHANES), the FSRG uses a computerized version of the five-step multiple-pass method that is not available for public use at this time. However, this technique can be duplicated using the computer-assisted method available from the Nutrient Data System for Research Software developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. The direct coding of the foods saves money in data-entry time, missing values, and standardization. Otherwise, after each interview, the time to enter the dietary information into an appropriate nutrient database must be considered.

The major drawback of the 24-h recall is the issue of underreporting *(6)*. Factors such as obesity, gender, social desirability, restrained eating, hunger, education, literacy, perceived health status, age, and race/ethnicity have been shown to be related to underreporting *(7–10)*. Common forgotten food items include condiments, savory snacks, cake/pie, meat mixtures, white potatoes, fat-type spreads, and regular soft drinks *(11)*. Harnack et al. *(12)* found significant underreporting of large food portions when food models showing recommended serving sizes were used as visual aids for respondents. Larger food portions have been observed over the past 20 to 30 yr *(13,14)*; this may contribute to underreporting, and methods to capture accurate portion sizes are needed. Some work addressing this issue has been reported *(15,16)*.

In studies comparing energy intake estimated from the triple-pass method with energy expenditure estimated from doubly labeled water (DLW) or accelerometers, underreporting of energy intakes ranged from 17% in low-income women *(10)* to 26% in overweight and obese women *(17)*. Comparisons in children under 11 yr of age are mixed. One study showed a 14% greater energy intake than DLW estimated energy expenditure *(18)* and another showed only group estimates of energy intake as being valid *(19)*. Under controlled conditions of weight maintenance, women underestimated energy intake by 13% during a self-selection period, but overestimated by 1.3% under more controlled conditions *(20)*. Men, on the other hand, underestimated 11% and 13% under both conditions. Among African-American women with type 2 diabetes, 58% or 81% of the women underreported energy intake depending on the criteria used for estimating energy expenditure *(21)*.

Two published reports comparing the dietary intake results from the five-step multiple-pass method with actual observed intakes are available at this time *(22,23)*. Conway et al. *(22)* recorded observed intakes in 42 adult men and compared the estimated energy intake from the observations with the energy intake estimated from a five-step multiple-pass 24-h recall. No significant differences were found for energy, protein, carbohydrate, and fat. Further, there was no association of body mass index (BMI) with level of reporting. For women following the same protocol *(23)*, the population was found to have overestimated its energy and carbohydrate intakes by 8–10%. No significant differences between mean observed and recalled intakes of energy and the macronutrients were found. Recalled fat intake was not significantly different from the observed intake across the BMI range studied *(23)*.

The five-step multiple-pass method was one of the dietary assessment methods included in one of the largest, most ambitious studies of biomarkers and dietary intake *(24)*. The Observing Protein and Energy Nutrition (OPEN) Study collected two 24-h dietary recalls approx 3 mo apart, as well as DLW and urinary nitrogen as a protein biomarker, in 484 adults. For men, underreporting of energy intake compared with total energy expenditure was 12–14% and for protein it was 11–12%. For women, these same comparisons indicated underreporting of 16–20% for energy and 11–15% for protein. In general, researchers using the 24-h recall should be aware of the potential for underreporting and be prepared to minimize the factors related to underreporting and, possibly, overreporting in children.

2.2. The Food Record

For the food record, participants are asked to record all food and beverages consumed throughout a 24-h period. To improve the accuracy of the food record, detailed instructions are provided to the participants and tools for measuring or weighing foods and beverages consumed must be provided. Because the food record depends on the individual's ability and desire to record foods eaten, the number of individuals completing records may be limited by motivation and literacy. In addition, the process of recording foods can alter how an individual eats *(25,26)*. Although no staff time is involved with interviewing subjects, as is the case with the 24-h recall, the time required for training subjects, telephoning with reminders to record, reviewing the records for discrepancies, and entering the dietary information into a nutrient database must be considered.

Because the food record does not require dependence on memory, this method is sometimes considered the reference standard with which other dietary assessment methods are compared *(1,2)*. The accuracy of reporting portion sizes can be improved by training the participants prior to starting the recording process *(27)*. Many of the same issues listed for the 24-h dietary recall with regard to underreporting also exist for the food record *(8,9,28–31)*. The food record is especially vulnerable to underreporting because of the complexity of recording food, and also because the process of recording food has been shown to be an effective technique for reducing food consumption *(25,26)*. The range of underreporting for energy intake as compared with energy expenditure as estimated by DLW is between 4 and 37% *(32)*.

The process of reviewing a food record and coding the foods for data entry requires trained individuals and can take a large amount of time. To decrease the burden on staff, some food-record methods provide a list of foods to check-off when consumed. As attractive as this may seem, the restriction in food choices makes this approach similar to a 1-d food frequency method, and limits the ability of investigators to make conclusions based on some foods and food groupings *(33)*.

Most individuals' diets vary greatly from day to day *(34)*. Therefore, it is not appropriate to use data from a single 24-h recall or a single food record to characterize an individual's usual diet. An example of day-to-day variation can be seen in Fig. 2. The figure shows the estimated daily dietary intakes of calcium from 6 d of food records collected from three girls prior to starting a metabolic study. The three girls in Fig. 2 represent the 10th, 50th, and 90th percentiles from 43 girls between the ages of 10 and 14 yr. Had the investigators only collected 1 d, and had that day been day 1 in the figure, the girl at the 10th percentile would have been assessed as having the highest calcium intake among the three, and the girl at the 90th percentile would have had the lowest intake. A single food record or 24-h recall can be used to describe the average dietary intake of a group; however, that single day cannot be used to assess achievement of dietary recommendations without special statistical applications *(34,35)*. Therefore, a minimum of two nonconsecutive days are recommended to make population inferences.

The number of days needed to estimate intake of a particular nutrient depends on the variability of the nutrient being assessed and the degree of accuracy desired for the research question *(2,36–38)*. Most nutrients require more than 4 d for a reliable estimate *(37,38)*. However, most individuals weary of keeping records beyond 4 d, which may decrease the quality of the records *(25)*. Block et al. *(39)* used an interesting approach, collecting 2 d of food records at four different times throughout the year to evaluate a food frequency questionnaire (FFQ). The advantages of this approach are that the collection of multiple days spaced far enough apart prevents record fatigue and captures seasonal variation. In developed countries, the within-person variation of day-to-day dietary intake for any one nutrient is usually greater than between-person variation; thus, collecting an inadequate number of days of intake would jeopardize a study's capacity to accurately describe intake and find important differences between persons *(37)*.

Beaton and colleagues *(38)* have developed guidelines for determining the number of days necessary to estimate an individual's true intake within a specified degree of error. Using the formula developed by Beaton *(38)* and values for the energy-adjusted within-person coefficient of variation from food records completed by US women as published

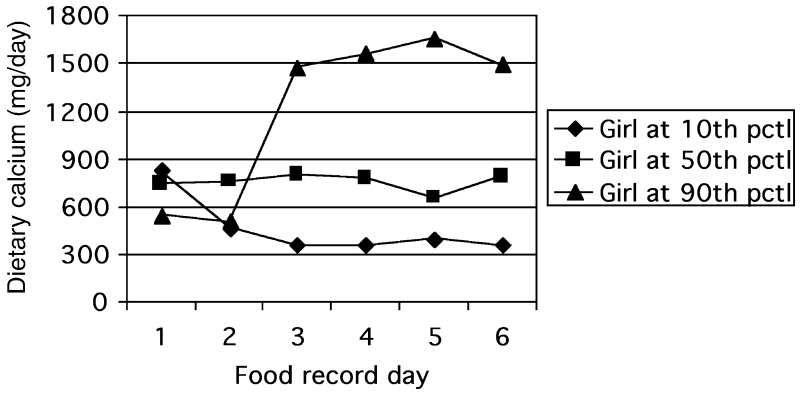


Fig. 2. An example of day-to-day variation in daily dietary intakes of calcium from 6 d of food records collected from three girls between 10 and 14 yr of age. The girls were selected from a larger group and each represented the noted percentile (pctl) in the group for average total dietary calcium intake.

by Willett *(2)*, the number of days needed to estimate a woman's calcium intake to within 20% of her true mean 95% of the time would be 13 d. The nonadjusted estimate would be 17 d. Similar principles by which to obtain the number of days to accurately assess usual intake for a nutrient have been reported by Nelson et al. *(37)* and Liu et al. *(36)*. For this approach *(37)*, the number of days of food records needed to ensure that $r \geq 0.90$ for calcium is 4 for toddlers and male children (5–17 yr of age). The number of days for adult males is 5, and for adult females is 8. The largest number of days needed, secondary to having the largest within-person variation, is 12 days for female children (5–17 yr of age). All of the estimates described here were derived from data collected prior to the 1990s, before fortification of the food supply with calcium became common. If the calcium-fortified foods are consumed only occasionally, then the number of days to accurately estimate calcium intake would increase (as within-person variability would increase). If these fortified foods are consumed regularly, then the estimates above would most likely still be valid.

2.3. Food Frequency Questionnaires

The FFQ estimates usual frequency of consumption of foods from a list for a specific period of time. Depending on the questionnaire used, estimates can be made for total diet or a specific nutrient or food. There are three basic types of FFQs: qualitative, semi-quantitative, and quantitative (*see* Fig. 3) *(40)*. Each style has its advantages and disadvantages based on the foods or nutrients being assessed, the objectives of the research, and the population being assessed. Some widely used and available FFQs are the "Block" FFQ, which is a quantitative FFQ, and the "Willett" or "Harvard" FFQ, which is a semi-quantitative FFQ *(41)*. Newly developed and available from the Risk Factor Monitoring and Methods Branch of the National Cancer Institute is the Diet History Questionnaire (DHQ), which is a quantitative FFQ. The performance of these three FFQ tools has been compared and found to be similar *(41)*. The qualitative FFQ, which attempts to classify individuals according to nutrient intake on the basis of frequency of consumption alone,

Qualitative	Semi-quantitative	Quantitative
How often do you eat the following vegetables? Green beans or peas O Never O A few times per year O Once per month O 2-3 times per month O Once per week O 2 times per week O 3-4 times per week O 5-6 times per week O Everyday	How often do you eat the following vegetables? Green beans or peas (1/2 cup) O Never O A few times per year O Once per month O 2-3 times per month O Once per week O 2 times per week O 3-4 times per week O 5-6 times per week O Everyday	How often do you eat the following vegetables? Green beans or peas (1/2 cup) O Never O A few times per year O Once per month O 2-3 times per month O Once per week O 2 times per week O 3-4 times per week O 5-6 times per week O Everyday How much each time O ¼ cup O ½ cup O 1 cup O 2 cups
Frequency only	Frequency plus addition of reference portion size	Frequency plus selection of usual portion size

Fig. 3. Examples of the three types of food frequency questionnaires.

has not been used routinely since the mid-1980s *(42)*. Recently, the qualitative FFQ was resurrected as a "food propensity questionnaire" to help derive usual intake over time from the 24-h dietary recalls collected in the NHANES *(43)*.

The FFQ estimates usual intake of foods and nutrients over a specified period of time, e.g., 1 wk, 1 mo, 1 yr. The FFQ is unique in that one can also specify a period of reference to recall, such as 5 yr ago or 10 yr ago. Like the 24-h dietary recall, the FFQ does not influence the eating behaviors of respondents. To complete the FFQ, there is a low burden on respondents. Almost all are optically scannable for easy data entry, some are available as interactive multi-media *(44)*, and some are moving to a Worldwide Web platform.

The FFQ is intended to rank or compare dietary intakes (e.g., foods or nutrients) among individuals *(45)*. In particular, the FFQ separates the "highs" from the "lows" with respect to intake of the specific foods in the FFQ and, to some extent, the nutrients in those foods. Because of the constraints imposed on the respondent with regard to food choices and portion sizes, the FFQ should not be used to assess the adequacy of dietary intakes of individuals or groups *(46)*. The foods in an FFQ are limited to foods representing the major contributors to a specific nutrient *(46)*, bioactive compound *(47)*, or food groups *(48)*, and rely heavily on differences in frequency of intake vs portion sizes. Foods are grouped together, thus limiting specificity if a respondent only eats one food in a group. As a result, the dietary estimates from an FFQ are not quantifiably precise and have a larger measurement error than the food record or 24-h dietary recall *(33)*. In addition, the use of an FFQ is limited to the populations for which the instrument was designed; thus, whole groups of foods central to a particular eating pattern may be missing from a particular FFQ *(49)*. Despite these limitations, the probability approach to estimate the prevalence of inadequate nutrient intakes in a regional population was successfully used with an FFQ developed to estimate total diet *(50)*.

Most FFQs have been designed to be self-administered and require 30–60 min to complete, depending on the instrument and the respondent. The process of completing an FFQ, although not burdensome, can be a high-level cognitive process. Subar et al. *(51)* attempted to address many of the cognitive processes involved with completing an FFQ when redesigning the DHQ. However, the mathematical and conceptual burden of calculating usual intake may be a particular challenge to individuals with lower education levels. As a result, a proportion of respondents report intakes that are implausible—either too high or too low. For example, the same interactive multimedia FFQ *(44,52)* was administered to middle- and upper-income adults, high-school seniors, and graduate equivalency diploma (GED) enrollees *(53)*. The prevalence of erroneous results (e.g., <600 kcal or ≥ 5000 kcal) ranged from 2.5%, 9%, to 19%, respectively. Low-literacy audiences are especially prone to difficulties with the FFQ that can be attenuated by using an interviewer as opposed to self-administration *(54)*.

The studies that have used a self-administered semi-quantitative FFQ most successfully include the Nurses' Health Study and the Health Professional Follow-Up study *(2,55)*. All of the respondents in these cohorts are well-educated, which likely contributes to more valid dietary estimates from the FFQ, compared with other study samples. This may explain the ability of the researchers affiliated with these cohorts to detect strong associations between FFQ dietary estimates and disease *(56)*, because dietary measurement error would be attenuated. When the estimated energy intake from the "Willett" FFQ was compared with total energy expenditure based on DLW in 10 young women

(mean age 25.2 yr) and 10 older women (mean age 75.0 yr), the FFQ gave significantly lower values for the young women, but not the older women *(9)*. On the other hand, the OPEN Study recruited highly educated subjects and found underreporting in men to be 31–36% for estimated energy intake from the DHQ compared with total energy expenditure as measured by DLW *(24)*. The equivalent comparison for women was underreporting of 34–38%. This is of great concern because underreporting in an FFQ contributes to severe attenuation in estimating disease relative risks. Schatzkin *(57)* and Kipnis and colleagues *(58)* provide excellent discussions of this problem.

3. MANAGING IMPLAUSIBLE DIETARY ESTIMATES

Because it is impossible to monitor the energy expenditure (EE) of every subject in a study, methods to evaluate under- and overreporters become necessary. Goldberg and colleagues *(59)* proposed a cutoff based on energy intake (EI) as estimated by a dietary assessment method, and EE as estimated by available formulas that include age, weight, height, and gender. Underreporters are considered to be those individuals in whom the EI is less than 0.76 of the EE. Acceptable levels are 0.76–1.24. Overreporters are considered to be those with an EI to EE of more than 1.24. The original formula assumes that everyone has a sedentary lifestyle, and this has been improved with the addition of physical activity level *(60,61)*. Several researchers have examined alternatives to identifying inaccurate dietary reports *(7,62)*. Although these cutoffs are based on energy, it is important to realize that underreporting and overreporting are selective, and not all nutrients or foods may be underreported or overreported the same way. Another approach is to identify absolute levels of energy intake that are improbable given the FFQ instrument used. Commonly used cut-offs are less than 600 kcal and 5000 kcal or more *(2)*. It is important to check that any individuals identified with implausible values do not differ from the entire sample, especially with respect to any parameters directly related to the study's objective. Completing data analysis with and without the extreme values can strengthen any conclusions made.

4. BRIEF DIETARY ASSESSMENT METHODS

The questionnaires for brief dietary assessment methods are developed specifically for measuring a single food group or nutrient *(63)*, or behaviors such as removing skin from chicken before eating or using low-fat salad dressing *(64)*. If an FFQ is shortened to 15–30 foods, then it is considered a brief dietary assessment method *(32)*. For example, to develop a brief "fat screener," Block identified 13 foods that accounted for most of the intake of fat of American women *(65)*, and used the same technique to develop a "fruit and vegetable screener" *(65)*. In the fruit and vegetable module for the Behavioral Risk Factor Surveillance System (BRFSS), two questions assess fruit intake, and four questions assess vegetable intake for a total of seven questions *(66)*. By reducing a 31-item FFQ to a seven-item questionnaire, a brief assessment method to estimate fruit, juice, and vegetable intake in an African-American population was created *(67)*.

Neuhouser and colleagues *(68)* developed and validated a useful brief dietary assessment tool blending features of the 24-h dietary recall and the FFQ, and coined the term *focused recall*. The focused recall is intended to produce detailed information that is focused on a specific group of foods eaten during the previous 24 h. The investigators'

purpose in developing the tool was to target the co-consumption of carotenoid-containing fruits and vegetables with savory snacks; however, the approach can be generalized to any specific group of foods, such as dairy or milk products. As a brief method, the tool holds promise when recent intake of a limited class of foods is relevant to a research project.

As with any dietary assessment method, the brief assessment method needs to be evaluated against some other measure of truth; as was done by Neuhouser et al *(68)*. The advantages of the brief methods are their inherent ease in completion by the respondent and their ease of analysis by the investigators. The method's advantage is also its disadvantage—the narrow focus may be limiting for many studies.

5. TYPES OF EPIDEMIOLOGIC STUDIES AND DIETARY ASSESSMENT METHODS

Other dietary assessment methods do exist, and traditional methods are being adapted; however, the four previously covered represent the major methods in use today. An excellent overview of available tools can be found in the comprehensive review done by Thompson and Byer *(1)* and later adapted *(32)*. Keeping in mind the primary principles of each method, their uses in different study designs are covered next.

5.1. Cross-Sectional Study Design and Surveillance Systems

One of the most common study designs is the cross-sectional study that provides a "snapshot" of the dietary practices of a population at a particular point in time as outlined in Fig. 4. In population study designs, one first determines the target population for which the conclusions of the study will be drawn. This will comprise the source population. Because it is nearly impossible to collect dietary and health information on a complete census, a sample from the source population is selected following recognized sampling techniques.

The source population can be the entire noninstitutionalized population in the country, as is the case with the sample drawn for the NHANES, or the residents of a state, as in the BRFSS, or targeted residents of a state, as in the Massachusetts Hispanic Elders Study (MAHES) *(69)*. For these types of studies, the investigators would assess the subjects' dietary characteristics, then, for analysis purposes, the individuals would be classified as either exposed to a dietary factor and "diseased," exposed to a factor and not "diseased," not exposed to a factor and "diseased," or not exposed to a factor or not "diseased."

This study design has its limitations when examining the association of diet and its role in the etiology of a disease. Any disease that has a long latency period, such as cancer or osteoporosis, would not work for this design. This design cannot be used with diseases that alter the exposure. For example, individuals diagnosed with osteoporosis may increase their dietary and supplemental calcium intake secondary to physician orders, thus leading to the false conclusion that high calcium intake is associated with osteoporosis. In these cases, results cannot distinguish if diet was a result of the disease or if the diet preceded the disease. Nonetheless, this is a valuable study design that can address many research questions using the appropriate dietary assessment methods and research questions for this design.

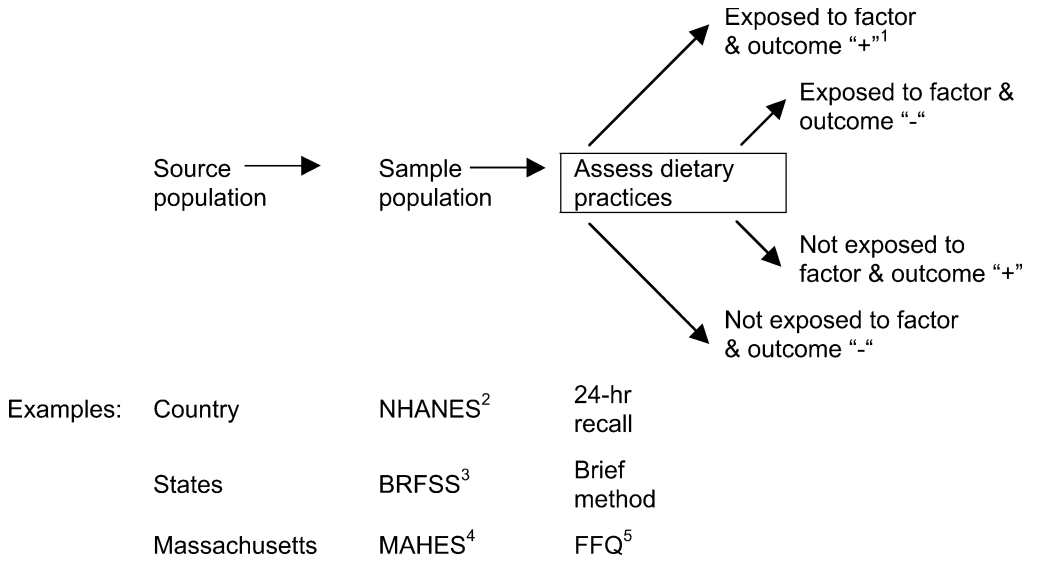


Fig. 4. Cross-sectional study and surveillance system basic design with examples of studies and dietary assessment methods used to assess dietary exposures for health outcomes (*see* text for references).
[1]"+" is positive, "–" is negative. Traditionally, outcomes have been disease-present or disease-absent, such as high blood pressure. However, outcomes can also be risk factors or measures of nutritional status, such as overweight, level of nutrient stores, hyperlipidemia.
[2]National Health and Nutrition Examination Survey, United States.
[3]Behavioral Risk Factor Surveillance System, United States.
[4]Massachusetts Hispanic Elders Study, a statewide survey conducted between 1993 and 1997 that included a representative sample of elderly Hispanics and a neighborhood control group of non-Hispanic whites.
[5]Food frequency questionnaire.

For the cross-sectional study, both the 24-h recall and the food record have the advantage of providing dietary intake information about actual foods eaten during the specified period of time of the cross-sectional analysis. The detail of foods consumed can be used for analysis according to nutrients and portion sizes, as well as dietary and food patterns. On the other hand, if the period of recall desired is months prior to the interview, the FFQ may be the more appropriate choice, as long as relative differences between groups is appropriate to answer the primary research question. The FFQ almost becomes the instrument of choice when study population size becomes large and/or if resources are limited. If the subjects are at remote sites in relation to the research center, the FFQ may be favored because it can be mailed to subjects. Alternatively, the 24-h dietary recall has been shown to work equally well in-person as well as over the telephone, allowing access to distant subjects *(70)*.

5.1.1. Using the 24-Hour Dietary Recall in a Cross-Sectional Study

A study by Novotny et al. *(71)* had as its primary purpose to identify contributors to differences in calcium intakes among Asian, Hispanic, and non-Hispanic White adoles-

cents. In selecting a dietary assessment method, the investigators considered the potential differences in dietary intakes secondary to cultural food practices; thus a method that allowed for specific foods to be recorded was necessary. Another factor was the age of the subjects. With ages between 11 and 19 yr, the investigators questioned the ability of the younger subjects to thoroughly complete food records. Based on these concerns and given that sufficient trained staff were available, the decision was made to use the 24-h dietary recall. Ideally, more than 2 d were desired; however, resources limited the final decision to two nonconsecutive days at least 1 wk apart, with the completion of approx 75% of the 24-h dietary recalls on weekdays and 25% on weekends. In the end, two 24-h dietary recalls were collected from 176 children of Asian, Hispanic, or non-Hispanic White background using the triple-pass method for the 24-h dietary recall. The multiple-pass method added the advantage of being a well-documented method with specific procedures that worked well, as the study sites encompassed five different states and identical procedures could be implemented.

After compiling the dietary data, Novotny and colleagues were able to ascertain that milk consumption was the most powerful indicator of calcium intake among each group of children despite their varied intakes. Further, the detail of the 24-h recall allowed for the observation that milk portion size was significantly associated with soda consumption, especially among the Hispanic adolescents, whereas the Asian children tended to consume higher-fat dairy foods with lower calcium content (e.g., ice cream and milk shakes). Thus, the detail of the two 24-h dietary recalls allowed the investigators to conclude that displacement of milk by soda among the Hispanic adolescents and filling-up on higher-fat milk products for the Asian adolescents may contribute to their lower calcium intakes than the non-Hispanic White adolescents, thus providing direction for targeted messages to youth concerning inadequate calcium intakes.

5.1.2. Using a Food Frequency Questionnaire in a Cross-Sectional Study

Application of an FFQ is limited to the populations for which the FFQ was designed. This becomes especially important with regard to cultural or regionally based foods. The MAHES is a cross-sectional study that was initiated to study issues of diet and health among Hispanic adults living in the northeastern United States *(69)*. To estimate dietary intake, the investigators adapted a version of the "Block" FFQ by modifying the food list and portion sizes based on data from the Hispanic Health and Nutrition Examination Survey and the Second NHANES. The revised FFQ was evaluated by comparing nutrient intakes between the FFQ and 24-h dietary recalls. Added foods included plantains, avocado, mango, cassava, empanadas, and custard. One of the published manuscripts from this cross-sectional study using the FFQ assessed fruit and vegetable intake and its association with total homocysteine (Hcy) and C-reactive protein (CRP) *(72)*. Significant dose–response relationships for both plasma CRP and Hcy concentrations with frequency of fruits and vegetable intake were observed. Had the investigators not made the initial investment to revise and test the FFQ, this significant relationship may not have been found in this cross-sectional analysis.

5.1.3. Using a Brief Dietary Assessment Method in Surveillance

The BRFSS is a telephone-based surveillance program conducted by the Centers for Disease Control and Prevention. As mentioned earlier, the fruit and vegetable brief dietary assessment questionnaire used by BRFSS has seven questions that have been

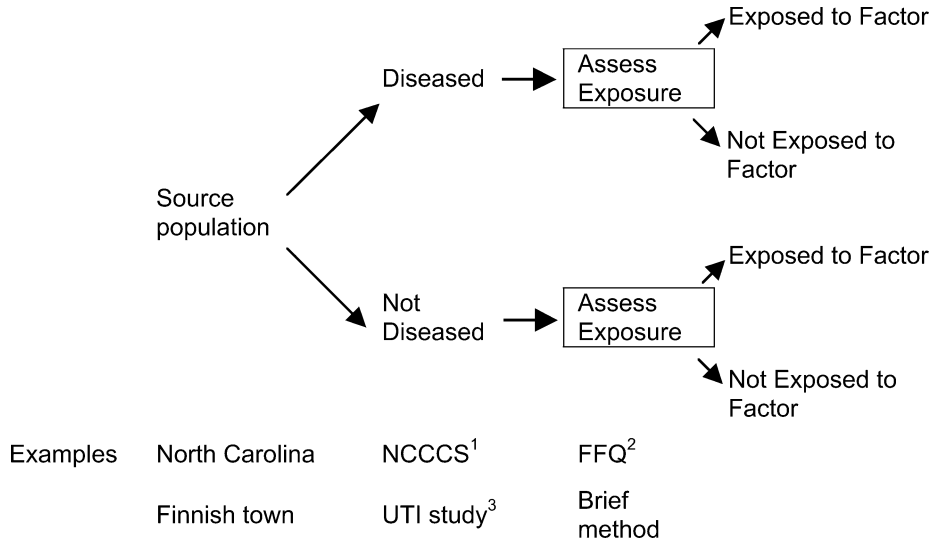


Fig. 5. Case–control study basic design with examples of studies and dietary assessment methods used to assess dietary exposures by disease outcome (*see* text for references).
[1]North Carolina Colon Cancer Study.
[2]Food frequency questionnaire.
[3]Urinary tract infection.

evaluated against other measures *(73)*. This questionnaire works well for the surveillance system because of its brevity especially in the context of other modules covered in one telephone interview. Keeping the interview within a reasonable amount of time improves participation. Each state uses the same set of questions, thus allowing comparisons of fruit and vegetable intake across states. The same questionnaire is used between years so that the achievement of five servings of fruits and vegetables daily over time can be monitored.

5.2. Case–Control Study Design

The case–control study design is often the study design of first choice when the disease of interest is a relatively rare event, such as cancer. Either all individuals with the disease of interest are recruited as being in the study population as "cases," or a random sample of individuals with disease are recruited as "cases." The comparison group or "controls" are selected as a random sample of the study population that represents the same community from which the "cases" were derived, as shown in Fig. 5. In matched case–control studies, each "case" is matched with one or more "controls" of the same age, gender, and possibly other factors that may confound the relationship of dietary exposure to disease.

Because the cases already have disease, the period of exposure of interest is the time before the onset of disease. Given this situation, the food record and 24-h recall, which reflect recent intake, are not applicable to this study design, because intakes are often altered by treatment regimens and the primary exposure may be in the distant past. That leaves the FFQ as the method of choice for a case–control study, with the reference period for food recall usually being 1 yr prior to the diagnosis of disease. Because the cases

generally are recruited fairly quickly after diagnosis of disease, the period of dietary recall reference for controls is often the year preceding the interview. Although there is the possibility of recall bias on the part of the cases having more at stake in remembering past events, there is otherwise no reason to think that the cases' and controls' abilities to recall over a past year would systematically vary. For some diseases, an individual's long-term dietary profile (e.g., the last 10 yr) would be an ideal time frame for dietary exposure; however, the ability of an individual to recall diet decreases when asked to recall 5 or 10 yr back *(1,74,75)*.

5.2.1. Using a Food Frequency Questionnaire in a Case–Control Study

The North Carolina Colon Cancer Study (NCCCS) examines risks for colon cancer in African Americans and whites *(76)*. The investigators of this study modified the "Block" FFQ to accommodate commonly eaten foods in North Carolina. For the analyses of the association of micronutrients to colon cancer risk *(76)*, only those nutrients that are reasonably captured by FFQs were included. These were identified as β-carotene, lutein, vitamins C and E, folate, and calcium. Supplement use was assessed with separate closed-ended questions. The researchers observed that in whites, the highest quartiles of β-carotene, vitamin C, and calcium intakes were associated with 40–60% reductions in colon cancer risk compared with the lowest quartiles. In African Americans, vitamins C and E were strongly inversely associated with a reduced risk for colon cancer. Despite the findings being consistent with previous research, the difference in risk by nutrients between the two groups was puzzling to the authors. Such observations could potentially result from errors in the measurement of diet by the FFQ and justifies further examination of diet and disease relationships and investigation of improved long-term recall methods.

5.2.2. Using a Brief Dietary Assessment Method in a Case–Control Study

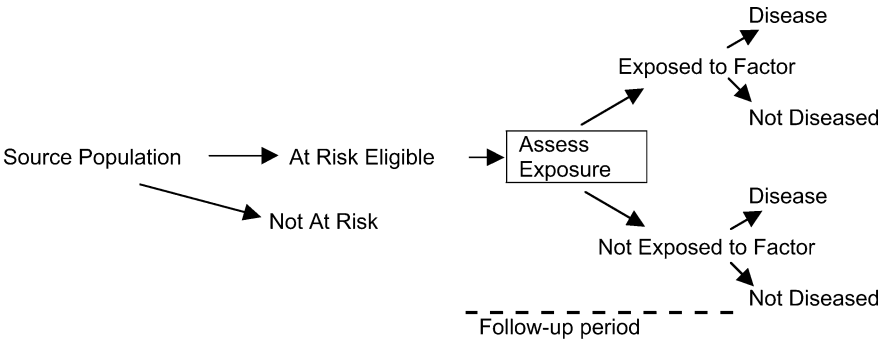
A brief dietary assessment method was implemented very cleverly by Finnish investigators examining the role of diet as a risk factor for urinary tract infection (UTI) with a case–control study design *(77)*. Because UTIs are believed to be caused by bacteria in the stool, dietary factors may affect the risk of contracting a UTI by altering the properties of the fecal bacterial flora. Women of an average age of 31 yr with a diagnosis of UTI were case-matched with women with no episode of UTI in the past 5 yr. A total of 107 case–control pairs were recruited. The investigators used a remarkably simple brief dietary assessment questionnaire that included 18 questions on milk and other dairy products, berries and berry juices, soft drinks, and coffee. For example, women were asked their frequency of consumption during the past month of milk (fresh or fermented with probiotics) and responses were never, less than one time per week, one to three times per week, and three or more times per week. Another question asked their average consumption during the past month of certain products, including milk, sour milk, and yogurt, as glasses (2 dL/d). The results showed an inverse association between mean daily use of fresh juice (fruit or berry, dL) and onset of UTI, odds ratio: 0.66 (95% confidence interval: 0.48, 0.92). For probiotic milk products, a significant inverse association was observed between frequency of consumption and occurrence of UTI. This particular tool was short and identified very specific dietary patterns; however, it performed its purpose of identifying possible mediators of UTI in this sample of women.

5.3. The Cohort or Prospective Study Design

The cohort study design starts with a healthy group exposed to varying extents to a given nutrient, and follows the group prospectively, counting the members who develop disease. It may be either concurrent (i.e., the observation is concurrent with the exposure) or nonconcurrent (i.e., the exposure precedes much or all of the observation). An example of the enrollment of participants that will be followed over a period of time is shown in Fig. 6. Closed cohort studies with fixed membership often include some type of dietary assessment. For a closed cohort, a source population is identified, then a sampling frame is created and only those individuals at risk for the disease or diseases under investigation are included at baseline. For example, the Nurses' Health Study was initiated to investigate the potential long-term consequences of the use of oral contraceptives *(78)*. So even though no single disease was identified, one needed to be a woman to be at risk for consequences due to oral contraceptives. The investigators selected nurses to follow, because they would provide a motivated population-base of women capable of completing detailed health questionnaires. The selection process was further narrowed to married registered nurses between 30 and 55 yr of age, who lived in the 11 most populous states and whose nursing boards agreed to supply their members' names and addresses. The final baseline cohort members were those women that responded to the initial questionnaire.

Once the members of a cohort have been established, baseline characteristics and risk factors of interest are measured, and then the members of the cohort are followed for onset of disease. There is a profound advantage with the cohort approach. Dietary intake is recorded prior to occurrence of any disease; therefore the dietary information is not biased by the diagnosis of disease. On the other hand, if the follow-up of the cohort members is a long period prior to the onset of disease, the diet may not appropriately reflect the average intake of the cohort members over time. Many cohorts in existence today have addressed this issue by collecting dietary intake at periodic intervals.

Because the purpose of the dietary assessment at baseline for a cohort is to estimate their current intake, almost every dietary assessment method can be considered. Recall that most nutrients have large day-to-day variation; thus, if a 24-h dietary recall or food record were selected, two or more days of each would have to be collected *(36,38)*. Given that cohorts must be large in order to detect any significant differences between exposed groups, the collection and analysis of 24-h dietary recalls and food records would prove to be expensive and impractical. The Nurses' Health Study previously described enrolled 122,000 women *(78)*—that would be too great a number of food records or 24-h dietary recalls to collect over the telephone. For this reason, the dietary assessment method of first choice in a cohort study is the FFQ. As an alternative, investigators can collect more detailed dietary information via multiple food records from a smaller sample of cohort members to assist with evaluation of the FFQ (if it had not been previously validated) and correction of possible measurement error in the FFQ *(79)*. However, sample selection from the cohort is not a trivial task, if one wants to ensure unbiased correlations between the two intake methods *(80)*. Some of the earlier initiated cohorts, such as the Framingham Heart Study *(81)* and the Honolulu Heart Study *(82)*, initially used a single 24-h dietary recall and later adopted study-specific FFQs.



Examples	Framingham, MA	Framingham Heart Study	FFQ[1]
	Birmingham, AL Chicago, IL Minneapolis, MN Oakland, CA	Coronary Artery Risk Development in Young Adults (CARDIA)	FFQ[2]
	Nursing boards in 14 states, women 25-42 years old	Nurses Health Study II	FFQ[3]

Fig. 6. Cohort or prospective basic design with examples of studies and dietary assessment methods used to assess dietary exposures prior to health outcomes (*see* text for references).
[1]Framingham Heart Study originally used a 24-h dietary recall to collect dietary information and switched to a semi-quantitative food frequency.
[2]A quantitative food frequency questionnaire is used.
[3]A semi-quantitative food frequency is used.

Another form of the cohort or prospective study design is the randomized trial. This is set apart from the observational cohort study in that the eligible subjects are randomized to receive an exposure of interest (e.g., vitamin A supplements or intensive dietary intervention). Figure 6 can be used to depict a randomized trial by replacing "Assess Exposure" with "Allocate Exposure at Random." Whichever dietary assessment method is used to measure effectiveness of an intervention, the subjects, in providing their responses, are more prone to social desirability, especially the treatment subjects *(83)*. Some approaches to counteracting this phenomenon include using more than one dietary assessment measure, using grocery shopping receipts, or using a biomarker. Much less work has been done on developing valid methods for measuring dietary change in population-based randomized trials than for any other study design *(83–85)*.

Another modification of the cohort method or prospective method is to use the prerecorded disease rates in a national or regional population for control purposes, rather than selecting a specially selected control group. This approach is appropriate when the exposure to the risk factor in the general population is negligible. Goulding and colleagues *(86,87)* adopted this approach using a nonconcurrent cohort design for 50 children 3–10 yr of age who had a history of avoiding the consumption of cow milk for less than 4 mo at some stage in their lives. Assuming that the exposure in the general population is minimal with regard to long-term avoidance of cow milk, the number of fractures in this group of 50 children was compared with the fracture rates in a pre-existing birth cohort

(87). In this analysis, the number of fractures in the avoidance group was compared with the numbers that would have been expected if subjects had experienced the same fracture rates specific for age and sex as the birth cohort (representing the general population). Although the exposure of cow milk avoidance was gathered retrospectively, this type of dietary exposure is not subject to the same recall bias as the detailed consumption of specific foods. In addition, among this young age group, the assumption that few children avoid cow milk for long periods of time is probably valid, thus allowing this approach of using the birth cohort as a general community control group.

5.3.1. Using a Semi-Quantitative Food Frequency Questionnaire in a Cohort Study

In 1980, the Nurses' Health Study started measuring dietary exposures with the addition of a semi-quantitative food frequency questionnaire to its battery of questionnaires (http://www.nurseshealthstudy.org/). The decision to use a self-administered FFQ worked well for this cohort because the questionnaire could be mailed to the respondents residing in 11 different states and easily returned upon completion with the other questionnaires. Because the members of this cohort are well-educated, they are in a position to appropriately calculate some of the mathematical problems posed by having one reference portion size. For example, the frequency would need to be increased if the usual portion size was double the portion size noted in the questionnaire.

The contribution of portion size to the ranking of individuals for vitamin A intake was examined by Samet et al. *(88)*. They compared the ranking of individuals by frequency alone and by "usual" portion sizes based on an in-person interview. The correlations between the methods were 0.86 for controls and 0.91 for cases. These results suggested that portion size questions provided little additional information and supported their decision to use the semi-quantitative FFQ with the cohort of nurses. Another decision was the period of recall. Women were asked to recall food intake for over the past year. An extensive evaluation was conducted by Willet and colleagues among a sub-sample of the cohort population *(89)*. The FFQ was completed followed by 28 d of food records spread out over 1 yr. At the end of the year, another FFQ was completed. The average nutrient intakes from the food records were compared to the estimated nutrient intakes from the FFQ. The correlation coefficients ranged between 0.5 and 0.7, indicating a satisfactory comparison between the two dietary assessment methods.

5.3.2. Using a Quantitative Food Frequency Questionnaire in a Cohort Study

The Coronary Artery Risk Development in Young Adults (CARDIA) Study was initiated to study the evolution of cardiovascular disease risk factors *(90)*. This is a multicenter population-based prospective study of black and non-Hispanic white young adults using four study centers in Birmingham, AL, Chicago, IL, Minneapolis, MN, and Oakland, CA. The goal for the dietary assessment measure was to assess patterns of food and nutrient intake relating to the development of coronary heart disease. The food frequency approach was appealing because it does not bias intakes and a minimum level of education is needed to complete the questionnaire. The investigators modified the 28-d dietary history used with the Western Electric study because this method had adequately defined intakes of saturated fat and cholesterol, which were found to be significantly associated with coronary heart disease *(79)*. Modifications included identifying foods frequently consumed from results of the NHANES II to reflect the current

food supply (at the time) and the intakes of a younger and more diverse population. The final format consisted of three parts: (1) questions about usual dietary patterns, (2) an assessment of sodium intake, and (3) a quantitative FFQ. The period of recall was set to the previous 28 d because this time-frame was found to be compatible with an achievable recall period and the period would correlate well with serum measures influenced by diet within this period of time. Rather than allow it to be self-administered, the decision was made to administer the questionnaire by interview, thus further minimizing the issue of differences in comprehension among study participants. The investigators recognized in advance that a 28-d recall period would not reflect seasonal changes in food intake. However, interviews were scheduled throughout the year so that seasonal intakes would be determined for the group. The relative validity of the final dietary assessment tool was evaluated by comparison with food records *(79)*. Even after 15 yr of follow-up, the detail incorporated into the questionnaire allowed the investigators to identify the frequency of dairy foods consumed by the subjects *(91)*. In overweight adults, the exposure to dairy foods was found to be inversely associated with insulin resistance syndrome, a risk factor for type 2 diabetes and cardiovascular disease.

6. CALCIUM-SPECIFIC FOOD QUESTIONNAIRES

There is a unique tool called the "Dietary Assessment Calibration/Evaluation Register" available on-line that catalogs the evaluation of dietary assessment methods. When selecting a dietary assessment method, this may be a useful first stop (http://www-dacv.ims.nci.nih.gov/) *(92)*. The comprehensive review by McPherson and colleagues in 2000 *(93)*, which evaluated dietary assessment methods among children 5–18 yr of age, would be useful for planning projects with children. Many calcium-specific FFQs have been evaluated among a variety of adult populations in the United States *(94–99)* with correlation coefficients ranging between 0.33 and 0.85. For Asian, Hispanic, and non-Hispanic white adolescents in the United States, a semi-quantitative FFQ has been extensively evaluated *(46,100)*. Calcium-specific FFQs have been developed and evaluated for use with adults in Sweden *(101)*, Italy *(102)*, Australia *(103)*, Malaysia *(104)*, and Mexico *(105)*, as well as children in New Zealand *(106)*. If one were to adopt one of these tools, it would be important to evaluate the tool if the populations differ dramatically. In addition, with the current level of calcium fortification in the food supply, tools developed in the distant past may not reflect these new sources of calcium.

7. EVALUATING DIETARY ASSESSMENT METHODS

The examples previously described highlight that the final choice of a dietary assessment method is driven not only by the study design, but by the target population, the study objectives, the outcome of interest, and the available resources *(45)*. The other issue alluded to throughout this chapter is the validity of any method for measuring the nutrient, food or food patterns of interest. Before closing this chapter, this issue will briefly be addressed. More detailed discussions can be found elsewhere *(2,24,26,32,107–110)*.

As with any measure, there is a desire to insure that the measure is reliable or reproducible and valid. With dietary assessment methods, this presents a challenge because the opportunities to directly observe and record what individuals eat over an extended period of time are limited (e.g., feeding studies). As a result, a common approach to evaluating

a dietary assessment method is to compare one method with a different type of method. For example, a food frequency may be compared with multiple food records; an advantage of comparing an FFQ to food records is that the records would not have the same memory bias as the food frequency. Because this type of comparison is basically relating one method to another, some have referred to this as "calibration" *(111)*. However, this may be confusing because calibration implies a resetting to a standard, which is not the intention of "calibration" with regard to dietary assessment. Thus, a recommendation has been made to refer to all aspects of testing the reliability and validity of dietary assessment methods as "evaluation" *(112)*. In general, correlation coefficients range between 0.4 and 0.7 for comparison between dietary assessment methods *(2,45)*. These coefficient ranges highlight the existence of measurement error in all dietary assessment methods, and thus various methods of energy adjustment *(2)*, methods of correcting for measurement error *(113)*, and investigations to better understand measurement error have been employed *(24)*.

It may be tempting to use a method previously evaluated as reliable and valid; however, one must recognize that a method validated with one group may not be applicable to another *(45,114)*. For example, Jensen et al. *(46)* evaluated a semi-quantitative food frequency among Asian, Hispanic, and non-Hispanic white adolescents primarily in the Western United States. The tool may not work as well with African-American adolescents in the southern United States, and an effort to evaluate the tool with this group would be prudent prior to adopting its use in a research study. All dietary assessment methods have some degree of measurement error *(115)*; therefore, efforts to keep these errors to a minimum must be implemented *(116)*.

The real challenge is comparing the results of the dietary assessment method with some measure of "truth." This is best achieved by identifying a biomarker of a nutrient or dietary factor *(26,117)*. The underlying assumption of a biomarker is that it responds to intake in a dose-dependent relationship *(2)*. The method that has widest consensus as a valid biomarker is DLW for energy *(26,118)*. Because DLW provides an accurate measure of total energy expenditure in free-living subjects, it has been successfully used to compare energy expenditure to estimated energy intake as determined by a dietary assessment tool. A biomarker does not rely on a self-report of food intake, thus theoretically the measurement errors of the biomarker are not likely to be correlated with those of the dietary assessment method. Another proposed biomarker is analysis of nitrogen from 24-h urine collections as an indicator for protein intake *(29,119)*. Other biomarkers collected from urine samples include potassium and sodium *(29)*. Plasma or serum biomarkers that have been explored are levels of ascorbic acid for vitamin C intake *(29,120)*, β-carotene for fruits and vegetables or antioxidants *(85,120,121)*. These latter markers are widely influenced by factors such as smoking status and supplement use, thus their interpretation as measures of absolute intake is limited.

Whereas a biomarker for a nutrient makes sense, a biomarker for an identified "healthy food pattern" may be unrealistic. Some nutrients are lacking in biologically valid biomarkers, such as calcium for adults. On the other hand, bone mineral content (BMC) as measured by dual-energy X-ray absorptiometry in young non-Hispanic white girls may be considered a cumulative historic marker. When comparing the estimated calcium intake from a semi-quantitative FFQ to bone measures in 14 non-Hispanic white females between 10 and 14 yr of age, a significant correlation coefficient ($r = 0.638$, $p = 0.014$) between total body BMC and calcium intake was observed *(100)*. However, this was not

found to be the case in African-American girls representing the same age group and following the same study protocol. The correlation coefficient was $r = -0.116$ ($p = 0.680$). This discrepancy could be due to differences in reporting, the FFQ not being appropriate for the African-American girls, and/or the BMC not being a biomarker for African-American girls. This highlights that any biomarker must be fully evaluated prior to its adoption in a particular study. Biomarkers cannot substitute for the dietary information collected from recalls, records, FFQs, or brief dietary assessment methods. Biomarkers can be used to validate the dietary information; however, the foods that contribute to a nutrient's presence can only be found by asking individuals what they eat.

8. CONCLUSIONS

Nutritional epidemiology is concerned with quantifying dietary exposures and their association with disease risk. There is no one dietary method that is considered a "gold standard." The choice of an appropriate dietary assessment method is dependent on the study design, the research objectives, the target population, and resources. Improved methods of collecting more accurate dietary information continue to be developed and refined. To corroborate results from dietary intake data, biological markers of nutritional exposures and nutritional status (referred to as biomarkers) are being developed to be used in tandem with dietary assessment methods. This chapter has described the reasons for adopting a particular dietary assessment method given a specific study design. Examples from the literature aid in outlining the decisions that investigators make to select or adapt a dietary assessment tool.

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5 Clinical Approaches for Studying Calcium Metabolism and Its Relationship to Disease

Connie M. Weaver

KEY POINTS

- Metabolic balance plus kinetic studies can pinpoint how calcium metabolism is perturbed (i.e., at the gut, kidney, or bone).
- Isotopic tracers can be used to quantitate calcium absorption efficiency and the bioavailability of calcium sources.
- Randomized, controlled trials are effective for showing the relationship of calcium intake to health outcomes.

1. INTRODUCTION

This chapter describes several types of clinical studies for studying the relationship of dietary calcium to health. Fractional calcium absorption studies are used to evaluate bioavailability from various sources or intrinsic calcium absorption capacity. The latter is an important risk factor for osteoporosis and possibly other disorders associated with low calcium status. The accuracy of different methods commonly used to determine fractional calcium absorption varies widely. Calcium retention measurements are useful to determine influences on bone mass in short-term studies. Randomized, controlled trials (RCTs) are the inferentially strongest approach to understanding the relationship between calcium intake and outcome measures of disease.

2. WHEN TO USE VARIOUS CLINICAL NUTRITION APPROACHES

Controlled feeding studies can provide valuable insights on how well calcium is absorbed and utilized from various sources by different individuals and under different conditions. They also may be used to determine the relationship between intake and risk for disease. Whereas the epidemiological approach to study the relationship between indices of health or disease risk (described in Chapter 4) can enroll large numbers of subjects, controlled feeding studies are typically more labor-intensive and necessitate smaller sample sizes. Well-done epidemiological studies can be representative of the

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general population and utilize the best outcome measures including disease endpoints. However, their ability to estimate calcium intake is imprecise and multiple confounding variables are not controlled, although better understood factors can be accounted for in the analysis. In contrast, the best-done clinical feeding studies can precisely control calcium intake and other dietary confounders. Some even control physical activity and use a crossover design to eliminate unforeseen confounders inherent in individuals. The more that diet and other variables are controlled, the more resources are required. This leads to reduced sample sizes and costs, and runs a risk of not representing the general population. Although the independent variable can be precisely controlled, the typical clinical study is too short to determine disease endpoints. With calcium, bone health is a frequent dependent variable of interest. Even changes in the best biomarkers for bone health, using bone densitometry or imaging, require longer periods than is feasible for a controlled diet study.

Clinical feeding studies are useful when quantitative information about calcium intake and an outcome is desired. The study design of choice depends on the particular outcome information sought. For any design, it is important to perform power calculations and to recruit sufficient subjects to ensure that meaningful effects are found.

In this chapter, various methods for determining calcium absorption are reviewed. Metabolic balance studies, which can provide quantitative data on calcium excretion and retention, are described. When calcium isotopic tracers are used in combination with metabolic balance studies, kinetic analysis, which provides information on rates of transfer among body compartments (*see* Chapter 6), can be performed. RCTs are also described briefly in this chapter. They represent an intermediate level of control between epidemiological studies and quantitative, controlled diet studies, and consequently require an intermediate number of subjects to determine the relationship between calcium intake and an index of health. The level of certainty of the diet–health relationships from cause-and-effect to associational usually also parallels types of studies from controlled, diet studies →RCTs → epidemiological studies. However, even changes observed in controlled feeding studies using a randomized-order, crossover design may not represent a cause-and-effect relationship if residual treatment effects remain because of an insufficient washout period.

3. METHODS FOR STUDYING CALCIUM ABSORPTION

Fractional calcium absorption from a fixed load is useful for determining physiological absorptive capacity or for determining bioavailability of calcium sources. There are many study designs that have been used to determine fractional calcium absorption. Most do not adapt subjects to a controlled diet, especially if calcium absorption is the only metabolic parameter of interest. When the question relates to calcium bioavailability from a particular source, the strongest design is to compare calcium absorption from that source with a referent source, such as milk or calcium carbonate, in a crossover design. This allows relative bioavailability to be determined with minimal influence of individual subject characteristics. Approximately 60% of the variance in cross sectional measures of calcium absorption can be accounted for by intrasubject absorption efficiency *(1)*. For menstruating women, this means studying each source at the same phase of the menstrual cycle, typically days 4–11 of the follicular cycle. When the calcium

source is the focus of interest, a controlled diet is often not necessary. The gastrointestinal environment should be similar between study phases, which is usually accomplished by an overnight fast. Similar results were found for bioavailability of calcium from tofu after an overnight fast or when the test meal was given in the middle a of the 4-d controlled diet period *(2)*. Longer feeding studies are required for measuring the influence of adaptation to the calcium source , level of calcium in the diet, or other dietary constituents on calcium absorption.

Examples of factors that are thought to alter calcium absorption through adaptation are nondigestible carbohydrates, which may influence lower gut bacterial fermentation or intestinal microvillar surface area (*see* Chapter 12), vitamin D and estrogen status (*see* Chapter 11), and chronically low calcium intakes.

3.1. Metabolic Balance Studies

On a controlled diet, net calcium absorption can be determined as

$$I - F,$$

where I = calcium intake and F = fecal calcium and each parameter is expressed in the same units (mg or mmol) and measured over the same time period (24 h, 1 wk, etc.). Net calcium absorption efficiency is determined as

$$(I - F)/I.$$

Balance studies are rarely the method of choice for determining calcium absorption for a number of reasons. Measured calcium in excreta from a test food cannot be distinguished from calcium in the rest of the diet nor from endogenous secretions. The former leads to uncertainty about bioavailability of calcium from the test source. The latter leads to underestimation of true bioavailability or absorption capacity. Furthermore, the large variability in fecal calcium even on controlled diets results in poor ability of the balance approach to discriminate between different sources. Further description of the conduct of metabolic balance studies and associated errors are discussed under Subheading 4.1. Even fecal markers, although necessary, are insufficient to deal adequately with the variability in daily fecal calcium excretion.

The metabolic balance approach for determining calcium absorption more often provides satisfactory results in animal models than in humans. Use of semi-purified diets enables all of the calcium to be provided by the test source of calcium. Use of inbred animals housed in metabolic cages under controlled conditions can reduce sources of variation. Calcium bioavailability in animal models has been shown to give similar rank order to humans and, dependent on the calcium load of the test meal, similar absolute values *(3)*. However, results from animal models (typically rats) can differ from those obtained using humans because the animals practice coprophagy, have intestinal phytases, and have substantially lower urinary calcium excretion, among other differences.

3.2. Calcium Isotopic Tracer Absorption

Isotopic tracer data are less variable than balance data. Thus, although fractional absorption determined by tracer studies results in conclusions similar to net calcium

Table 1
Calcium Isotopic Tracers

Atomic number	Symbol and mass number	Radioisotopes			Stable isotopes
			Maximum radiation energies		
		Half-life	β (Mev)	E (Mev)	Natural abundance (%)
	^{41}Ca	10^5y	–	–	10^{-15}
20	^{42}Ca	–	–	–	0.646
	^{43}Ca	–	–	–	0.135
	^{44}Ca	–	–	–	2.083
	^{45}Ca	164d	0.255	–	–
	^{46}Ca	–	–	–	0.0033
	^{47}Ca	4.53d	1.98	1.29	–
	^{48}Ca	–	–	–	0.18

absorption determined by balance studies *(4)*, more subtle differences can be discriminated with isotopic tracer studies, and sample sizes can be much smaller. Fractional calcium absorption and total absorbed calcium determined from kinetic modeling using data collected in metabolic balance and tracer studies is described in Chapter 6 and is not covered in this section. When the whole spectrum of calcium metabolism parameters is not needed, there are several approaches available for determining calcium absorption using isotopic tracers that require substantially fewer resources. However, caution must be exercised when only absorption is determined because a dietary component being tested may have an impact on another aspect of calcium metabolism that can either augment or minimize an apparent effect on absorption (*see* Chapter 9).

A list of isotopic tracers of calcium appears in Table 1. Useful radiotracers of calcium are ^{47}Ca and ^{45}Ca. ^{47}Ca is a γ- emitter, and therefore can be used for whole-body counting in studies of calcium absorption and retention in animals or humans in facilities where animal or human γ-counters are available. Its short half-life limits the length of the experiment and is a reason for its scarcity and relatively high expense. A limitation of whole-body counting is that mechanisms cannot be investigated because the organs affected (i.e., gut, kidney, or bone) cannot be inferred from whole-body retention curves. Another limitation is how long it takes the oral isotope to clear the gut. If the study is too short, unabsorbed calcium tracer in the colon will appear as if it were retained calcium. As a β-emitter, ^{45}Ca is measured in a liquid scintillation counter and is appropriate for biological fluids or samples that can be converted to fluids. Although ^{47}Ca can also be measured in biological fluids, the lower costs and longer half-life typically make ^{45}Ca the preferred radioisotope for tracer studies. Precision of analysis with radioisotopes depends on the counting rate, but samples can usually be counted to 1–2% precision.

There are many more nonradioactive (stable) isotopes of calcium than radioisotopes. These isotopes, of heavier mass than ^{40}Ca—which represents almost 97% of calcium in nature—are measured as isotopic ratios by mass spectroscopy. The methods of choice currently are high-resolution, inductively coupled plasma mass spectrometry (HR-ICPMS) *(5)* and thermal ionization mass spectrometry (TIMS) *(6)*. The former has the advantage of greater sample throughput and the latter has the advantage of greater precision (<0.1–0.2% vs 1–2%). Stable isotopic tracers offer the advantage of not exposing

subjects to radioactivity and not having to time experiments around a short half life tracer. They have the disadvantage of being more expensive to purchase and analyze. Use of calcium stable isotopes for clinical studies of calcium metabolism was first proposed in 1983 *(7)*.

The long-lived radioisotope ^{41}Ca can be used in such small doses ($\leq$100 nCi) that it can be considered to be radiologically benign. A single dose of this size labels the skeleton for life, which poses a lifetime radiation exposure of <0.1 mre. The benefits of this tracer are that the tracer can be monitored for long experiments in contrast with the upper limit of approx 2 wk with other isotopes. Urinary appearance of ^{41}Ca after 100 d from dosing, when the ^{41}Ca can be considered to be coming from the skeleton, provides a direct, sensitive measure of bone resorption. Changes in bone loss can be accurately measured following an intervention. The disadvantage of this approach is that ^{41}Ca is measured with an accelerator mass spectrometer (AMS), which is not available in most research centers. There are two in the United States—one at Purdue University and one at Lawrence Livermore National Laboratory. Opportunities involving AMS in nutrition have been recently reviewed *(8)*.

The design of the study using calcium isotopic tracers depends on available resources and capacity of the laboratory to measure and administer isotopic tracers in humans. The ideal approach (short of full kinetic modeling, described in Chapter 6) is the use of double isotope tracers described by de Grazia et al. *(9)*. One isotope is administered orally to label dietary calcium and the other intravenously to measure calcium removal from the blood. Oral isotopes take longer to enter the plasma pool than intravenous doses, so we give the oral isotope 1–2 h prior to giving the intravenous isotope. The two isotopes track identically after 20 h *(10)* (*see* Fig. 1). Precise timing sequence of the oral and intravenous tracers is not important if total urinary recovery is measured, as is necessary for a single collection timepoint. Ratios of the oral to intravenous tracers can be made in the first 24-h urine sample postdose, or in a single sample of urine or serum after 20 h, although the later may be less accurate *(11)*. When adjusted for dose, this represents fractional calcium absorption as:

Fractional true absorption:

$$\frac{\int_0^t T_{OR}\,dt}{\int_0^t T_{IV}\,dt} \bullet \frac{DOSE_{IV}}{DOSE_{OR}} \cong \frac{T_{OR}}{T_{IV}} \bullet \frac{DOSE_{IV}}{DOSE_{OR}}$$

where T = tracer concentration in serum or urine calcium.

A variation of this method uses a single oral calcium isotopic tracer to label the diet without the intravenous tracer. This method is most reliable when used in a crossover design to compare relative bioavailability, as it is more likely that calcium clearance, a product of body pool size and turnover rate, would be similar between two test phases in the same individual. The most common application of this method is to measure the oral tracer in a single blood draw 5 h postdose. This method has been highly correlated with the double-isotope tracer technique *(12,13)*.

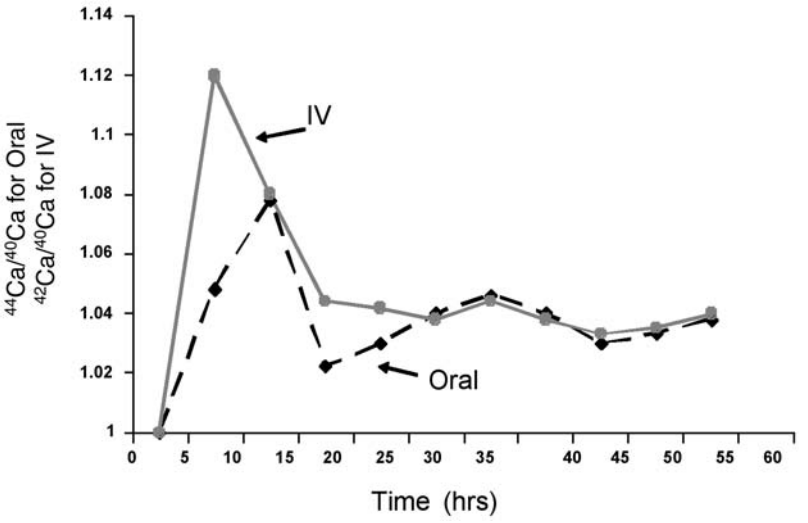

Fig 1. Enrichment of tracer isotopes in urine after a 77-kg male received 13 mg of ^{44}Ca orally and 4 mg of ^{42}Ca intravenously. (Adapted from ref. *10*.)

Using the single tracer method in adults, fractional calcium absorption can be calculated by adjusting for body size area as:

$$FxAbs = (SA_5^{0.92373}) \mu [BSC \mu (H_t^{0.52847}) \mu (W_t^{0.37213})]$$

where Fx Abs = fractional absorption, SA_5 = 5-h serum calcium specific radioactivity, Ht= height in meters, Wt = weight in Kg. BSC is body size correction, and has a value of 0.3537 in women and 0.3845 in men.

This method was used to determine calcium bioavailability of most of the sources reported in Chapter 9. A 5% difference in fractional calcium absorption can usually be detected with 10–15 subjects using a crossover design. Other investigators have used a 1-h *(14)* or 3-h *(15)* blood draw. However, these shorter sample times do not correlate as well with true calcium absorption as does the 5-h timepoint *(12)*. Any single point in time poses the risk of a shifting serum profile, which could alter apparent bioavailability from one test period to the next. Consequently, some investigators prefer to measure total tracer appearance in the urine *(16)* or feces *(17)*. The accuracy of these approaches depends on completeness of collection, unlike single point sampling. A further problem with the 1-h *(14)* approach is the use of a very small test load (e.g., 20 mg). Such small test loads may provide insights on the active absorption component, but do not provide otherwise nutritionally relevant information.

Good agreement has also been reported between the double tracer method and the fecal recovery method from a single isotope *(9)*. When absorption is calculated from unabsorbed tracer appearing in the stools, the diet must be controlled long enough to encompass the transit time of the tracer *(18)*. When tracer appearance in blood or urine is used to monitor fractional calcium absorption, often the tracer is given at breakfast following an overnight fast. Typically, the diet is not controlled except for the breakfast, when blood is collected or for just 1 d when urine is collected *(19)*. A 24-h urine collection may be

sufficient, but when a response delay is expected, as occurs in the presence of nondigestible fiber *(20)*, urine might need to be collected for several days. In this example, a sufficient prefeeding period is also needed to allow microbial gut adaptation.

When determining physiological absorption capacity, important considerations are the size of the calcium load and the chemical form of calcium to be administered. As fractional absorption is inversely related to load, all comparisons should be made using the same load. Frequently, loads of between 100 and 300 mg calcium are tested. Some choose the load equivalent to one-third of the daily intake. When fractional absorption is being compared across experiments, it is better to include a common source as a reference. Radioisotopes typically are purchased as $CaCl_2$. This soluble isotope can be mixed with milk or juice for consumption or converted to another salt. It is not recommended to give pure $CaCl_2$, as it is a stomach irritant. Alternatively, a capsule of a preweighed calcium salt containing the tracer can serve as the oral dose. This is common with stable isotopes of calcium that are purchased as calcium carbonate.

The method chosen to incorporate an isotope into the food being tested for bioavailability deserves thoughtful consideration. Intrinsic labeling techniques (which incorporate isotopes during growth of plants or animals as previously described *[21]* or during the synthesis of a supplement *[22]*), attempt to prepare the label in the same physical and chemical form as the native calcium of the tested source. Extrinsic labeling of calcium sources is simpler and frequently, but not always, allows a good approximation of calcium absorption from intrinsically labeled sources *(23)*. This approach involves premixing a soluble form of the calcium isotope with the food to be tested prior to consumption. It assumes that the tracer has adequately exchanged with endogenous calcium, a point that generally must be validated before proceeding.

Calcium isotopic tracer absorption can also be determined from whole-body ^{47}Ca retention curves. An example of this approach is shown in Fig. 2 of Chapter 12. This is an excellent method for determining calcium absorption, but few laboratories have the capacity to administer ^{47}Ca to humans and to measure subsequent whole-body retention. The use of this special method and exposure to radioactivity from a γ-emitter is better justified for more complicated problems that measuring fractional absorption.

3.3. Serum Profiles of Calcium, Parathyroid Hormone, and Vitamin D Metabolites

Calcium bioavailability from a number of sources has been estimated from areas under the curve (AUC) of profiles of total or ionized serum calcium or its regulators, that is, parathyroid hormone (PTH) or vitamin D metabolites following an oral load after an overnight fast. This approach is useful for determining calcium absorption from preformulated commercial products, where intrinsic labeling cannot be used. Some examples of this approach comparing calcium salts to a blank are shown in Fig. 2. This approach may be more easily achievable for many laboratories in that isotopic tracers are not required. However, the method is crude compared with isotopic tracer approaches. Changes in serum calcium are small, as serum calcium is tightly regulated so that subtle changes would likely be missed. Note that there is only an approx 5% rise in serum calcium on a 500-mg load (Fig. 2A). Similarly, changes in regulators of calcium absorption are difficult to observe (Fig. 2B).

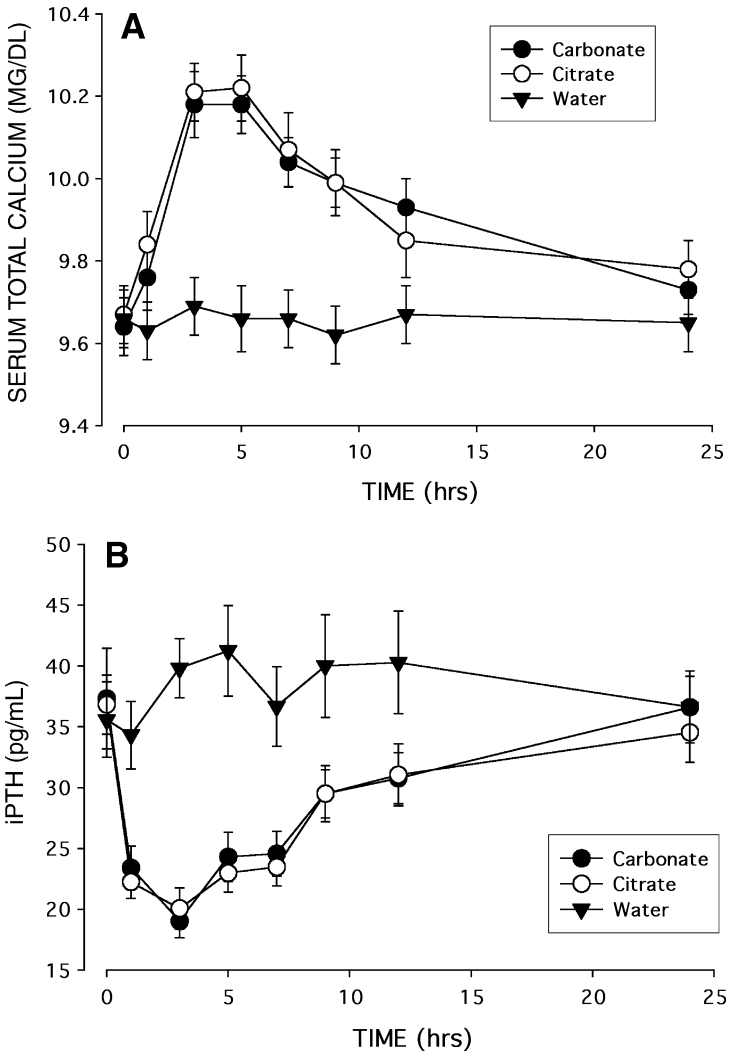

Fig 2. (A) Time course of the total serum calcium, both as absolute values for two calcium sources and for the blank load. Error bars are 1 SEM. **(B)** Time course of serum iPTH following ingestion of two calcium sources and for the blank load, both as absolute values. Error bars are 1 SEM. (Copyright Robert P. Heaney, 2004. Used with permission.)

AUCs have been successfully used to determine responses in large doses, i.e., a 500-mg calcium supplement vs placebo *(24)* (Fig. 2A) or fractional calcium absorption efficiency differences in vitamin D-deficient and -sufficient individuals *(25)*. The acute PTH suppression method *(26)* can be made more sensitive by adapting subjects to a low calcium diet the week before they receive the test meal. Using this approach, AUC for serum PTH was significantly altered for test meals containing 500 mg Ca as milk, $CaCO_3$, or fortified orange juice, but not for serum Ca *(27)*. If serum AUC for any measured variable is used to estimate calcium absorption, it is important to collect data points long enough to avoid misclassification.

3.4. Urinary Calcium

Urinary calcium excretion following a bolus dose has been used to estimate calcium absorption, but this method is plagued with even more uncertainties than serum calcium profiles. As for observing changes in serum AUC (discussed in the previous section), test loads must be large. Following a 500-mg Ca load, the postprandial increase in urinary calcium excretion was only approx 5% of the load over a 5- to 8-h period in elderly men and women *(27)*. Postprandial increments in urinary calcium excretion were approximately twice as variable as for serum calcium in a study comparing calcium carbonate and calcium citrate in postmenopausal women (77–99% vs 38–60% CV, respectively) *(24)*. In addition to reflecting absorbed calcium spilled over into the urine, urinary calcium is influenced by diuretics, the prior day's salt intake, and size and turnover of the exchangeable pool. That makes use of a crossover design and standard conditions especially important for this method.

Urinary calcium can derive from diet or bone, and without tracers the source cannot be distinguished. This may explain why relative calcium absorbability estimated from urinary calcium excretion does not always agree with calcium tracer data. For example, urinary calcium excretion was greater for calcium citrate than for calcium carbonate *(28)*, whereas the calcium tracer approach showed no difference in fractional calcium absorption between these salts *(29)*. Suppressed serum ionized calcium with calcium carbonate suggests a slight alkalosis, which would be expected to reduce urinary calcium excretion compared to calcium citrate *(24)*. The increased urinary calcium from calcium citrate likely comes from bone rather than increased absorption from the diet. Other studies reporting enhanced calcium absorption that have utilized urinary calcium increments, such as for coral calcium *(30)*, must be repeated using isotopic tracer methods.

Use of urinary calcium excretion to estimate calcium absorption would be especially problematic for growing animals and children because of the low correlation between calcium intake and urinary excretion. In adolescent girls, only 6% of the variation in urinary calcium was explained by calcium intake *(31)*.

4. METHODS FOR DETERMINING CALCIUM RETENTION

Cumulative calcium retention can be determined from total body calcium content measured by bone densitometry. This is a reasonable index of calcium status. Shorter term calcium retention studies while subjects are fed controlled diets can be used to quantify factors that can offset calcium retention. Two such methods are metabolic balance and whole-body ^{47}Ca retention.

4.1. Metabolic Balance

Balance studies calculate net retention as intake minus excretion:

$$\text{Calcium retention} = I - (F + U)$$

where I = intake, F = feces, and U = urinary calcium.

Balance studies are sufficiently sensitive, when rigorously controlled, to distinguish differences when large effects are expected as exists when comparing pubertal growth with that of adults *(32)*, lactating state with nonlactating state *(33)* racial differences *(34)*, the effects of skeletal unloading *(35)*, and some diet effects such as calcium intake *(36)*.

The treatment differences in these examples can exceed 200 mg calcium retention per day. Power calculations show that sample sizes of five to six subjects per group are sufficient to find significant differences of this magnitude at an α of 0.05 with 80% power even though the variances were large. The ability to determine smaller effects of diet depends on the magnitude of the effect and the specific population. We have been able to show treatment effects on calcium retention of 40 mg/d with 10–15 adolescent subjects in crossover studies. However, for treatment differences in calcium retention of approx 40 mg/d in postmenopausal women using the variance that we observed in a recent study, power calculations suggest 180 subjects would be needed to show significance using a crossover design.

Balance studies can also be used to determine calcium requirements as the response of calcium retention to calcium intake reaches a plateau when calcium intake is no longer limiting maximal calcium retention, that is, bone accrual (*see* Chapter 7). The errors associated with measuring balance are not symmetrically distributed. Errors associated with incomplete consumption of the diet or collection of urine and fecal excretion and failure to measure other, including cutaneous, losses are often cited limitations, because they often bias results toward more positive retention values. However, useful information about the role of other dietary factors or lifestyle choices in shifting the location of the inflection point of the maximal retention curve (which, thus, shifts calcium requirements higher or lower) can be determined. This application has the advantage of not depending on actual values of calcium retention. The maximal retention approach seeks the intake where a plateau occurs rather than focusing on absolute retention. Finally, analytical procedures typically have a CV of 5% or more.

Metabolic balance studies involve feeding a controlled diet, collecting excreta, and measuring calcium input and output. Intake cannot be estimated from food composition tables. All foods and beverages containing calcium must be prepared by weighing ingredients to the nearest 0.1 g. Prepared commercial foods can be used if their composition is homogeneous. Duplicate collections of all of the foods and beverages consumed in a 24-h period are analyzed for calcium and other constituents that influence calcium balance, including protein, phosphorus, fiber, and electrolytes. Diets should be designed to be constant in these constituents throughout the study period. Foods, beverages, and oral health care products, including tap water, which contain calcium, inhibitors of calcium absorption such as tea, which contains oxalate, or hypercalciuric ingredients such as salt cannot be allowed to vary from day to day.

If the metabolic study is not conducted in all subjects simultaneously, but rather as a rolling enrollment, it may not be practical to analyze a duplicate sample of each day. In that case, dietary composites representing each cycle day from a dietary intervention should be prepared in intervals throughout a study period to track the variability that occurs over time and the variability between the daily diets. Dietary composites should be measured for calcium and those nutrients that could potentially affect calcium metabolism, e.g., protein, sodium, potassium, and phosphorus. Dietary homogenates representing each day of the menu cycle, for example, 7 d for a 7-d menu cycle, should be freeze-dried and aliquoted in triplicate for all nutrient analysis. Variation among these triplicate samples is an indication of homogeneity in the sample and analytical precision. Replicate analysis of dietary composites prepared over the entire study period demonstrates variation owing to variability in food items, dietary preparation, and laboratory analysis. Analysis across cycle menus represents daily variability within the diet.

Analysis of dietary composites from a metabolic balance study in our laboratory, which used a 7-d menu cycle over a 19-mo period, demonstrated 3% variation in calcium from triplicate analysis of dietary samples. The variation in calcium from the replicate analysis of dietary composites, which were collected at quarterly intervals over the 19-mo study period, was 5%. Daily variation in calcium across the 7-d cycle menus was 6%.

Urine and feces are collected in acid-washed containers for later analysis of total calcium by atomic absorption or ICPMS. In adolescents, a 1-d lag is used when calculating intake minus fecal excretion to account for the approx 19-h transit time in the gut. (That transit time can rise to 11–12 d in mature or older adults.) Menstrual losses of calcium can generally be ignored. Cutaneous losses are often ignored but can be measured by extracting acid-washed clothing worn for 24 h in addition to whole-body washdown procedures before and after the collection. Using this method, we have determined cutaneous losses of approx 52 mg/d in adolescents. Cutaneous losses determined by patches overestimated calcium losses by almost eightfold *(37)*. These approaches do not measure losses from hair and nails. Cutaneous losses in adults have been estimated to be 60 mg/d by the difference between whole-body retention of ^{47}Ca and excretion in urine and feces *(38)*.

To determine the effect of a variable on calcium retention within a population, the best approach is the use of a crossover design in the same subjects to minimize confounding effects that are constant within an individual, such as hormonal status, gastrointestinal and kidney function, mucosal mass, transit time, and vitamin D status. Randomized-order assignments of treatment can minimize seasonal effects of vitamin D status. Nevertheless, the presence or absence of an order effect should be tested statistically. Subjects can also be pretreated with vitamin D supplements before and throughout the study period to ensure normal vitamin D status.

The length of the run-in period needed for a subject to adapt to the study diet and the length of the balance period once steady state is reached must be carefully considered. Misinterpretations have occurred when subjects have been switched from high to low calcium intake periods without an appropriate adaptation period, as the higher calcium intakes spill into the feces during the lower calcium period for several days. When a nonabsorbable fecal marker such as polyethylene glycol (PEG) is given at every meal, the fecal calcium:PEG ratio can be used to determine when steady state is achieved. We have found that the Ca:PEG ratio becomes constant after 6 d in adolescents and most adults (Table 2, adapted from ref. *39* as well as unpublished data). Similarly, when adult black and white women were switched from a diet containing 2000 mg/d for 3 wk to 300 mg/d for 8 wk, whole-body retention of ^{47}Ca varied from week 1 to week 2, but not from week 2 to week 8 *(40)*. Thus, determining balance during the run-in period can give useful information about when steady state is achieved in calcium balance or another dietary constituent being tested for its effect on calcium retention. Subjects cannot adapt to a low calcium intake, that is, cannot come into balance, as the homeostatic control mechanisms is inefficient. Malm *(41)* studied prisoners for up to 2 yr and found continued negative calcium balances on low intakes.

Balance periods should be sufficiently long to evaluate trends over multiple periods. Some investigators make collections in several day pools and monitor multiple periods. We typically make collections in 24-h periods for 2 to 3 wk after a 1-wk adaptation period. Calculating daily balances for multiple periods allows an error term to be determined so that differences from zero balance can be determined for each individual. In pubertal

Table 2
Fecal Calcium:Polyethylene Glycol Ratios (mg/mg) During a 3-wk Balance Period*

Group	*Calcium Intake (mg/d)*	*Week 1*	*Week 2*	*Week 3*
Adolescent girls				
	800	0.25 ± 0.13^a	0.21 ± 0.06^b	0.19 ± 0.04^b
	1300	0.82 ± 2.20^a	$0.32 \pm 0.06b$	0.38 ± 0.72^b
	1800	0.53 ± 0.08^a	0.48 ± 0.09^b	0.48 ± 0.07^b
Adults				
	1300	1.49 ± 5.20^a	0.36 ± 0.09^b	0.36 ± 0.08^b

*Different letter superscripts within rows indicate means are significantly different for each level of calcium intake at $p < 0.05$. (Data from ref. *39* and unpublished data.)

children, balance periods should not exceed rapid hormonal shifts, which can outweigh the influence of diet on calcium retention *(42)*.

Methods to assess compliance of urine and fecal collections and to adjust for discrete 24-h periods are helpful in reducing variation in balance data and in interpreting the quality of data. However, errors can be made in measuring compliance markers so that corrected data may be less accurate than uncorrected data for any given day. Thus, all components of any calculation should be carefully inspected. Especially troublesome is the apparent overcorrection of fecal calcium when using a marker to adjust for low compliance.

Adjustment of urine is usually made with creatinine. Subjects excrete a rather constant level of creatinine proportional to lean body mass. The mean daily creatinine excretion of a subject over the study period can be used to adjust each day to a more precise 24-h period, as it is difficult to completely empty one's bladder at precise regular time periods, especially for children. Twenty-four-hour pools with creatinine values less than 11 mg/kg should be discarded as substantially incomplete (except, perhaps, in emaciated individuals).

Daily fecal calcium output is highly variable despite constant conditions, as a result of variable gut transit times that do not segregate into discrete periods and because calcium flows forward and backward in the intestine *(43)*. A number of nonabsorbable fecal markers have been employed to evaluate compliance and transit time, and to convert individual stools collected at irregular intervals to daily fecal calcium output. We use PEG 4000 as a continuously administered, nonabsorbable marker as developed by Wilkinson *(44)*, who demonstrated clearly a reduction in daily variation by correcting stool samples by recovery of this marker. Capsules are prepared containing PEG weighed to the nearest mg and consumed at each meal throughout the study. The ratio of Ca to PEG in each 24-h pool is multiplied by the amount of PEG consumed during 24 h in order to determine daily fecal calcium. PEG excretion can also be used to estimate fecal lag (or intestinal transit) time. In the first few days after starting a PEG-labeled balance study, fecal PEG may be negligible, reflecting the fact that current fecal collections reflect prestudy diet residue. The time required for PEG excretion to approximate PEG input is the fecal lag time. The week 1 data in Table 2 show the effect of fecal lag clearly.

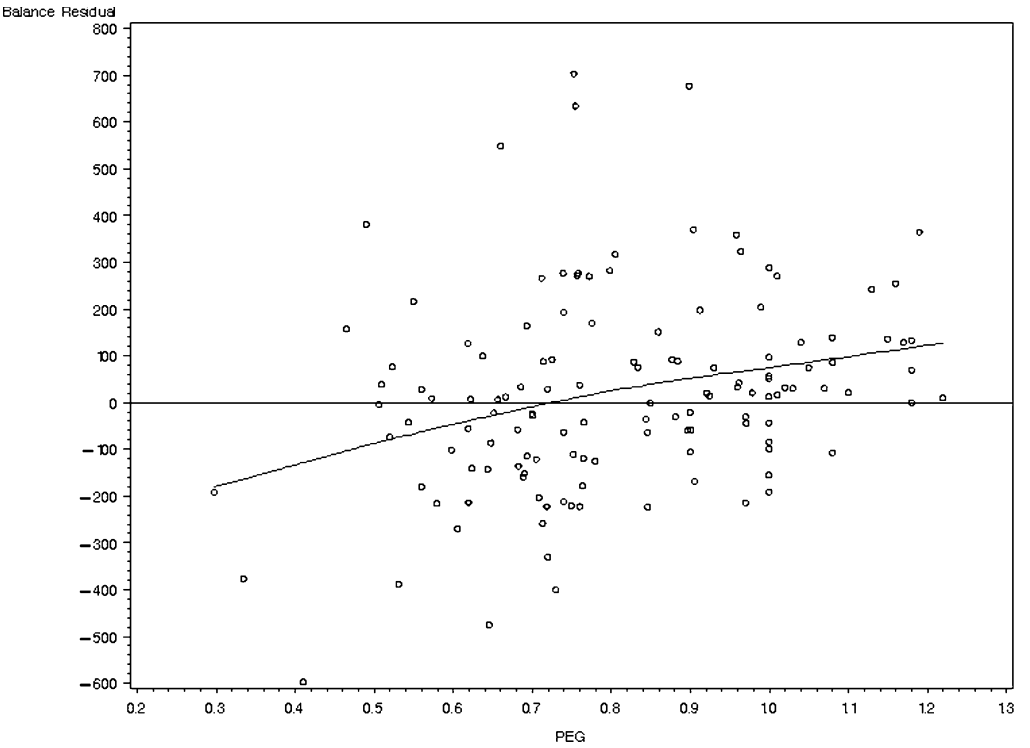

Fig 3. Calcium balance residuals vs fractional polyethylene glycol recovery in one study of postmenopausal women *(46)*.

The week 1 fecal collections were dominated by prestudy food (prevailing calcium plus zero PEG). It is important to measure fecal lag when performing kinetic studies (*see* Chapter 6), in order to time the fecal recovery of the intravenous tracer correctly.

Dissolved minerals and minerals as part of particulates do not move through the gut at the same rate. For water-soluble dietary constituents such as calcium, PEG is superior to previously used markers which more closely match the insoluble materials, such as Cr_2O_3 and barium sulphate, although recovery of all three markers was 98–100% *(44)*. It should be noted that PEG analysis is a tedious and difficult method.

Adjusting fecal calcium as described above supposedly corrects for incomplete stool collections. However, Eastell et al. *(45)* reported a PEG recovery of only 81% compared with 95% using ^{51}Cr in the same experiment. Therefore, we suspect that adjusting fecal calcium with PEG may overcorrect, which becomes worse with decreasing compliance. To examine this issue, we used data from one of our studies in which we calculated calcium balance using the PEG adjustment. Each group was studied three times, and we computed residuals for calcium balance by subtracting the group mean from each individual observation. A plot of these residuals vs PEG is given in Fig. 3. The plot includes a center line at zero (the mean of the residuals) as well as a smooth fit to the data. There appears to be a positive association between the PEG value and the residual. This means that observations with low values of PEG tend to be associated with balance values that are low relative to the group mean and, similarly, high PEG values are associated with

balance values that are high relative to the group mean. This association is consistent with a scenario in which the PEG overcorrects the fecal calcium values: when the PEG is low, the corrected fecal values are too high and, therefore, the balance values are too low. The effect of compliancy on treatment effect can be determined by examining the F statistic when data are evaluated by using various cutoffs for % PEG recovery as inclusion criteria.

4.2. Whole-Body ^{47}Ca Retention

Whole-body retention of the γ-emitter ^{47}Ca can be determined with a precision of 2.6% of the administered dose *(47)*. With doses of 1–4 μCi ^{47}Ca, whole-body retention has been followed for 1 wk (*38*; Chapter 24, Fig. 2, this volume) to 4 wk (*48*; Chapter 12, Fig. 2, this volume).

Whole-body ^{47}Ca retention is more precise than retention determined by metabolic balance. However, it lacks the ability to determine the mechanism of the impact of an intervention (i.e., gut, kidney, or bone). Mechanisms are best understood by kinetic modeling (*see* Chapter 6).

5. RANDOMIZED, CONTROLLED TRIALS

An RCT is a strong study design for determining the relationship between calcium supplementation and a health outcome measure. Typically, subjects are randomized to the test calcium source or placebo. Ideally, the RCT is double-blinded to the subject and researcher. This requires the placebo to be indistinguishable from the calcium source. This is not always possible—for example, when the trial is milk or another source for which there is no feasible placebo.

The length of the trial and number of subjects depends on the outcome measures of interest. For changes in bone density, power calculations typically show that to detect a mean change of 0.7 to 1.1% or a group difference of 1.0 to1.5% in bone mineral density of the spine or total hip in adults, 50 subjects per group is necessary for 80% power. Shorter time periods may be acceptable in growing children. For other outcome measures such as insulin resistance or body weight changes, a few weeks may be satisfactory (*see* Chapters 20 and 26).

RCTs can directly assess response to changes in diet that might be confounded in epidemiological approaches. A classic example of this was recently reported *(49)*. Cross-sectional analysis of the relationship between dairy calcium intake and total hip bone mineral density showed a positive relationship for elderly men, but not women. Others have also shown no relationship between calcium intake and bone measures in postmenopausal women *(50)*. However, an RCT of 750 Ca/d in the same individuals showed a similar positive response in men and women *(51)*. Perhaps women's self-reports underestimated dietary calcium intakes more than did men's. Regardless, this shows the need to confirm hypotheses generated by epidemiological approaches with controlled feeding experiments or RCTs.

6. CONCLUSIONS

Controlled feeding studies are important to establish cause-and-effect relationships between calcium intake and health outcome measures. Studies of fractional calcium absorption also are used to determine bioavailability of calcium from various food sources. Calcium retention studies are useful for setting calcium requirements.

Accurate information depends on careful consideration of study design and measurement methods. Mechanistic information can be obtained with controlled diets and isotopic tracers. This requires a dedicated laboratory and special attention to ethical issues. As we learn more about health outcomes that are related to calcium intakes, the nature of clinical studies expands.

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6 Kinetic Studies

Meryl E. Wastney, Yongdong Zhao, and Connie M. Weaver

KEY POINTS

- Kinetic studies employing tracers can be used to calculate rates of calcium metabolism at sites not accessible for direct measurement.
- The design of a tracer study must take into account the question being addressed, because this will influence the tracer dose, sampling sites, and length of the study.
- Different mathematical approaches can be used to analyze the data. Compartmental analysis uses pools and pathways that are analogous to physiological processes and can therefore be used to investigate sites where metabolism changes during different nutritional or disease states.
- Short-term kinetic studies (days–weeks) provide a snapshot on calcium and bone metabolism at one point of time. Multiple kinetic studies over the course of a therapeutic intervention can tell us the continuing effect on calcium metabolism.
- With increased computing power, and dedicated modeling software, we can now measure dynamic properties of calcium metabolism to increase our understanding of calcium homeostasis.

1. INTRODUCTION

Kinetic studies have been used to calculate changes in rates of calcium absorption, excretion, and bone turnover in children, adolescents, and adults. The studies have shown how absorption and bone turnover change with intake. For example, adolescents absorb more calcium on higher calcium intakes, sparing bone resorption. Different approaches to analyzing kinetic data have been compared, and in the future, linking kinetics with biochemical and endocrine indices may be necessary in order to understand dynamic changes in calcium homeostasis during health and in disease.

2. TRACER STUDIES: WHAT THEY CAN TELL US

For many in vivo studies, it is not possible to access pathways of metabolic interest, so we need to rely on indirect measures. Tracers provide such a window into metabolism. By literally "tracing" an element or compound and applying mathematical techniques,

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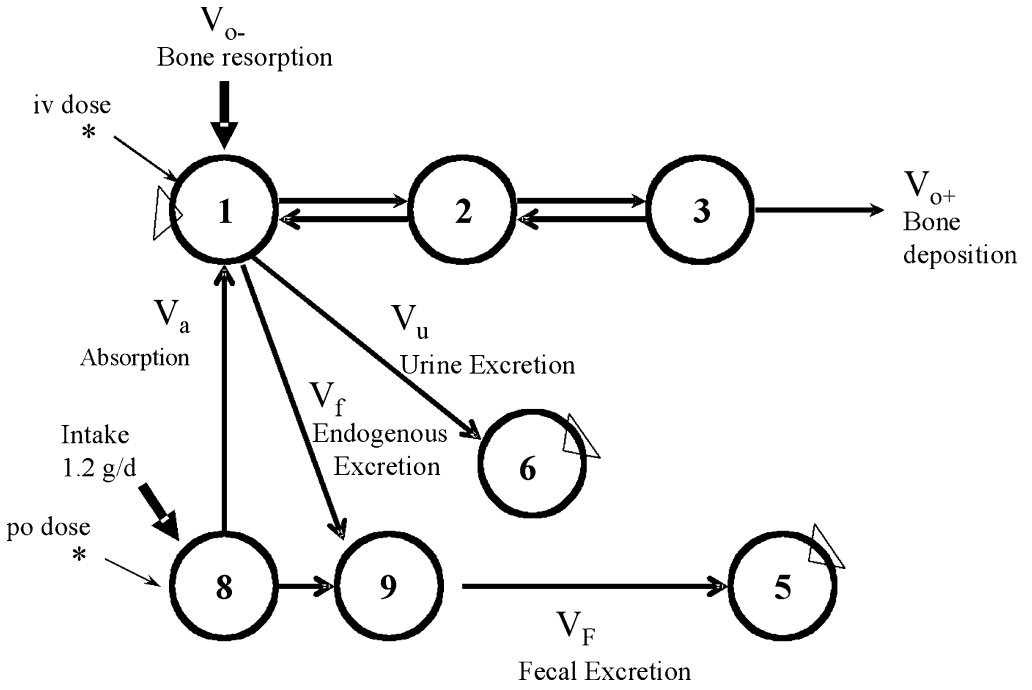

Fig.1. Model for calcium metabolism. Circles represent compartments; numbers in circles represent compartment number; arrows represent movement between compartments; thick arrows represent entry of calcium via the diet, or bone resorption (Vo-). Asterisks indicate entry of tracer and triangles identify sampled compartments. Compartment 1 contains blood, compartment 2, soft tissue, and compartment 3, exchangeable calcium on bone. (Adapted from Wastney et al., 1996 *[1]*.)

we can deduce how the compound moves through the body without actual direct measurements (Fig. 1).

Examples of body processes not susceptible to direct measurement, but which readily yield their secrets to kinetics methods, include measurement of rates of calcium deposition in and removal from bone, rate of entry of calcium into the gut through digestive secretions, exchange rates between cellular and extracellular calcium, and many other, similar variables.

Use of tracers implies making assumptions, an important one being that the tracer exactly follows the path of the compound being traced, while itself not perturbing the system.

3. EXPERIMENTAL CONSIDERATIONS

The adage that you "can't get something for nothing" certainly applies to tracer kinetics. The tissues sampled, the length of the study, and the frequency of sampling will determine the type of information that can be obtained from a study. The more data collected, the more information obtained and the more reliable the results. It is sometimes said that one well-designed kinetic study is more valuable than a large number of studies with limited data per subject. That is because a large amount of data, collected from

different sites, on one (representative) subject provides a more comprehensive view of the system working as an integrated whole, and ensures that the calculated model parameter values are well-determined, with low associated error.

3.1. Type, Dose, and Site of Tracer Administration

The type of isotope selected is usually determined by the cost of the isotope and the analytical capability for detection of the tracer in samples. Some of these issues are discussed in more detail in Wastney et al. *(2)*. Tracers of calcium may be radioactive (e.g., ^{41}Ca, ^{45}Ca, ^{47}Ca) or stable (^{42}Ca, ^{43}Ca, ^{44}Ca, ^{46}Ca, ^{48}Ca) (*see* Chapter 5). The dose administered must be small, so the added tracer does not contribute sufficient mass to perturb the system. Multiple doses can be given if the system is to be traced for a long period, or an isotope such as ^{41}Ca (which can be measured with high sensitivity) can be used to trace bone resorption for years.

The questions to be addressed influence the site of tracer administration. If the interest is absorption, doses are given orally, whereas if the interest is bone turnover, intravenous (iv) administration is preferable because it circumvents absorptive variability. Often, two tracers are administered, one orally and one intravenously. This enables both absorption and bone turnover to be determined simultaneously, assuming, as always, that both isotopes, once in the system, are handled in the same way.

3.2. Tissues Sampled

Generally, serum, urine, and feces are sampled, although saliva can also be useful in representing serum, because the calcium tracer in saliva reflects that of serum *(4)*. With γ-emitting tracers, such as ^{47}Ca, even whole-body regions, such as an arm or a leg, can be measured.

3.3. Length of Study

When kinetic modeling is used to investigate bone turnover, the length of a study may influence the results. For adolescents, sampling for 14 d vs 7 d provided more reliable estimates of bone turnover, whereas continuing sampling for 21 d did not provide more information than 14 d (Fig. 2 and Table 1) *(3)*. However, for adults, in whom bone turnover is slower, it was determined that studies of 20 d were necessary to define rate of bone resorption *(5)*. On the other hand, sampling beyond 7 d did not improve calcium absorption measures (Table 1) *(3)*.

When a tracer is used to follow the changes in bone resorption due to therapeutic interventions, kinetic studies can be extended for years with the long-lived ^{41}Ca and sensitive detection using accelerator mass spectrometry (AMS) (*see* Table 1 in Chapter 5).

3.4. Frequency of Sampling

The sampling frequency depends on the purpose of the study. The serum tracer concentration drops very rapidly during the few hours following an intravenous introduction of a tracer, which makes early collection time points important in determining initial compartment sizes. On the other hand, after an oral dose, serum tracer concentration usually reaches the peak around 5 h postdose, which has been proven to be the most relevant time for sampling to estimate calcium absorption. Thus, serum collection has to be at least 5 h long in most calcium absorption studies. When bone turnover is the primary

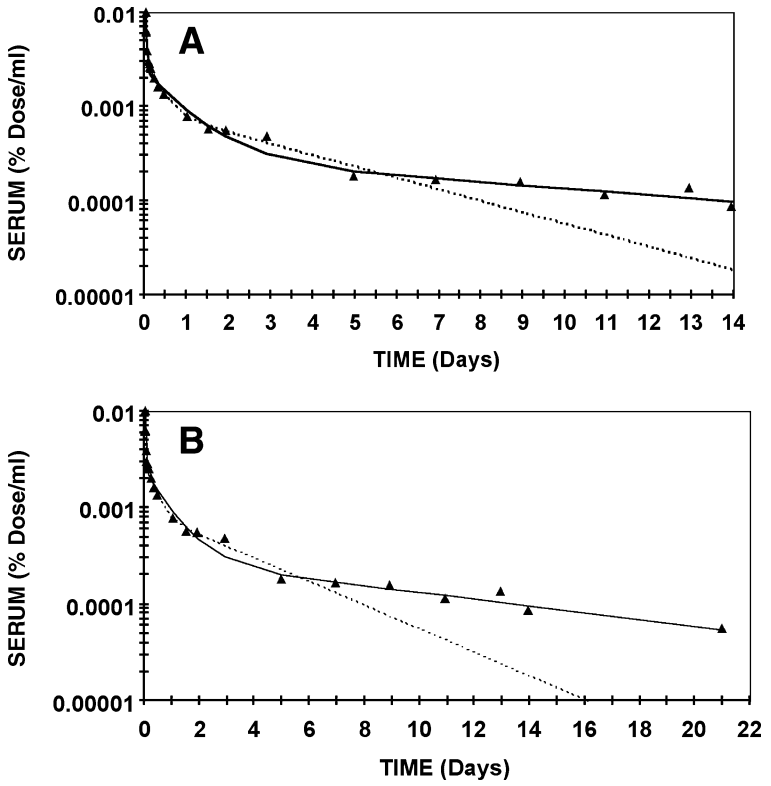

Fig. 2. The effects of length of study on serum disappearance curves of intravenous stable calcium isotopic tracer in an adolescent girl. Symbols are observed data, lines are values calculated by the model shown in Fig. 1 for 7 d (dotted line) compared with **(A)** data collected for 14 d and **(B)** 21 d. (From Weaver et al., 2003 *[3]*.)

Table 1
Results of 7-, 14-, and 21-d Study in Teenaged Girl (1300 mg Ca/d intake)

	7 d	*14 d*	*21 d*
L(0,3) fract/day	0.355	0.090	0.085
Absorption (%)	52	49	49
Vo^+ (mg/d)	2282	1583	1557
Vo^- (mg/d)	2273	1472	1447
Balance (mg/d)	9	111	110
V_u (mg/d)	113	113	113

Parameters refer to the model in Fig 1. L(0,3) is the fraction of the calcium in compartment 3 (Fig. 1) that is incorporated into bone each day. Vo^+ is bone formation rate; Vo^- is bone resorption rate; V_u is urinary calcium excretion rate.

Table 2
Informational Content of Serum Samples After Tracer Administration as Calculated by WinSAAM

IV					*Oral*				
Sample priority	*Information content*	*Sample time*			*Sample priority*	*Information content*	*Sample time*		
		Days	*Hours*	*Min*			*Days*	*Hours*	*Min*
1	0.455	14	336	20160	*1*	0.954	0.125	3	180
2	0.230	3	72	4320	*2*	0.471	0.042	1	60
3	0.226	1	24	1440	*3*	0.162	3	72	4320
4	0.214	13	312	18720	*4*	0.160	0.045	1.08	65
5	0.198	11	264	15840	*5*	0.157	0.049	1.17	70
6	0.183	5	120	7200	*6*	0.154	1	24	1440
7	0.154	1.5	36	2160	*7*	0.134	0.055	1.33	80
8	0.142	9	216	12960	*8*	0.067	2	48	2880
9	0.128	7	168	10080	*9*	0.066	5	120	7200
10	0.126	2	48	2880	*10*	0.065	1.5	36	2160
11	0.121	0.417	10	600	*11*	0.061	0.417	10	600
12	0.100	0.333	8	480	*12*	0.029	0.333	8	480
13	0.073	0.146	3.5	210	*13*	0.018	0.250	6	360
14	0.070	0.208	5	300	*14*	0.011	0.208	5	300
15	0.064	0.250	6	360	*15*	0.009	0.083	2	120
16	0.062	0.167	4	240	*16*	0.005	0.167	4	240
17	0.058	0.104	2.5	150	*17*	0.004	0.146	3.5	210
18	0.056	0.125	3	180	*18*	0.004	0.104	2.5	150
19	0.046	0.083	2	120					
20	0.034	0.055	1.33	80					
21	0.020	0.049	1.17	70					
22	0.007	0.045	1.08	65					

Information content is a relative value (with no units).

focus of the study, serum collection points after the first week become critical, as shown in Table 1. Experience provides an empirical basis for decisions regarding collection points. However, once a model is established with preliminary data, there are formal ways to quantify the timepoints at which it is more important to satisfy the research purpose *(6)*. One of these approaches is informational analysis (using the INFO command) in WinSAAM *(6)*. An example is shown in Table 2. Parameter values for young women *(1)* were used to determine the information content of serum samples taken following iv or oral tracer administration. The program determines the relative contribution of each sample. In Table 2, these values have been prioritised according to their informational content. Because sampling was extended over a wide time range, the times are shown as days, hours, and minutes after tracer administration for clarity.

The prioritization of samples according to informational content differed depending on whether the tracer was administered intravenously or orally, i.e., whether the research purpose related to bone or to absorption. It can be seen that following iv dosing, samples with the highest information are those taken 1–3 d and 11–14 d after dosing; whereas after

oral dosing, the samples with the most information are those taken within 1–3 h. Using this approach, an investigator can design a sampling schedule to maximize the information obtained from a study.

Although it is apparent that, in measuring absorption, samples taken during actual absorption will be more useful than samples taken days later, and that in measuring bone mineralization, it would be best to allow sufficient time for tracer to exchange with the various exchangeable compartments, these facts can prove invaluable to the investigator for determining which points within the respective time windows are most useful. For absorption, is a 3-h value better than a 5-h value? For bone mineralization, how long should a study go? When is the earliest timepoint that provides useful information? And so forth.

4. DATA ANALYSIS AND MODELING

Modeling is the process of representing pathways of metabolism by mathematical equations. A number of studies have been analyzed using kinetic models with time-invariant rate constants. The review by Heaney *(8)* describing principles of tracer kinetics is still highly relevant. What has changed is the software and computing power available to analyze kinetic data *(9)*.

Both compartmental and noncompartmental approaches have been applied, with the power-function the most common of the latter category. By contrast, compartmental models are expressed as a series of differential equations. Comparison of compartmental vs noncompartmental analysis has been reported by Jung et al. *(5)*. The noncompartmental approach does not provide insight into the underlying biological processes of calcium deposition into bone *(8)*. The compartmental approach has evolved, based on more extensive sampling, from a single compartment up to five compartments *(10)*. The differences among and pitfalls of these models have been extensively discussed elsewhere *(8)*. The description of an approach for analyzing calcium kinetic data by compartmental analysis using WinSAAM is provided in Wastney et al. *(2)*.

4.1. Absorption

Methods for measuring calcium absorption have been discussed in Chapter 5. Here, we will elaborate on tracer-based methods. Each of the methods for determining calcium absorption has limitations and involves assumptions, but generally with more data it becomes possible to test the assumptions. Fecal recovery of an oral tracer is the least invasive method. However, this method can only determine apparent calcium absorption, not true calcium absorption. Fecal tracer consists both of unabsorbed tracer and tracer absorbed and then excreted in the digestive secretions. Unless the endogenous component of the total fecal tracer can be measured (usually requiring a different tracer, given intravenously), true absorption will be underestimated. By contrast, the double-tracer method can determine true absorption, but it requires two tracers, and an intravenous injection.

In the double-tracer method, the ratio of the two tracers at 24 or 48 h postdose is used to estimate the absorption fraction, because the iv tracer parallels the oral tracer closely after 20 h. In an outpatient setting, a much easier method is the single-tracer, single-sample, 5-h specific activity method, developed and validated in women by Heaney and Recker *(11)*. Following a practical calcium load (e.g., approximately one-third of daily

calcium intake), serum tracer specific activity peaks around 5 h postdose. This value, after adjustment for height and weight, explains 93% of the variance of calcium absorption measured by the double-tracer method. An even more accurate, but resource-demanding, method uses kinetic modeling with a series of serum, urine, and fecal samples postdose. Serum profiles of iv and oral tracers can be integrated to determine true calcium absorption.

The research purpose dictates the best method. The various purposes for measuring absorption include: (1) determining relative bioavailability of foods and supplements; (2) measuring active calcium absorption to identify malabsorbers or to study mechanisms; and (3) characterizing absorption as a component of calcium metabolism. If comparative bioavailability is the goal, reproducibility of the absorption test within subjects is more critical than the accuracy of the method. In contrast, when the vitamin D-mediated, active component of calcium absorption is the desired information, a small calcium dose is preferable so as not to saturate the first order kinetics of the absorptive apparatus. In this type of assessment, early serum samples (i.e., 1-h or 3-h) must be taken rather than a 5-h serum sample because, with a small load, calcium absorption would have finished by 3-h. But results using this method do not correlate well with *total* calcium absorption. Finally, when calcium absorption is assessed as one of the principal components of whole-person calcium metabolism, as for comparing treatments or defining different populations, or when actual quantities absorbed are needed (as in nutrition studies), absolute values are critically important.

Several approaches to determining calcium absorption have been compared from data collected in a double-tracer study in which serum samples were collected from 1 h postdose up to 12 h on 23 subjects *(12)*. Serum data were fitted to calculate calcium absorption by deconvolution, a type of kinetic modeling. A 24-h urine and a spot urine were also collected and used to calculate calcium absorption. Yergey et al. *(12)* found no difference between absorption determined from the ratio of tracers excreted in urine after 24 h and deconvolution, whereas the value determined from a spot urine sample differed significantly. We have compared values determined from our own data sets for young women (Table 3). Calcium absorption was estimated by determining the ratio of oral and iv tracers in urine collections, oral tracer in 5-h serum sample, and kinetic analysis by compartmental modeling *(1)*. The estimate of absorption from the double-isotope ratio in urine collections increased with the length of collection period, with the increase being larger in some subjects than in others (e.g., Subject Xb vs Xa, Table 3). This is a result of differing kinetics between subjects. The 5-h serum value was not different from the values determined from 24- or 48-h urine samples. The absorption determined from the breakfast test meal (44%) was higher than from dietary calcium (22%, not shown in Table 3) because the latter reflects the effect of different bioavailabilities of the various calcium sources making up a mixed diet as compared with a sole calcium source (usually a drink or tablet) in a test meal *(1)*.

4.2. Bone Deposition and Bone Resorption

With tracer kinetics, bone deposition is determined from the final slope of the exponential curve, which is influenced mainly by movement of calcium in and out of bone. If we can assume that the system is in steady state (pool sizes, apart from bone, remain constant), we can multiply the fractional loss by the mass of the slowest exchangeable

Table 3
Values Calculated for Absorption Following Tracer Administration

	Fractional Ca Absorption				
	Double isotope (urine)			*Oral isotope specific activity (serum)*	*Kinetics (serum, urine, feces)*
Subject	*24-h*	*48-h*	*14-d*	*5-h*	*Model from 14-d*
Xa	0.255	0.269	0.257	0.252	0.481
Xb	0.106	0.178	0.335	0.244	0.447
Xd	0.193	0.201	0.291	0.255	0.364
Xe	0.236	0.247	0.364	0.274	0.456
Xf	0.313	0.338	0.429	0.260	0.486
Xg	0.285	0.296	0.309	0.261	0.431
Xh	0.319	0.427	0.355	0.291	0.588
Xi	0.255	0.263	0.321	0.243	0.355
Xj	0.241	0.295	0.350	0.247	0.374
Xk	0.290	0.344	0.388	0.276	0.385
Xl	0.183	0.174	0.203	0.241	0.452
Average	0.243[a]	0.276[b]	0.327[c]	0.259[ab]	0.438[d]
Std Dev.	0.063	0.077	0.062	0.016	0.068

Paired *t*-test was used for statistics. Different letters indicate significant differences ($p < 0.05$).

pool to determine the rate of bone deposition termed "A" (for accretion) by Heaney *(8)*, or "Vo^{+}" in SAAM. Bone resorption is the rate of calcium release from bone termed "R" or "Vo^{-}", and it represents the calcium required to enter blood from bone to maintain a constant pool size.

Most calcium tracers can only be followed for days or weeks, either because, if radioactive, they decay, or, if stable, they cannot be given in large enough quantity without perturbing the system. Thus, multiple times of introducing tracers are required for studying bone resorption over a long period. In contrast, ^{41}Ca can be tracked for years mainly because of the sensitivity of its detection using AMS. From ^{41}Ca studies, urinary ^{41}Ca specific activity decreases by a single exponential after approx 100 d *(13)*. This is considered to reflect skeletal calcium loss. In addition to acting as a clinically useful tool for assessing anti-resorptive therapy, ^{41}Ca, after prolonged periods, may also provide useful insights into bone metabolism, such as the sizes and interactions of intraskeletal compartments.

Changes in bone resorption and deposition from short-term studies may not accurately reflect long-term changes in bone balance. Because bone is constantly being remodeled (areas of bone are resorbed and then replaced with new bone), any intervention that slows down remodeling will first appear as a decrease in resorption. There is a delay before deposition will slow to match the reduced resorption rate. Heaney *(14)* has described this phenomenon as the "remodeling transient." Kinetic studies are essential for defining, delimiting, and characterizing the processes that underlie this transient. In studies that do not involve a treatment intervention, short-term kinetic studies may accurately predict long term changes in bone turnover and balance because the remodeling transient is not

present. Heaney *(14)* cites studies in which conclusions on the magnitude of the effects of treatment (estrogen) on bone loss would differ if the study had ceased after 1 yr vs 2 yr vs 3 yr, even though any of those time points would have demonstrated the overall protective effect of estrogen on bone. Short-term kinetic studies at several time points could have characterized these dynamic changes.

4.3. Excretion: Urinary, Fecal, and Endogenous

Urinary and fecal excretion of calcium can be measured. Endogenous excretion, by contrast, can only be determined from the amount of iv tracer excreted in feces, adjusted for the time delay in fecal calcium excretion. The appearance of iv tracer in feces is a function of tracer concentration in serum calcium, and this in turn is determined by how rapidly serum calcium turns over. Therefore, either a compartmental approach that accounts for fecal, urine, and serum data simultaneously *(15)*, or calculation of the integral of serum- or urine-specific activity values *(16,17)* must be used. In any event, adjusting for fecal lag time so as to match the fecal collection to the corresponding serum interval that governs tracer appearance in feces is also necessary. In compartment modeling, this is achieved by adding a fecal delay compartment.

4.4. Retention

Retention, the difference between calcium absorbed and calcium excreted, can be determined by balance studies or by the difference between bone deposition and bone resorption from kinetic analysis. The value obtained for retention from kinetic studies is underestimated from shorter kinetic studies (7 d vs 14 and 21 d) (Table 1)

5. USING MODELS TO EXPLORE METABOLISM: RELATIONSHIP OF KINETICS TO OTHER MEASURES OF CALCIUM METABOLISM

Models can be used to explore metabolism by comparing kinetics in a healthy (or treated condition) with those in a disease (or untreated state) *(18)*.

The degree to which kinetic parameters relate to other measures of calcium metabolism has been investigated in a number of studies. Lauffenburger et al. *(19)* compared histomorphometry, kinetics, and biochemical parameters in patients with either low (osteoporosis) or high (Paget's disease) bone turnover. They found high correlation between the results of the different approaches *(19)*. O'Brien et al. *(20)* studied differences between generations of females in families with or without histories of osteoporosis using compartmental modeling analysis. Bone turnover rates were determined from stable calcium isotopic tracer kinetics. Although exact values for bone resorption cannot be determined with confidence in this study, which did not control diet or collect feces, bone formation rates can be estimated from serum tracer profiles. Bone turnover increased more in families with a history of osteoporosis in response to higher calcium intakes than in healthy families. This is an example of the kind of question that can be addressed with tracer kinetics.

6. CONCLUSIONS: THE NEXT GENERATION OF MODELS

Calcium kinetic models will be expanded in the future to represent dynamics, and the homeostatic mechanisms *(21)*. This means linking models for parathyroid hormone,

vitamin D, and other calcitropic hormones to calcium metabolism. Several dynamic models have been proposed for humans *(22,23)*. Most have been theoretical and compared only with limited, if any, data. With the additional computing power, more powerful software packages and accumulated data now available for calcium and bone metabolism, there is a need for a dynamic model to integrate knowledge on calcium regulation. Results from kinetic studies could be combined with data from balance studies, bone scans, biomarkers, biochemical indices, and hormonal regulators of calcium to aid our understanding of the temporal and quantitative relationships.

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7 Requirements for What Endpoint?

Robert P. Heaney and Connie M. Weaver

KEY POINTS

- The official calcium intake requirement is pegged to a bony endpoint.
- Adequate calcium intake supports many health outcomes in addition to bone.
- For some ethnic groups and for some life stages in all groups, optimal calcium intake may relate to nonskeletal endpoints.
- Hence, current recommendations, although generally satisfactory for bone, may not be adequate for optimal total body health.

1. INTRODUCTION

In Chapter 2, we noted that calcium was a threshold nutrient and we introduced the term "minimum daily requirement" (MDR), defined as the intake just sufficient to get an individual up to the bone retention threshold, i.e., the point at which no further increase in bone mass will occur despite further increases in calcium intake. The concept of an MDR has been largely abandoned for many other nutrients, but it remains apt for calcium, as Fig. 1 in Chapter 2 shows graphically.

In defining the calcium intake requirement, the Calcium and Related Nutrients panel of the Food and Nutrition Board used the notion of maximal calcium retention, that is, the retention plateau at or above the threshold intake *(1)*. In doing so, they explicitly chose bone mass as the functional indicator for calcium nutritional adequacy. It was recognized then that calcium plays a role in other disorders (*see* Part VI of this book), but information was insufficient to allow the panel to peg the requirement to the optimal functioning of systems other than bone. Much information has been accumulated since the recommendations of the Calcium and Related Nutrients panel were submitted to the Food and Nutrition Board, and we discuss some of that new information in Part VI. Here, we review the considerations that went into setting the calcium requirement, show how the MDR may itself not be optimal, even for bone, and set forth the physiology that undergirds the fact that, for certain disease endpoints, the calcium intake requirement may be substantially higher than that needed for skeletal health.

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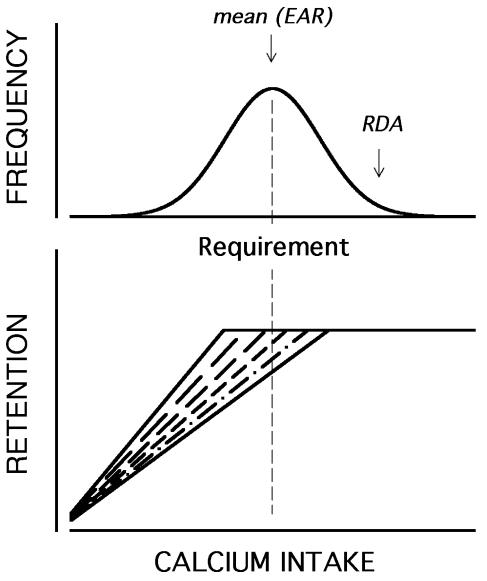

Fig. 1. Schematic depiction of varying utilization efficiencies for calcium (lower panel) and the distribution of such efficiencies (upper panel). For each of the utilization efficiencies, the maximal retention value is the same, but some individuals reach maximal retention at a lower calcium intake than others, while still others require more. The upper panel schematically presents the distribution of such individual intake requirements. The mean of that distribution is the Estimated Average Requirement (EAR), and the Recommended Dietary Allowance (RDA) for calcium would, accordingly, be set roughly two standard deviations above that mean value. (Copyright Robert P. Heaney, 2004. Used with permission.)

2. SETTING THE REQUIREMENT

For most or all nutrients, the published requirement represents the least intake an individual can get by on and still attain some desired health outcome or reach some target value for a functional indicator of nutritional status. Because of differences in absorption or utilization efficiency from individual to individual, there will be a range of requirements, with some individuals able to achieve the desired outcome at lower intakes and others requiring more to produce the same effect. This concept is illustrated for calcium in Fig. 1. In the bottom panel are depicted what a range of requirements means in terms of individual threshold diagrams. Individuals reach their particular bone retention thresholds at various intakes. The top panel presents, schematically, what the distribution of such individual requirement values might look like. The mean value of this distribution is the Dietary Reference Intake (DRI) called an "Estimated Average Requirement" (EAR). By contrast, a "Recommended Dietary Allowance" (RDA) is an intake sufficient to meet the needs of roughly 97% of the population, a value that would be located about two standard deviations to the right of the EAR in the top panel of Fig. 1.

The currently recommended intake values *(1)* for calcium are the so-called "Adequate Intakes" (AI). These happen to be identical to the EAR for calcium, and hence represent an intake that is below the threshold for roughly half the population.

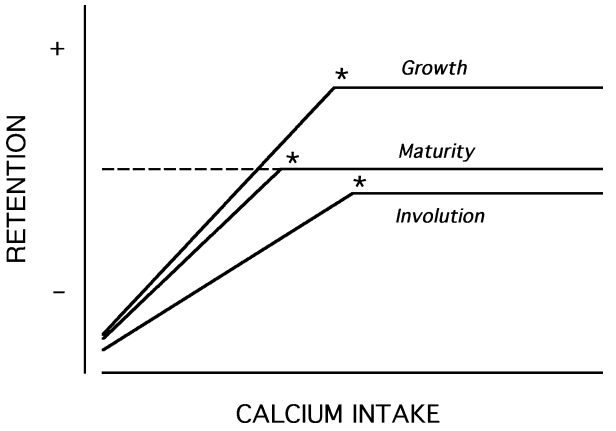

Fig. 2. Retention curves for three life stages. The dashed horizontal line represents zero retention and hence maintenance of bone mass, whereas during growth one would expect positive retention, and during involution, some degree of bone loss, irrespective of calcium intake. For each curve, the asterisks indicate the minimum daily requirement (MDR). (Copyright Robert P. Heaney, 1998. Used with permission.)

Taking the "least to get by on" approach to nutritional recommendations inevitably leads to different recommendations for different ages and physiological states. For example, during a woman's reproductive years, when she has high circulating estrogen levels (and correspondingly better conservation of calcium), she can "get by on" a lower calcium intake than is possible in a postmenopausal, estrogen-deprived state. At least, her bones can get by on less calcium (discussed later).

These age- and state-specific differences in the requirement are illustrated in Fig. 2, once again schematically, but for three age-states: growth, maturity, and involution. There are three key features about each of the retention diagrams in Fig. 2: (1) the steepness of the ascending limb of the retention curve; (2) the location of the threshold point along the range of calcium intakes (horizontal axis); and (3) the location of the plateau region along the range of retention values (vertical axis). The *steepness* of the ascending limbs of the curves is a reflection of the efficiency with which the organism uses dietary calcium; the threshold *intake* is the MDR; and the threshold *retention* is the desired physiological state, that is, bone accumulation during growth, bone maintenance during maturity, and minimization of bone loss during involution.

The desired retention values for growth and maturity are intuitively obvious, but the negative value for retention in the involutional phase of life deserves comment. It reflects the fact that there are other factors operating in the body during involution which lead to diminution in bone mass. These factors cannot be countered by calcium, because they are not caused by insufficient calcium intake. They include decreased physical activity, declines in the production of various hormones that are trophic for bone, and intercurrent illnesses and infections, among others. From the standpoint of nutrition during this phase of life, the goal is to achieve a calcium intake during involution that minimizes bone loss and ensures that nutritional inadequacy is not contributing to whatever decline in bone mass may otherwise be occurring.

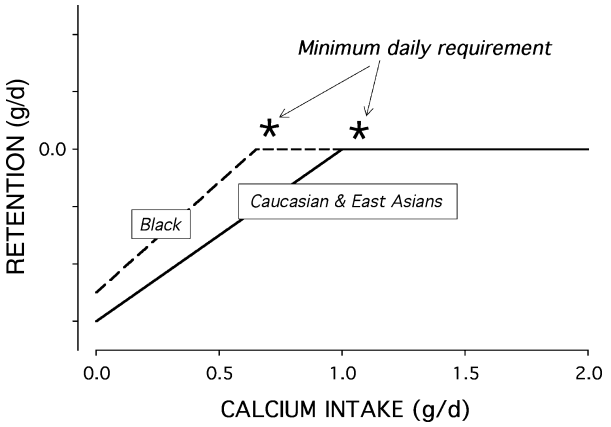

Fig. 3. Calcium retention curves for blacks, as contrasted with Caucasians and east Asians, with an approximate estimate of the quantitative differences in minimum daily requirement (MDR). (Copyright Robert P. Heaney, 2001. Used with permission.)

3. ETHNICITY AND THE REQUIREMENT

In animals, it is possible to perform dose–response experiments, controlling calcium intake at various life stages for long enough to determine the location of the intake thresholds. An example is the work of Forbes et al. *(2)* depicted in Fig. 1 in Chapter 2. For the most part, comparable studies have not been done in humans of any ethnic or racial background. Partial exceptions can be found in the work of Matkovic and Heaney *(3)* and of Jackman et al. *(4)*. Using calcium balances measured across a range of intakes, these investigators have provided estimates of the location of the intake thresholds. However, these data have been accumulated mainly in Caucasians. From the limited evidence that is available, it appears that East Asians have approximately the same requirements as do Caucasians, particularly when diet calcium is corrected for differences in body size. However, two lines of evidence indicate that blacks have a lower requirement for the skeletal endpoint, as illustrated schematically in Fig. 3. The evidence comes from two sources: adult bone mass values are higher in African Americans than in Caucasians, and at the same time, the distribution of their calcium intakes is shifted to the left of that for Caucasians. This means that, despite a lower calcium intake, they acquire more bone than Caucasians or East Asians. The second line of evidence, discussed briefly in Chapter 10, lies in the fact that the bony resorptive apparatus of blacks is relatively resistant to parathyroid hormone (PTH). This means that, in order to maintain extracellular fluid (ECF) $[Ca^{2+}]$ in the face of lower intake, they must secrete more PTH and maintain higher levels of 1,25 dihydroxyvitamin D ($1,25[OH]_2D$), which, in fact, is found to be the case *(5)*. As a consequence, they make better use of dietary calcium: through most of life by reduced urinary calcium loss, and, at some stages, by more efficient intestinal absorption as well. It is as a consequence of these adjustments that the slope of the ascending limb of the retention curve is steeper in blacks, and the retention maximum is reached at a lower calcium intake. The precise value of the difference in the requirement between blacks on the one hand and whites and East Asians on the other can only be roughly estimated, but is probably on the order of 300 mg/d, as Fig. 3 suggests.

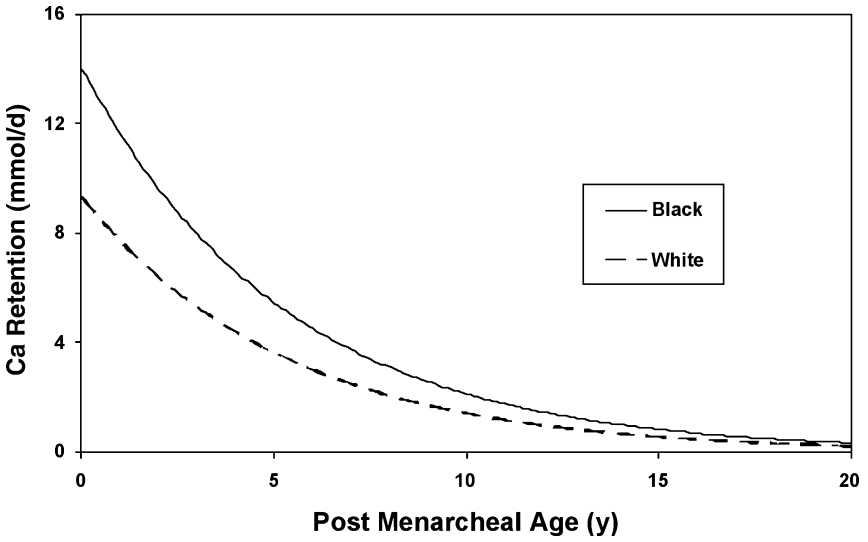

Fig. 4. Model fit for calcium retention, as a function of postmenarcheal age, in black and white females. The cumulative racial difference in bone mass, based on calcium accretion from onset of menarche to 20 yr postmenarche, is predicted to be 12%. (Reprinted from ref. *6*.)

Evidence of more efficient calcium absorption and suppressed bone resorption in black compared with white pubertal girls is shown in Chapter 17. The racial difference in calcium retention during formation of peak bone mass appears to be greatest at onset of menses and diminishes as peak bone mass is reached. Figure 4 *(6)* shows calcium retention as a function of postmenarcheal age in black and white women. The model was developed using data from whites that spanned the whole age range. The curve for blacks was created using data from adolescent black girls projected using the model developed on white women. An estimate of the cumulative difference in retained calcium from the area between the two curves converted to bone mass is 12%, consistent with the 10 and 13% higher femoral neck bone mineral content and density, respectively, observed in black compared with white women from National Health and Nutrition Examination Survey (NHANES) III 1990–1994 *(7)*. Thus, much of the difference in adult mass can be accounted for by the differences in calcium handling during growth. Accordingly, one would expect that the racial differences in calcium retention depicted in Fig. 3 would be most pronounced during adolescence and diminish after peak bone mass has been achieved. Consistent with this expectation are the similar whole-body ^{47}Ca retention curves for adult black and white women in Fig. 2 in Chapter 19.

4. IS THE MDR OPTIMAL?

As Fig. 2 makes clear schematically, and as the very name MDR suggests, this intake is "the least one can get by on." Simply expressing the requirement in this way suggests automatically that the MDR may not be optimal, even for the bony endpoint which currently serves as the functional indicator for calcium nutrition. There is, in fact, a body of evidence suggesting that somewhat higher intakes would be optimal.

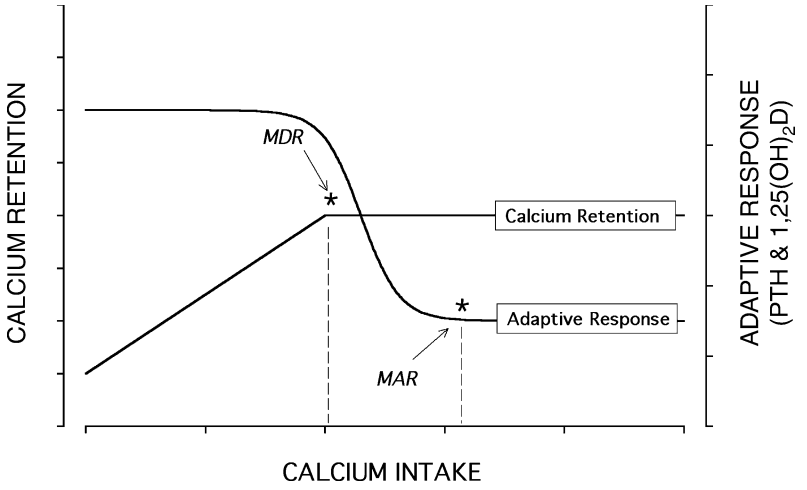

Fig. 5. A curve for the adaptive response to insufficient calcium intake, superimposed on the calcium retention curve, showing that the minimal adaptive response (MAR) is not achieved until an intake somewhat in excess of the minimum daily requirement (MDR). (Copyright Robert P. Heaney, 2004. Used with permission.)

As is evident from an understanding of the physiology involved (*see* Chapters 10 and 11), any intake located along the ascending limb of the retention curve will tend to evoke an adaptive response from the organism (i.e., increased PTH secretion) with its cascade of effects. But simply getting up to the threshold itself requires continuing adaptation. Although increasing intake beyond the threshold point will not lead to higher bone mass, it will lead to decreased adaptation because, as the diet becomes richer and richer in calcium, less and less compensation will be required to permit obtaining all the calcium that might be needed both for growth and to offset obligatory losses (i.e., to remain on the plateau of the retention curve). Thus, there is a phase lag between calcium retention and the adaptive response, depicted schematically in Fig. 5.

There are two implications for optimal calcium nutrition that flow from this insight. The first relates to the bony endpoint and the second to nonskeletal disease. For bone, even though maximal calcium retention may be achieved, the still somewhat elevated PTH levels would be expected to elevate the level of bone remodeling which, as discussed in Chapter 2, is a fragility factor in its own right. This is shown, for example, in the fracture experience of patients with untreated, mild primary hyperparathyroidism *(8)* in whom, despite no appreciable difference in bone mass, fracture risk is approximately threefold greater than in age-matched normal controls. Thus, one would predict that fracture risk would decline somewhat as calcium intake increases past the threshold intake. The precise amount required to produce this level is unclear, and probably rises with age. For example, McKane et al. *(9)* were able to reduce PTH levels in healthy women over age 65 yr to young adult normal values at an intake of 2400 mg Ca/d, a value well above the current AI. Importantly, this seemingly high intake did not depress PTH levels below the young adult normal range. Such an intake, although high by contemporary standards, would probably be in the mid range of calcium intakes for hunter–gatherer populations (adjusted for differences between primitive and contemporary body sizes) *(10)*, and

hence may be close to the intake for which human physiology has been adapted over the course of evolution. Additional evidence supporting this conclusion is seen in the calcium homeostatic response to a challenge such as sodium-induced hypercalciuria (*see* Chapter 10). This behavior illustrates beautifully how the fine-tuning of the calcium economy presumes an intake such as that employed by McKane et al.

The second facet of this phase lag of Fig. 5 relates to the calcium paradox diseases discussed in Part VI, and introduced briefly in Chapter 19. There we note that diseases such as hypertension may be aggravated or initiated as a consequence of high circulating levels of $1,25(OH)_2D$. Thus, to the extent that a threshold intake may still be associated with some elevation of serum $1,25(OH)_2D$, susceptibility to the calcium paradox diseases will be aggravated. This is probably most clearly seen in the case of blacks who, as noted above, have lower calcium intakes than whites, higher circulating levels of PTH and $1,25(OH)_2D$ *(5)*, and a lower bone threshold intake than whites or East Asians. At the same time, African Americans are known to be at increased risk of hypertension and cardiovascular disease, and they have been shown to respond with significant blood pressure reductions to a diet high in calcium and fruits and vegetables (*see* Chapter 28) *(11)*. The calcium intake that produced this benefit was approx 1200 mg/d, well above the bony retention threshold for blacks illustrated in Fig. 3. Thus, although the Food and Nutrition Board selected bone calcium retention as the functional indicator of calcium nutrient adequacy for all persons, newer evidence indicates that, for at least some population groups (e.g., African Americans) blood pressure and/or cardiovascular status is a more appropriate functional indicator.

The evidence supporting use of a non-bony functional indicator is clearest for hypertension, particularly in blacks, and much work needs to be done in order to clarify the optimal calcium intake for the remaining nonskeletal health outcomes. However, it can be noted that the calcium intakes associated in observational studies with minimizing the expression of the nonskeletal diseases related to low calcium intake are all in the range of 1100–1800 mg/d. These values are above the AI for all individuals up to age 50 yr, and at or above the AI for older individuals. Hence, when the requisite data are finally accumulated and the calcium requirement is once again revisited by the Food and Nutrition Board, it would not be surprising to see intake recommendations which may be higher than those required simply for the bony endpoint.

5. CONCLUSIONS

An adequate calcium intake is necessary to ensure optimal functioning of many body systems. Yet current intake recommendations were pegged exclusively to a skeletal endpoint and represent the lowest intake an individual can ingest without compromising the mechanical function of the skeleton. Available evidence indicates that, for certain physiological states and certain ethnic groups, nonskeletal functions of calcium may be more sensitive indicators of the requirement and thus, the optimal *total body* requirement may be higher than the current, bone-related DRIs.

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8 Dietary Calcium

Recommendations and Intakes Around the World

Anne C. Looker

KEY POINTS

- Many countries have published calcium intake recommendations since 1988. These recommendations vary by as much as 900 mg/d.
- Calcium recommendations published after 1997 tend to be higher than those published during 1988–1996.
- Data on calcium intakes in children are too scanty to draw conclusions about adequacy.
- Young men are the only group among adolescents and adults that appears unlikely to have inadequate calcium intakes.
- Data on calcium intakes above the upper limit of 2500 mg/d are too scanty to draw firm conclusions; nonetheless, the risk of inadequate intakes is likely much higher than the risk of excessive intakes.

1. INTRODUCTION

The critical role of calcium in human health has been recognized for many years, as reflected by a long history of calcium intake recommendations *(1)*. Although the need for an appropriate intake of calcium is well recognized among health authorities, data on calcium intakes suggest that a large percentage of the population in most countries does not consume recommended amounts. The objective of the present chapter is to review calcium recommendations and intakes in various countries to provide a current snapshot of calcium nutrition around the world.

To meet this goal, several methods were used to locate published information on calcium recommendations and dietary intake data collected in the 15-yr period from 1988 to 2003. These included a Medline search and use of several Internet search engines to identify papers or other relevant sources of information. The International Reference Guide on Health Data *(2)* was used to identify 13 countries that conduct national surveys that include some type of dietary information. Internet websites of several regional and national health agencies were also searched. Finally, reference lists and professional contacts were used to identify additional sources of information. Nonetheless, this chap-

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ter is not meant to be an exhaustive list of all existing recommendations or datasets on calcium intakes worldwide, but rather to provide selected examples that can illustrate the variability in recommendations and intakes around the world.

2. CALCIUM RECOMMENDATIONS

2.1. Recent Calcium Recommendations in Different Countries

Calcium recommendations published since 1988 were located for 33 countries or organizations using the methods described in the Introduction. A detailed summary of these recommendations is given in Table 1 (for summaries of recommendations published prior to 1988, *see* refs. *3* and *4*). Approximate age groupings have been used to summarize the recommendations, because the exact definitions of the age categories differ between countries. As illustrated in Fig. 1, the absolute amounts of recommended calcium vary widely between the different countries. For example, 75% of the recommended intakes for adolescent males fall between 850 and 1200 mg/d, but the range varies from a low of 500 mg/d (recommended in Sri Lanka) to a high of 1300 mg/d (recommended in the United States and Venezuela and by the Food and Agriculture Organization [FAO] of the World Health Organization [WHO]). In general, the range of recommended values in these 33 countries tended to be narrower in infants, toddlers and younger children than in older children, adolescents and adults: the average difference between the highest and lowest amount recommended was 537 mg/d in the younger groups versus 820 mg/d in the older groups, respectively.

The recommendations also vary depending on how recently they were developed. An upward trend in calcium recommendations has been noted in the past 15 years in some European and North American countries *(5–9)*. This trend is illustrated in Fig. 2, which shows that recommendations shown in Table 1 for adults published after 1997 are significantly higher than those published in 1989–1996. Recommendations for older children and adolescents published after 1997 also tended to be higher than those published earlier, but the differences were not statistically significant.

Possible reasons for the variability in calcium recommendations include differences in their conceptual basis (e.g., avoidance of deficiency vs prevention of chronic disease), the endpoint being used (calcium balance vs bone mineral density), assumptions about the percent of ingested calcium that is absorbed, inclusion of insensible loss of calcium via skin, hair, or nails, and the possibility that the calcium requirement itself may vary from culture to culture for dietary, genetic, body size, lifestyle, and geographical reasons *(10)*. Recommendations may also vary as a result of different interpretations of the same data *(3)*. Some recommendations are based solely on review of original research, others are based solely on a review of other recommendations, while still others may use a combination of both approaches *(3)*.

Some countries choose to adopt recommendations from other countries or from authoritative bodies (such as FAO/WHO or European Community [EC]) rather than developing their own unique recommendations *(11,12)*. There is a growing trend toward harmonization of recommendations across countries, as witnessed by the joint development of recommendations for the Nordic countries *(13)*, and the D-A-CH 2000 (Austria, Germany, and Switzerland) *(14)*. The European Union has compiled a set of recommendations for use across the EC *(15)*. Canada and the United States have collaborated

recently to develop Dietary Reference Intakes for use in both countries *(9)*. Other regions, such as Southeast Asia, are also moving toward greater harmonization *(16,17)*. Reasons to consider harmonization include similarities between populations in some countries, expense and lack of resources to undertake nutrition research, reduction in consumer confusion, increase in world trade, and creation of a global food supply *(12)*.

2.1.1. Upper and Lower Limits for Calcium Intakes

In addition to identifying a target amount of calcium to consume, calcium recommendations from some countries also include a tolerable upper limit (UL) and a lowest acceptable level for calcium intake. The UL is defined as "the highest average daily nutrient intake level likely to pose no risk of adverse health effects for almost all individuals in the general population" *(9)*. Intakes that rise above the UL are believed to carry an increasing risk of adverse effects. Several countries or organizations have identified an upper limit for calcium of 2500 mg/d; examples among those listed in Table 1 include Belgium, EC, Japan, the Netherlands, the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden), Taiwan, and the United States *(9,13,15,18–23)*.

The lowest acceptable level for calcium has been defined as "the intake below which there may be cause for concern for a substantial section of the population" *(15)*, or an amount of the nutrient that is enough for only the few people in a group who have low needs *(24)*. As these definitions imply, the lowest acceptable level is intended to be used for assessment of results from dietary surveys, rather than in assessing an individual's diet *(13)*. Selected examples of countries that have set a lowest acceptable level for calcium include the Nordic countries and the EC *(13,15)*. Both groups defined 400 mg/d as the lowest level for males and females, but the EC indicated that this value applied to adults only *(15)*, whereas the Nordic recommendation covers ages 15–50 yr *(13)*. The United Kingdom has defined a Lower Reference Nutrient Intake (LRNI) for calcium, equal to two standard deviations below the Estimated Average Requirement (EAR), for several age groups: 200–275 mg/d for infants and young children, 325 mg/d for older children, 450–480 mg/d for adolescents, and 400 mg/d for adults *(24)*. Ireland has defined a Lowest Threshold Intake for adults as 430 mg/d *(25)*.

3. CALCIUM INTAKES

3.1. Calcium Intakes in Different Countries

Calcium intake from food in 20 selected countries around the globe are summarized in Table 2. National data have been included whenever possible; however many countries either do not routinely collect dietary data from a nationally representative sample, have not collected it recently, or do not report individual intake data *(2,26,27)*. To provide a more complete picture of calcium intakes, Table 2 also includes regional data for selected countries for which nationally representative data could not be located. Because data were located for only 20 (10%) of the 192 independent states in the world *(28)*, the information on calcium intakes is not intended to be an exhaustive review, but rather to illustrate the variability in calcium intake that exists in different countries. It should also be noted that these data do not include calcium intake from nonfood sources, such as vitamin-mineral supplements, antacids, hard water, or medicines that contain calcium as an excipient or inert ingredient.

Table 1
Recommended Calcium Intakes (mg/d)[a] From Selected Countries Published Since 1988

Country/organization	*Infants*	*Toddlers*	*Young children*	*Older children*	*Adolescents*	*Young adult*	*Middle-aged adult*	*Older adult*	*Pregnant*	*Lactating*
Australia (1991) *(65)*										
Male	300–550	700	800	800	1000–1200	800	800	800	–	–
Female	300–550	700	800	900	800–1000	800	800	1000	+300	+400
Austria[b] (2000) *(14)*										
Male & Female	220–400	600	700	900	1100–1200	1000–1200	1000	1000	1000	1000
Belgium (2000) *(18)*										
Male & Female	400–600	800	800	800	1000–1200	900	900	1200[c]	1200	1200
Canada[d] (1997) *(9)*										
Male	250–500	550	600	700	900–1100	800	800	800	–	–
Female	250–500	550	600	700	700–1100	700	800	800	+500	+500
China (2001) *(66)*										
Male & Female	300–400	600	800	800	1000	800	800	1000	800–1200[e]	1200
Colombia (1992) *(67)*										
Male & Female	400–550	450	450	450	550–650	500	500	500	+300	+400
European Community (1993) *(15)*										
Male	400	400	450	550	1000	700	700	700	–	–
Female	400	400	450	550	800	700	700	700	700	1200
Denmark[f] (1996) *(13)*										
Male & Female	360–540	600	600	700	900	800	800	800	900	1200
FAO/WHO (2002) *(10)*										
Male & Female	300–400	500	600	700	1300	1000	1000	1300[c]	1200	1000
Finland (1998) *(13,68)*										
Male & Female	360–540	600	600	700	900	800[g]	800	800	900	900
France (2000) *(69,70)*										
Male & Female	–	500	700–900	1200	1200	900	900[h]	1200	1000	1000
Germany[b] (2000) *(14)*										
Male & Female	220–400	600	700	900	1100–1200	1000–1200	1000	1000	1000	1000

Iceland[f] (1996) *(13)*										
Male & Female	360–540	600	600	700	900	800	800	800	900	1200
Indonesia (1994) *(71,72)*										
Male & Female	400–600	500	500	500–700[i]	600–700	500	500	500	+400	+400
Ireland (1999) *(25)*										
Male & Female	800	800	800	1200	800	800	800	1200	1200	
Italy (1996) *(73)*										
Male	600	800	800	1000	1200	1000	800	1000	–	–
Female	600	800	800	1000	1200	1000	1200–1500/1000[j]	1200–1500/1000[j]	1200	1200
Japan (2002) *(19)*										
Male	200–500	500	500	600–700	800–900	600–700	600	600	–	–
Female	200–500	500	500	600–700	700	600	600	600	+300	+500
Mexico (1994) *(74)*										
Male & Female	450–600	800	800	800	1200	800–1200	800	800	1200	1200
The Netherlands (2000) *(20)*										
Male	210–450	500	700	1200	1200	1000	1000–1100	1200	–	–
Female	210–450	500	700	1100	1100	1000	1000–1100	1200	1000	1000
Norway[f] (1996) *(13)*										
Male & Female	360–540	600	600	700	900	800	800	800	900	1200
The Phillipines (2002) *(75)*										
Male	200–400	500	550	700	1000	750	750	800	–	–
Female	200–400	500	550	700	1000	750	800	800	800	750
Poland (1998) *(76)*										
Male & Female	–	–	900	1000	1100–1200	1000	1000	1000	1500	2000
Singapore (2002) *(77)*										
Male & Female	300–400	500	600	700	1000	800	800	1000	1000	1000
Spain (1994) *(78)*										
Male & Female	500	600	650	650	800–850	850	600	700	1200	1300
Sri Lanka (1998) *(79)*										
Male & Female	500	400	400	400	500–600	400	400	400	1000	1000
Sweden (1996) *(21)*										
Male & Female	360–540	600	600	700	900	800	800	800[k]	900	1200

(continued)

Table 1 (Continued)

Country/organization	*Infants*	*Toddlers*	*Young children*	*Older children*	*Adolescents*	*Young adult*	*Middle-aged adult*	*Older adult*	*Pregnant*	*Lactating*
Switzerland[b] (2000) *(14)*										
Male & Female	220–400	600	700	900	1100–1200	1000–1200	1000	1000	1000	1000
Thailand (1989) *(80)*										
Male & Female	360–480	800	800	800–1200	1200	800	800	–	1200	1200
Taiwan (2002) *(23)*										
Male & Female	200–400	500–600	800–1000	1200	1000	1000	1000	1000	+0	+0
United Kingdom (1991) *(24)*										
Male	525	350	450	550	1000	700	700	700	–	–
Female	525	350	450	550	800	700	700	700	–	+550
United States[d] (1997) *(9)*										
Male & Female	210–270	500	800	1300	1300	1000	1200	1200	1000–1300[l]	1000–1300[l]
Venezuela (2000) *(81)*										
Male	210–270	500	500	800	1300	1000	1000	1200	–	–
Female	210–270	500	500	800	1300	1000	1000	1300	1300	1300
Vietnam (1996) *(82)*										
Male & Female	300–500	500	500	500	700[m]	500	500	500	1000	1000

FAO, Food and Agriculture Organization; WHO, World Health Organization.

[a]Calcium intake ranges reflect different recommendations for age subgroups within an age category except where noted. For infants, range may also reflect different recommendations for breast- vs bottle-fed infants.

[b]Austria, Germany, and Switzerland share the same recommended intakes (DACH 2000).

[c]Applies to postmenopausal women.

[d]Canada and the United States share the same recommendations (IOM, 1997).

[e]Amount depends on trimester.

[f]The Nordic Countries (Denmark, Iceland, Norway, and Sweden) share the same recommended intakes.

[g]900 mg/d recommended for 19- to 20-yr-old individuals.

[h]1200 mg/d recommended for women age 55+ yr.

[i]600–700 mg/d recommended for 10- to 12-yr-old individuals.

[j]Higher amount (1200–1500 mg/d) recommended for postmenopausal women who do not use estrogen therapy.

[k]A supplement of 500–1000 mg/d may, to a certain extent, delay bone loss.

[l]1300 mg/d recommended for girls ≤ 18 yr.

[m]600 mg/d recommended for 16- to 19-yr-old girls.

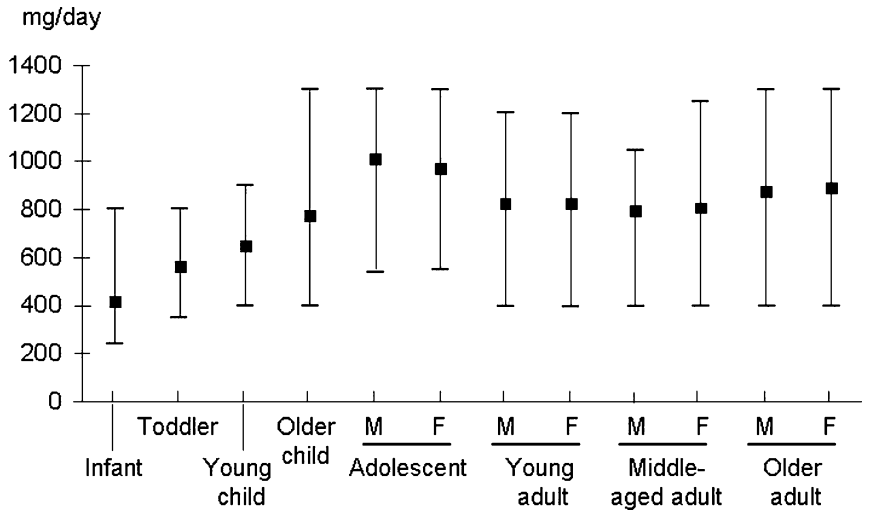

Fig. 1. Range of calcium recommendations from 33 countries. Highest value is at the top of each bar; black box indicates mean value; lowest value is at the bottom of each bar.

Some additional caveats arise when comparing calcium intakes in Table 2 between world regions or individual countries. The small number of countries for which calcium data were obtained limits regional comparisons because some regions either are not represented or are represented by a few countries only. Use of different dietary methods in the different studies may also affect comparisons: mean intakes are generally similar when based on questionnaires vs diet records, but one study found a difference of 125 mg between the two methods *(29–34)*. Finally, differences in the presentation of the data in the different published reports (e.g., use of different age groups, means vs medians, or combined vs sex-specific estimates) also complicate a comparison of the data from different countries.

With these caveats in mind, some general trends emerge. For example, mean intakes among adolescents and adults vary considerably in the different countries, with the highest versus lowest mean intakes differing by as much as 900 mg/d in some age groups (Fig. 3; data for younger age groups are not included in Figs. 3 and 4 because there were less than 10 observations for these ages). When world regions for which there were data for at least two countries in the sample were compared, calcium intakes generally appeared highest in Scandinavian countries, lowest in Asian countries, and intermediate in western European, Oceania (Australia/New Zealand), and North American countries (data not shown).

3.1.1. Adequacy of Calcium Intakes

Published estimates of the prevalence of inadequate calcium intake in different countries suggest that in many countries, a large proportion of the population fails to consume a sufficient amount of this mineral *(35–49)*. However, precise estimates of inadequacy are difficult to define because of the complicated nature of assessing dietary adequacy. This complexity results from several factors, but a major difficulty stems from the conceptual basis that underlies the recommended intakes. For example, a Recommended Dietary Allowance (RDA) is typically defined as the amount of a nutrient that covers the

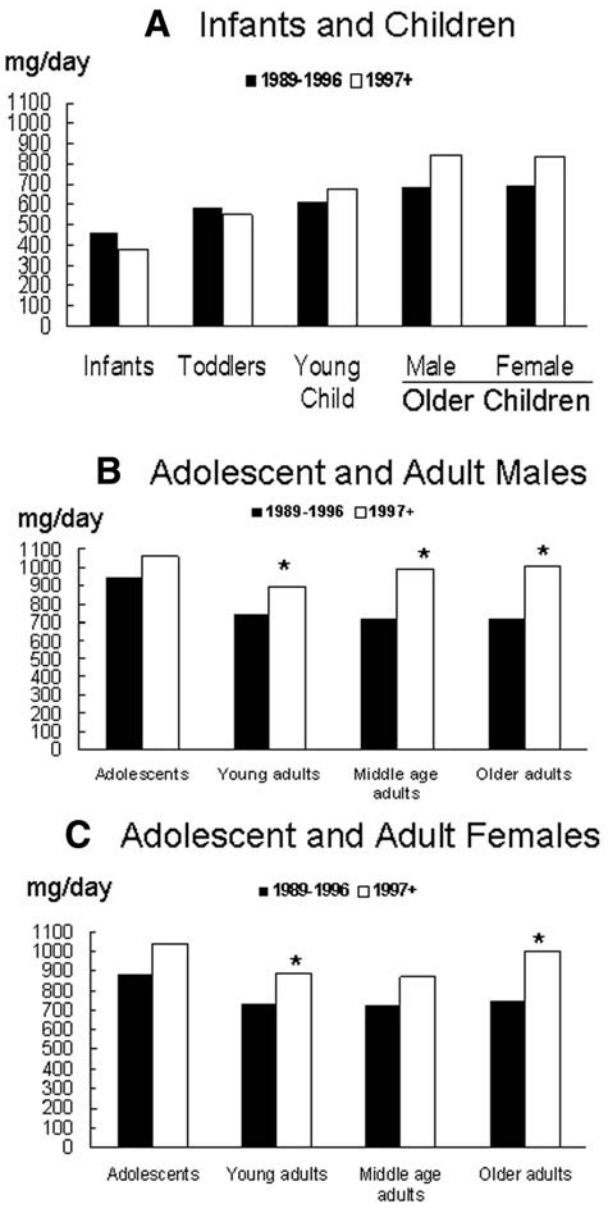

Fig. 2. Time trends in calcium recommendations in the United States. *$p < 0.05$. (From refs. *6–9*.)

needs of 97–98% of healthy individuals *(9)*, so failure to consume the full RDA does not necessarily mean that intakes are deficient in that nutrient. One approach to addressing this issue has been to calculate the percentage of the group that consumes some proportion of the RDA, generally ranging from 50 to 77% *(35–37,50)*. Another approach has been to calculate the prevalence with intakes that fall below an EAR, e.g., the amount of nutrient that is estimated as the requirement, as defined by a specified indicator of adequacy, in 50% of the individuals in a particular group *(9)*. Unfortunately, EARs for calcium are not available for many countries.

Table 2
Calcium Intake Data

Country	*Children*	*Adolescents*	*Young adults*	*Middle-aged adults*	*Older adults*
Australia: national data	√	√	√	√	
Austria: national	√	√	√	√	√
Britain: national data	√	√	√	√	
Canada: regional data	√	√	√		
Denmark: national data	√	√	√	√	√
Finland: national data	√	√			
France: national data	√	√	√	√	√
Germany: national data	√	√	√		
Hong Kong: regional data	√	√			
Hungary: regional data	√	√	√		
Iceland: national data	√	√	√	√	
Ireland: national data	√	√			
Italy: national data	√	√	√	√	√
The Netherlands: national data	√	√	√	√	√
New Zealand: national data	√	√	√	√	
Norway: national data	√	√	√	√	√
Singapore: national data	√	√	√		
Spain: regional data	√	√	√	√	√
Sweden: national data	√	√	√		
United States: national data	√	√	√	√	√

Mean or median calcium intake (mg/d) in selected countries collected since 1988

Community/country	*Survey name and/or areas covered*	*Sample size*[a]	*Year of data collection*	*Dietary method used*	*Age (yr)*	*Calcium intake*		
						Both sexes	*Male*	*Female*
I. National data								
Australia *(83)*	National Nutrition Survey	3399[b] (61.4%)	1995	24-h recall	10–15	–	1054	794
					25–64	–	983	760
Australia *(41)*	National Nutrition Survey	13,858 (61.4%)	1995	24-h recall	4–7	–	800[c]	675[c]
					12–15	–	1006[c]	732[c]
					19–24	–	1005[c]	691[c]
					19+	–	866[c]	688[c]
Austria *(42)*	Vienna and lower Austria	3590	1998	Weighed 7	4–6	1095	–	–

(continued)

Table 2 (Continued)

Community/country	*Survey name and/or areas covered*	*Sample size*[a]	*Year of data collection*	*Dietary method used*	*Age (yr)*	*Calcium intake*		
						Both sexes	*Male*	*Female*
	(Austrian Study on Nutritional Status)			day records	7–9	770	–	–
					10–12	747	–	–
					13–14	726	–	–
					15–19	743	–	–
					20–25	870	–	–
					26–35	867	–	–
					36–45	840	–	–
					46–55	804	–	–
					56–65	774	–	–
					> 65	734	–	–
Britain *(43,84)*	National Diet and Nutrition Survey	1640	1992–1993	4 weighed food records	1.5-2.5	–	682	643
					2.5–3.5	–	642	628
					3.5–4.5	–	625	595
		1724 (47%)	2000–2001	7 weighed food records	19–24	–	867 (825)[c]	706 (669)[c]
					25–34	–	1030 (951)[c]	736 (718)[c]
					35–49	–	1049 (1017)[c]	814 (789)[c]
					50–64	–	1035 (1002)[c]	903 (850)[c]
		1687	1994–1995	4 weighed food records	65–74, free living	–	852	704
					75–84, free living	–	813	680
					65–84, institutionalized	–	935	900
					85+, free living	–	764	647
					85+, institutionalized	–	981	828

Denmark *(85)*	Danskernes Kostvaner 1995	3098	1995	7-d dietary record	1–3	–	910 (886)[c]	996 (886)[c]
		(66%)			4–6	–	1053 (957)[c]	890 (874)[c]
					7–10	–	1224 (1177)[c]	1093 (1056)[c]
					11–14	–	1266 (1196)[c]	1061 (1007)[c]
					15–18	–	1362 (1423)[c]	1121 (988)[c]
					19–24	–	1379 (1151)[c]	1100 (958)[c]
					25–34	–	1121 (1162)[c]	1015 (935)[c]
					35–44	–	1027 (993)[c]	901 (927)[c]
					45–54	–	983 (978)[c]	947 (916)[c]
					55–64	–	1051 (901)[c]	885 (844)[c]
					65–74	–	954 (977)[c]	912 (904)[c]
					75–80	–	822 (834)[c]	864 (861)[c]
Finland *(86)*	National FINNDIET 2002	2007 (63% of invited)	2002	Two 24-h recalls	25–34	–	1391	1001
					35–44	–	1203	986
					45–54	–	1137	954
					55–64	–	1075	946
France *(87)*	L'enquête INCA,1999	3003	1998–1999	7-d dietary records	3–5	790	–	–
					6–8	836	–	–
					9–11	833	–	–
					12–14	835	–	–
					15–24	817	–	–
					25–44	884	–	–
					45–64	856	–	–
					65+	857	–	–

(continued)

Table 2 (Continued)

Community/country	*Survey name and/or areas covered*	*Sample size*[a]	*Year of data collection*	*Dietary method used*	*Age (yr)*	*Calcium intake*		
						Both sexes	*Male*	*Female*
Germany *(88)*	German Nutrition Survey	4030 (56% of invited)	1998	Dietary History	18–24	–	1395[c]	1129[c]
					25–34	–	1319[c]	1118[c]
					35–44		1189[c]	1116[c]
					45–54		1211[c]	1114[c]
					55–64	–	1117[c]	1066[c]
					65–79	–	949[c]	973[c]
Iceland *(45)*	Dietary Survey of The Icelandic Nutrition Council 2002	1366 (70.6%)	2002	24-h recall	15–19	–	1355	1004
					20–39	–	1377	1034
					40–59	–	1133	871
					60–80	–	1032	835
Ireland *(89)*	North/South Ireland Food Consumption Survey (63%)	1379	1997–1999	7-d food record	18–35	–	996[d] (957)[cd]	682[d] (673)[cd]
					36–50	–	962[d] (928)[cd]	742[d] (713)[cd]
					51–64	–	840[d] (803)[cd]	722[d] (666)[cd]
Italy *(44)*	Nationwide Nutritional Survey of Food Behaviour INN-CA 1994–1996	2734 (47% of contacted households; 72% of surveyed individuals)	1994–1996	7-d food diary	1–9	797	826	742
					10–14	862	933	780
					18–64	893	946	850
					>64	845	936	795
The Netherlands *(90,91)*	Dutch National Food Consumption Survey DNFCS-3	5958 (71%)	1997–1998	2-d diet record	1–4	–	846	790
					4–7	–	872	858
					7–10	–	914	901
					10–13	–	1006	912
					13–16	–	1045	904
					16–19	–	1095	908
					19–22	–	1114	865
					22–50	–	1068	963
					50–65	–	1112	995
					65+	–	1024	959

New Zealand *(46,92)*	National Nutrition Survey	4636 (50% of originally selected; 85% of invited)	1997	24-h recall	15–18	–	957 (894)[c]	783 (740)[c]
					19–24	–	938 (875)[c]	760 (713)[c]
					25–44	–	998 (908)[c]	759 (714)[c]
					45–64	–	864 (809)[c]	712 (676)[c]
					65+	–	799 (751)[c]	670 (636)[c]
Norway *(93)*	Kosthold blant 4-åringer Ungkost 2000 (National dietary survey of 4-yr-old children)	391 (52%)	2000		4	–	673	687
Norway *(94)*	Ungkost-2000 (National dietary survey of 9- and 13-yr-old pupils)	Age 9: 815 (80%)	2000		9	–	914	751
		Age 13: 1009 (85%)			13	–	933	785
Norway *(95)*	Nationwide study on dietary behavior of adolescents	1564 (88%)	1993	Food frequency	18	–	1626[c]	1048[c]
Norway *(96)*	NORKOST 1997	2672 (54%)	1997	Food frequency	16–19	–	1400	1000
					20–29	–	1300	900
					30–39	–	1100	800
					40–49	–	1000	800
					50–59	–	900	800
					60–69	–	800	800
					70–79	–	900	800
Singapore *(37)*	National Nutrition Survey 1998	2400	1998	24-h recall	18–29	480 (420)[c]	510 (443)[c]	455 (390)[c]
					30–39	480 (430)[c]	523 (475)[c]	446 (402)[c]
					40–49	486 (418)[c]	513 (471)[c]	462 (389)[c]
					50–59	506 (455)[c]	509 (473)[c]	503 (444)[c]
					60–69	447 (386)[c]	445 (395)[c]	448 (371)[c]

(continued)

Table 2 (Continued)

Community/country	*Survey name and/or areas covered*	*Sample size*[a]	*Year of data collection*	*Dietary method used*	*Age (yr)*	*Calcium intake*		
						Both sexes	*Male*	*Female*
Sweden *(97)*	Riksmaten	1215	1997–1998	7-d food record	17+	–	1069 (1010)[c]	927 (904)[c]
					17–24	–	1201 (1163)[c]	937 (886)[c]
					25–34	–	1090 (1035)[c]	973 (970)[c]
					35–44	–	1029 (977)[c]	888 (872)[c]
					45–54	–	1041 (999)[c]	901 (868)[c]
					55–64	–	1035 (1013)[c]	927 (904)[c]
					65+	–	1064 (962)[c]	937 (912)[c]
United States *(98)*	National Health and Nutrition Examination Survey	8604 (71% of originally selected sample; 93% of examined)	1999–2000	24-h recall	<6	853 (768)[c]	916 (809)[c]	785 (708)[c]
					6–11	889 (821)[c]	915 (843)[c]	860 (812)[c]
					12–19	938 (787)[c]	1081 (956)[c]	793 (661)[c]
					20–39	909 (762)[c]	1025 (856)[c]	797 (684)[c]
					40–59	853 (720)[c]	969 (834)[c]	744 (621)[c]
					60+	721 (619)[c]	797 (716)[c]	660 (563)[c]
II. Regional data								
Canada *(99)*	Nova Scotia (Nova Scotia Nutrition Survey)	2200 (80%)	1990	24-h recall	18–34	–	1161	738
					35–49	–	913	624
					50–64	–	822	582
					65–74	–	776	595

Canada *(47)*	Prince Edward Island (Prince Edward Island Nutrition Survey)	1995 (80%)	1995	24-h recall	18–34	–	1151 (969)[c]	714 (621)[c]
					35–49	–	838 (771)[c]	637 (581)[c]
					50–64	–	791 (706)[c]	600 (530)[c]
					65–74	–	785 (691)[c]	547 (496)[c]
Canada *(100)*	Québec (Québec Nutrition Survey)	2118 (69%)	1995	24-h recall	18–34	–	1114	788
					35–49	–	922	658
					50–64	–	736	622
					65–74	–	771	574
Canada *(101)*	Saskatchewan (Saskatchewan Nutrition Survey)	1798 (46%)[e]	1993	24-h recall	18–34	–	1251	822
					35–49	–	994	761
					50–64	–	793	651
					65–74	–	812	633
Hong Kong *(48)*	Hong Kong Chinese	1010 (40%)	1995–1996	Food frequency	34–55	–	605	570
Hungary *(102)*	Budapest and 7 other counties	2559	1992–1994	Dietary record, 24-h recall, food frequency	18–34	–	868	630
					35–54/59	–	659	579
					≥55/60	–	699	613
Spain *(38)*	Canary Islands (Encuesta de Nutrición de Canarias)	1189 (69%)	1997–1998	Two 24-h recalls	6–10	1019	1093	959
					11–17	1027	1092	952
					18–24	922	987	859
					25–34	900	996	810
					35–44	974	1101	874
					45–54	938	936	940
					55–64	926	956	901
					65–75	936	930	940

[a]Response rate given in parentheses when available.
[b]Subset of total sample created to be comparable with 1983 survey sample.
[c]Median.
[d]Food sources only.
[e]Not considered representative of the population *(47)*.
h, hour; d, day.

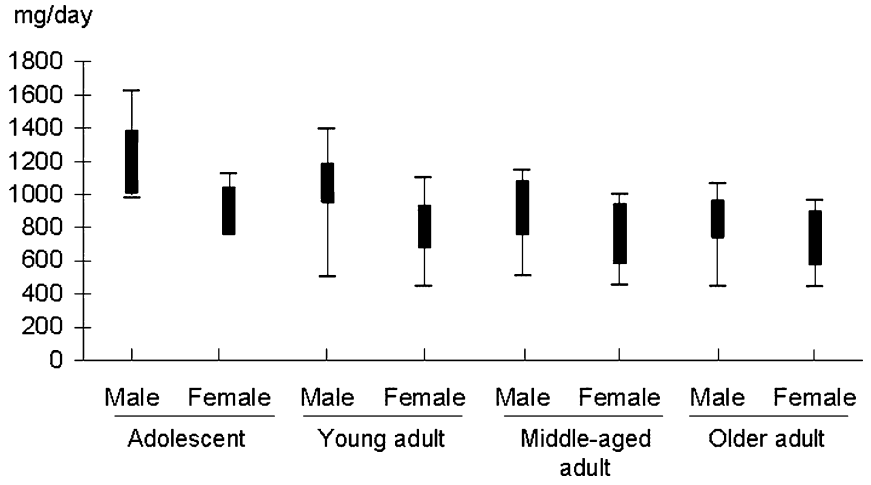

Fig. 3. Range of mean calcium intakes from 20 countries. Highest value is at the top of each bar; black box indicates range in which 75% of mean values fall; lowest value is at the bottom of each bar.

A third, more indirect approach to evaluating calcium adequacy is to assess whether the population or group has a mean or median intake at or above the recommended intake; if so, it is likely there is a low prevalence of inadequate intakes *(51)*. This approach is used in Fig. 4, which summarizes the percent of adolescent and adult population subgroups with a mean or median intake that meets or exceeds country-specific recommendations in 20 selected countries. Only young adult men appeared highly likely to have a low prevalence of inadequate intakes: mean intakes in this age group in approx 94% of the 20 countries met or exceeded the country-specific calcium recommendation. The percent with mean intakes at or above recommended levels ranged from roughly 50 to 71% in the other age and sex groups. The exact extent of inadequate calcium intakes in these groups may be uncertain, but it is probably reasonable to assume that their risk is not negligible.

3.1.1.1. Excessive Calcium Consumption

Excessive calcium intake can also have detrimental effects on health. The UL of 2500 mg/d for calcium described earlier was suggested primarily to avoid these effects. The most extensively studied adverse effects include nephrolithiasis, hypercalcemia, and renal insufficiency (milk-alkali syndrome) *(9,52)*. The possibility that calcium may have negative effects on the metabolism of other minerals, such as iron, magnesium, or zinc, has also been studied *(53–58)*. Results from these studies have been inconclusive. For example, high calcium intake has been linked to poorer magnesium status in rats *(53)* but not in humans *(54)*. Likewise, high calcium intakes in single meal studies reduce iron absorption, but the effect is diminished when the total diet is studied, and up to 12 wk of calcium supplementation did not produce changes in iron status *(58)*.

Estimates of calcium intakes that reach or exceed the UL of 2500 mg/d were only located for a few countries. For example, according to the 1994 Continuing Survey of Food Intakes of Individuals, approx 1% of adolescent boys aged 14–18 yr in the United States consumed more than 2500 mg/d from food alone; no other age or sex group had

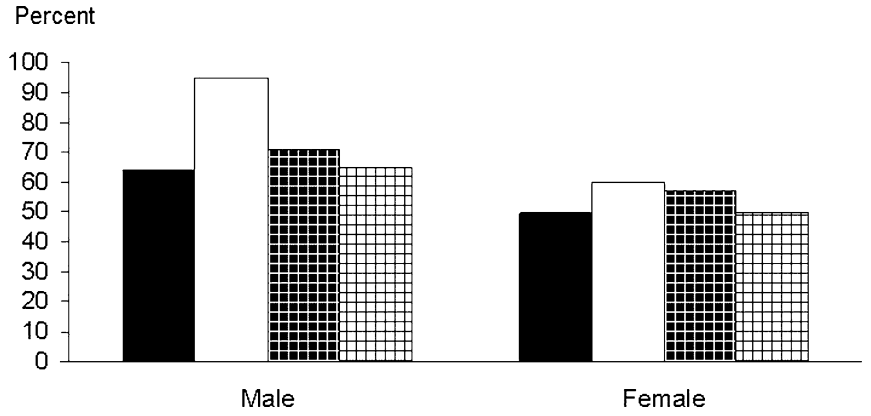

Fig. 4. Percent of adolescents and adults in 20 countries meeting country-specific calcium recommendations. ■ Adolescent; □ Young adult; ▩ Middle-aged adult; ⊞ Older adult.

intakes that exceeded the UL *(9)*. Data from the 1997–1998 Food Habits of Canadians Survey indicated that the prevalence with calcium intakes above the UL in the total population of Canadian men aged 18–65 yr was 1.4% when based on food alone and 2.1% when supplements were included *(59)*. Comparable figures for Canadian women were less than 1% regardless of whether supplement intake was considered or not. Interestingly, if supplement users were considered exclusively, the prevalence with intakes above the UL rose to 7% among Canadian men and 2% among Canadian women *(59)*. A greater prevalence of high intakes from food alone was found among adult Finnish men: data from the 1992 FINDIET indicated that approx 10% had calcium intakes that were 2300 mg/d or higher *(60)*. These data are too scanty to draw any firm conclusions about the possibility of excess calcium intakes worldwide. But the likelihood of potentially excessive calcium intakes appeared to be low in two of the three countries for which relevant data were located. Young adult and adolescent males appeared to be most likely to exceed the UL.

Nonetheless, concerns about possible excessive calcium intakes exist in light of the increasing number of calcium-fortified food products that are available. For example, informal market surveys in the United States found that availability of calcium fortified foods increased between 1994 and 1996 *(61)*, and nearly four times more foods and beverages with added calcium were introduced in 1999 than in 1995 *(62)*. Policies regarding calcium fortification (e.g., amounts, food vehicles, voluntary vs mandatory) vary in different countries; for example, calcium fortification is currently voluntary in the United States, whereas fortification of flour with calcium is mandatory in Britain *(63)*. The amount of calcium that can be added to foods is not controlled in the United States, whereas discussions are ongoing among members of the European Commission regarding controls on the amounts of vitamins and minerals in supplements and fortified foods *(63,64)*.

A few studies have assessed the potential ability of these calcium-fortified products to contribute to excessive calcium intakes. For example, Whiting and Wood *(52)* illus-

trated how calcium intake by a hypothetical 25-yr-old man could increase from 2000 mg/d to 3800 mg/d if some currently available calcium-fortified foods were substituted for their unfortified versions. Johnson-Down et al. *(59)* performed simulations using different fortification scenarios and found that any scenario sufficient to increase the mean intake of Canadian women close to recommended levels led to 6–7% of men having calcium intakes above the UL. Suojanen et al. *(60)* found that calcium intakes would reach the UL of 2500 mg/d among approx 10% of Finnish women and exceed 3000 mg/d among 10% of Finnish men if all unfortified foodstuffs were replaced by their counterparts that were either already calcium-fortified or for which an application to fortify had been submitted. It should be noted that the UL was judged to be conservative by the Dietary Reference Intake (DRI) panel, and that "for the majority of the general population, intakes of calcium from food substantially above the UL are probably safe" *(9)*. Nonetheless, these findings lend support to the recommendation made by the Food and Nutrition Board *(9)* regarding the need to maintain surveillance of calcium-fortified products in the market place and monitor their impact on calcium intake.

4. CONCLUSIONS

Several countries have published recommendations for calcium intake since 1988. These recommendations vary by as much as 900 mg/d, with differences being greater for older children, adolescents, and adults than for infants, toddlers, and younger children. Recommendations published in 1997 or later tend to be higher than those published in 1988–1996. Among adults and adolescents in the 20 countries considered, only young men appeared to be highly likely to be at low risk for inadequate calcium intake. Data for younger age groups were too scanty to draw conclusions about adequacy. Published data on calcium intake above the UL of 2500 mg/d are scanty for all age groups, so firm conclusions on the prevalence of excess calcium intakes are not possible. More data on the prevalence with intakes above the UL are needed, given the increased number of calcium-fortified products in the food supply of many countries. At present, however, the risk of inadequate intakes is probably much higher than the risk of excessive intakes.

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9 Food Sources, Supplements, and Bioavailability

Connie M. Weaver and Robert P. Heaney

KEY POINTS

- Most of the calcium in the American diet comes from dairy products.
- Calcium intake is a marker for diet quality.
- Without adequate dairy products, calcium requirements can only practically be met by consuming fortified foods or supplements.
- Calcium absorption is inversely related to the calcium load of the meal.
- Calcium bioavailability is influenced by the presence of inhibitors and enhancers of calcium absorption in the food or meal.
- Calcium absorption from various salts has at most only a weak relationship to solubility.

1. INTRODUCTION

Early humans are thought to have consumed a diet rich in calcium from a wide range of plant sources *(1)*. With cultivation of plants, a few staple cereal crops became the major source of energy for modern man. Botanically speaking, cereal grains are the fruit of the plant, which is the part of the plant that accumulates the least amount of calcium. Since the agricultural revolution, the main food source of calcium in the diet of most populations is dairy products. Calcium adequacy in the diet became directly related to dairy consumption. In the last few years, an enormous increase in diversity of food sources of calcium has become available in North America through extensive fortification. Now, calcium requirements can be met through consumption of dairy products (primarily milk); through fortified foods; or through supplements.

The choice of source or combination of sources to meet the calcium needs of an individual depends on many factors and has implications for overall health. Some individuals do not consume sufficient milk to meet their calcium needs because of health reasons such as milk protein allergies or perceived milk intolerance, taste preferences, or philosophies. Others simply never acquired a habit of drinking milk as the beverage of choice. Milk-drinking habits track from early age and are related to milk-drinking habits of the mother *(2,3)*. Habits, once formed, are difficult to change. Fortification of foods already being consumed has the advantage of probable compliance if the indi-

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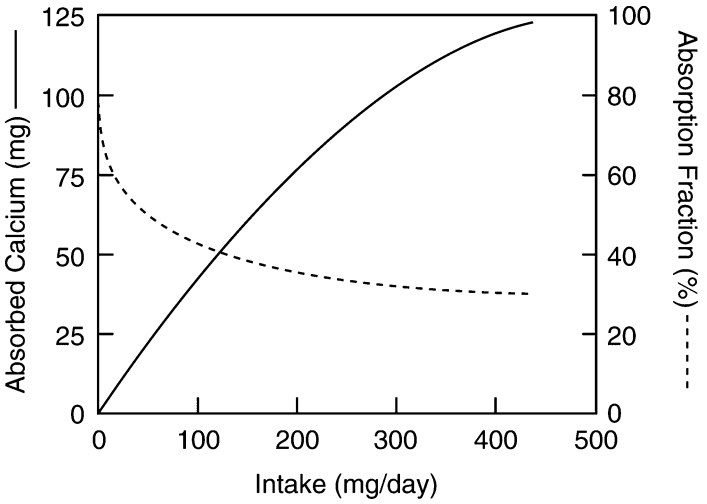

Fig. 1. Theoretical relationship between calcium intake and net calcium absorbed (solid line) and absorption efficiency (dashed line). (Reproduced from ref. *52*, with permission from ILSI Press.)

viduals whose intakes are most inadequate are actually being targeted. Using calcium-fortified foods to meet calcium requirements requires more attention to ensure adequacy because of the varied levels of fortification among sources and the generally lower frequency of consumption of any one fortified food in contrast with milk as the beverage of choice among milk drinkers. Supplements may be effective for meeting calcium needs on an individual basis, but reliance on supplements has limited effectiveness for a whole population because of issues with adherence.

The choice of calcium source influences not only the amount of consumed calcium but also of that of other nutrients. Furthermore, the source of calcium can vary in cost and bioavailability or absorbability. The rest of this chapter focuses on these issues. Dietary factors that influence postabsorptive retention of calcium are discussed in Chapter 12.

2. PHYSIOLOGICAL FACTORS AFFECTING CALCIUM ABSORPTION

Regardless of the source of calcium, calcium absorption efficiency decreases with increasing intake, as depicted in Fig. 1. However, total calcium absorbed keeps increasing with load. Consequently, calcium absorption efficiency is greater if calcium is ingested in divided doses throughout the day. However, with a high enough intake at one time, the calcium need for the day can be met from an increasing proportion of paracellular absorption. This is the concept used by General Mills for manufacturing Total® cereal, which supplies 100% of the recommended daily intake of calcium per serving.

Calcium status of the individual, as determined by habitual calcium intake, influences calcium absorption efficiency. Girls on low calcium intakes had higher calcium absorption efficiencies *(4)*. Figure 2 illustrates the adaptive efficiency based on low compared with adequate calcium intakes in adult women. The ability to adapt to chronically low calcium intake is insufficient to protect bones in most individuals of Caucasian or East Asian origin.

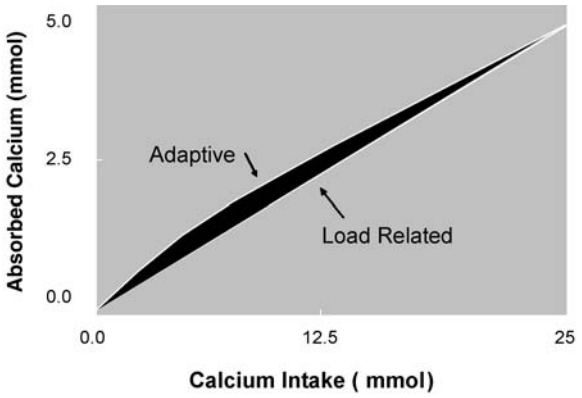

Fig 2. Relationship between calcium intake and absorbed calcium in women tested on their usual calcium intakes (adaptive) and in women tested with no prior exposure to the test load (load-related, a physiochemical effect). (Reproduced from ref. *53*, with permission from Raven Press.)

Life stage is another physiological factor that influences calcium absorption from a given source. This topic is discussed in detail in Part V of this book. Briefly, noteworthy stages that affect calcium absorption efficiency are adolescence, pregnancy, and aging. The high calcium absorption that occurs with rapid bone accretion during puberty is little affected by calcium load. Calcium absorption efficiency is also upregulated in the third trimester of pregnancy. Age-related declines in calcium absorption efficiency are the basis for increased requirements of older individuals. Disorders that influence calcium absorption include hyperparathyroidism and diseases of the kidney, which compromise active calcium absorption. Achlorhydria does not lead to a decrease in calcium absorption if calcium is consumed with food *(5)*.

Assuming an obligatory calcium loss of the average adult of 5 mmol (200 mg)/d, net calcium absorption (intake minus fecal output), with no consideration for bone accretion, must be at least this amount to prevent negative calcium balance. The calcium intake to produce this level of net calcium absorption at various calcium absorption efficiencies is given in Fig. 3. Zero active calcium absorption represents only passive absorption. In this state, with zero active calcium absorption, a calcium intake greater than 60 mmol (2400 mg) is required to prevent negative calcium balance.

Lactose intolerance is a reason given by many individuals for avoiding dairy foods. In many individuals, levels of functioning intestinal lactase declines in childhood. However, lactose nonpersistence is not a reliable indicator of lactose intolerance symptoms associated with consuming large quantities of lactose. Even those individuals with verified lactose intolerance can digest lactose-containing foods without evidence of intolerance by consuming up to 2 cups of milk or equivalent amounts of lactose together with food at a meal *(6)*.

3. FOOD SOURCES OF CALCIUM

Milk and other dairy foods provided 84% of the calcium from foods in the United States in 1989–1993 *(7)*. Unfortunately, milk is being displaced as a beverage of choice

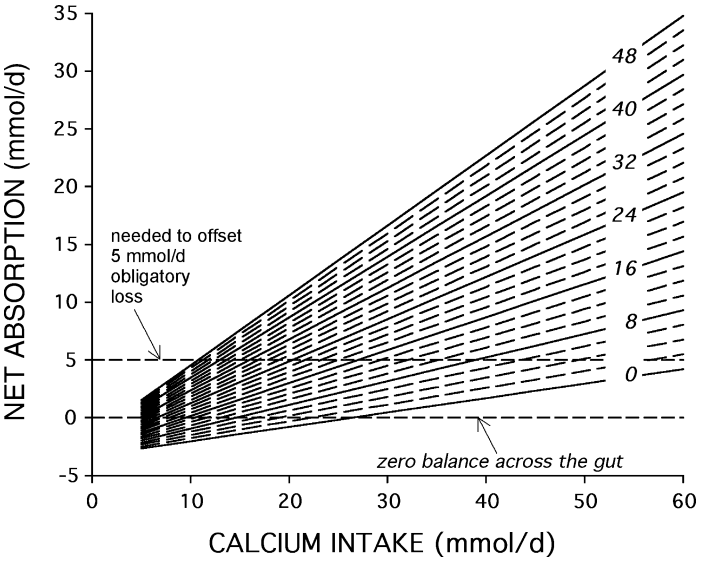

Fig. 3. Relationship between calcium intake and net absorption for varying levels of active absorption (indicated at the right of the contour lines). Net absorption is defined as the difference between oral intake and fecal output. The various contour lines are plots of the equation: NetAbs = (Intake + 3.75) × (PassAbs + ActAbs) – 3.75, where PassAbs = passive absorption fraction (=[0.125], and ActAbs = active absorption fraction). (Copyright Robert P. Heaney, 1999; with permission.)

by sweetened soft drinks and juices (Fig. 4). Americans drank more than four times as much milk as carbonated soft drinks in 1945; in 2001, they drank nearly 2.5 times more soda than milk. Table 1 shows the nutrient contribution of 1 c of milk to the diet. Milk is a nutrient-dense food in that it supplies concentrated nutrients relative to calories. Clearly, milk is a rich package of nutrients, and drinking milk is the most economical strategy for achieving sufficiency of a broad range of nutrients. Limiting milk in the diet necessitates dietary adjustments beyond meeting calcium needs. This often is not accomplished in the general population. Low calcium intakes from limiting milk in the diet have been associated with low intakes of magnesium, riboflavin, vitamins B_6 and B_{12}, and thiamin. The degree to which calcium intakes serve as a marker for total diet quality from one study *(8)* is shown in Fig. 5. Using 7-d diet records in 272 healthy Caucasian premenopausal women, scores were assigned for 9 nutrients: 0, if the nutrient was consumed in quantitites less than two-thirds of recommended intakes for that nutrient, and 1 if intake exceeded that level. A maximum possible score for each women was 9, and scores of 4 or below were considered poor diets. Of the women who had calcium scores of 0, 53% had poor overall diet quality, that is, five or more nutrients ingested at less than two-thirds recommended levels. Only 10% of women with calcium scores of 1 had overall poor quality diets.

The effectiveness of a particular source depends on the calcium content in a serving and its absorbability. Generally, calcium content varies more widely than bioavailability. Table 2 (expanded from Weaver et al. *[9]*) gives both these parameters for a variety of foods in addition to a comparison of how many servings are needed to supply the same amount of absorbable calcium as a glass of milk. Figure 6 demonstrates graphically the

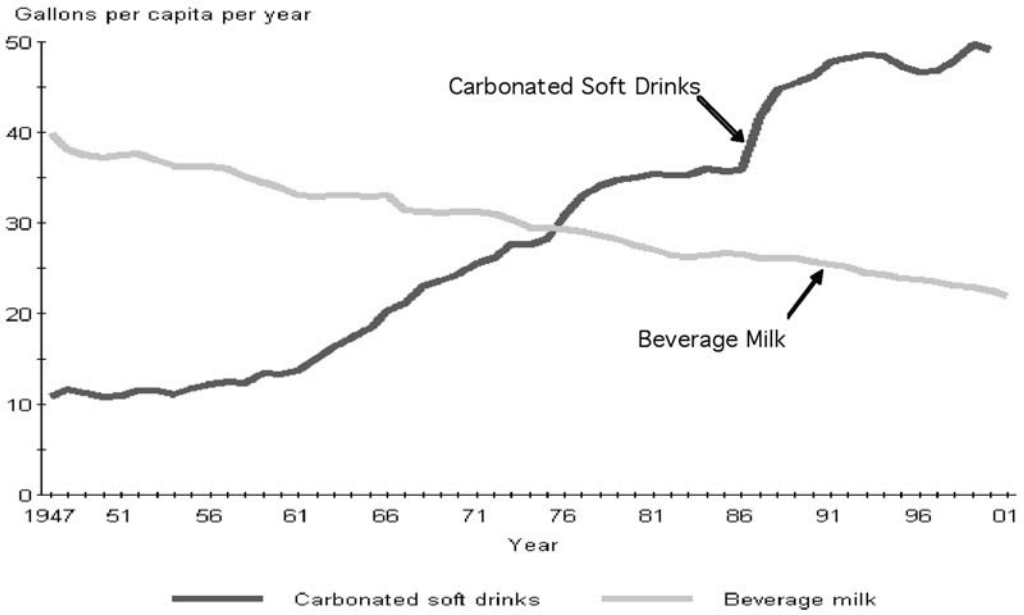

Fig. 4. Trends in milk and carbonated soft drink consumption from 1945 to 2001. (From US Department of Agriculture/Economic Research Service, courtesy of Patricia Britton.)

wide range of calcium absorbed per serving. Calcium absorption from dairy, milk, yogurt, and cheddar cheese is similar and is not affected by flavorings such as chocolate, fat content, or removal of lactose *(12,24)*.

Bioavailability of calcium from the foods in Table 2 was determined using foods intrinsically labeled with either stable or radioactive isotopic tracers. Intrinsic labeling of milk was accomplished by intravenously injecting a stable calcium isotope into the jugular vein of a cow and collecting the milk over 3 d. The milk was pasteurized, homogenized, and aseptically packaged. A portion of the labeled milk was processed into cheese or yogurt at Kraft, Inc. Plant foods were labeled with isotopes of calcium through their administration into the nutrient solution of hydroponically grown plants or direct insertion, i.e., into the petioles of wheat. Calcium fractional absorption was determined in humans using either a single 5-h blood sample (which has been shown to correlate highly with the double isotope technique, as described in Chapter 5) or fecal recovery of unabsorbed isotope.

In order to estimate the amount of calcium from a standard serving in Table 2, the calcium load has to be adjusted from the actual test dose to the level in a typical serving. This is because absorption efficiency is inversely related to load (Fig 1). The equation for adjusting the load is given in a footnote in Table 2. In most of our studies of calcium bioavailability from foods, our reference food was milk. Thus, once absorption efficiency was adjusted to the load in a serving, the ratio of efficiency of the test food compared with milk could be used to determine absorption efficiency at that load. Failure to adjust for calcium load has led to nonsensical reports on the literature *(25)*, such as the same absorption fraction for fresh and frozen broccoli, when frozen broccoli has twice the calcium content per half-cup serving.

Table 1
Nutrient Contributions of 1 Cup of 1% Milk

Nutrient	*Amount*[a]	*% AI/RDA*[b]
Calcium	290 mg	29
Phosphorus	231 mg	33
Protein	8.2 g	18
Potassium	366 mg	9
Magnesium	27 mg	8
Riboflavin	0.45 mg	10
Vitamin D (fortified)	127 IU	32
Energy	102 kcal	

[a]Source: ARS Nutrient Data Base for Standard Reference, Release 16-1.
[b]For adult female aged 31–50 yr.

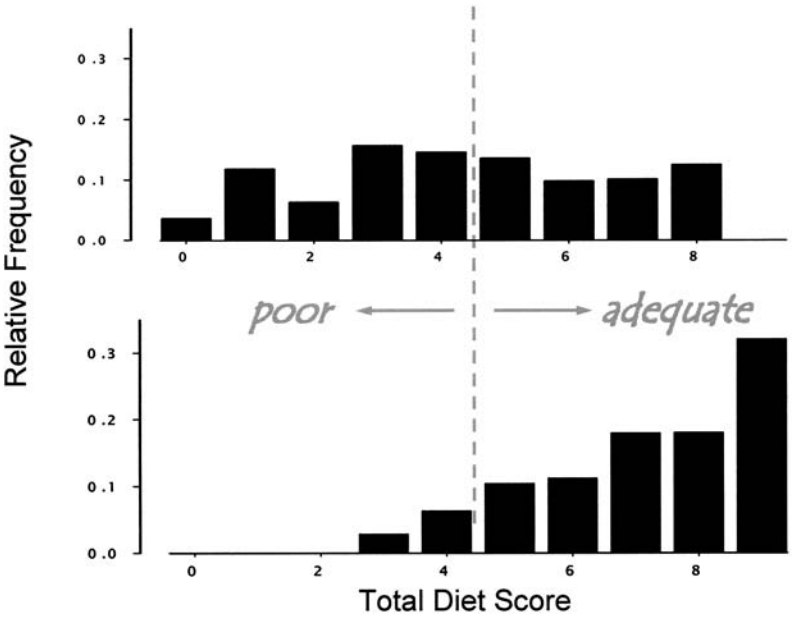

Fig. 5. Distribution of diet scores for nine total nutrients (calcium, iron, magnesium, vitamins A, C, B_6, thiamin, and riboflavin) for 151 premenopausal women with calcium intake less than two-thirds of the recommended dietary allowances (RDA) (top panel) and 121 premenopausal women with calcium intakes greater than two-thirds of the RDA (bottom panel). (Adapted from ref. *8*.)

A few foods that contain appreciable amounts of calcium have not been tested for calcium absorption. These include small fish with bones and some ethnic foods.

Differences in calcium absorption between sources, once load is accounted for, relate to the food matrix. The matrix may contain enhancers or inhibitors of calcium absorption. Although solubility at neutral pH has little effect on calcium absorption except at extreme limits outside 0.14 m*M*/L for calcium carbonate to 7.3 m*M*/L for calcium citrate *(26)*, some enhancers and inhibitors to calcium absorption work by affecting calcium solubility, and therefore, availability to the enterocyte, within the gut.

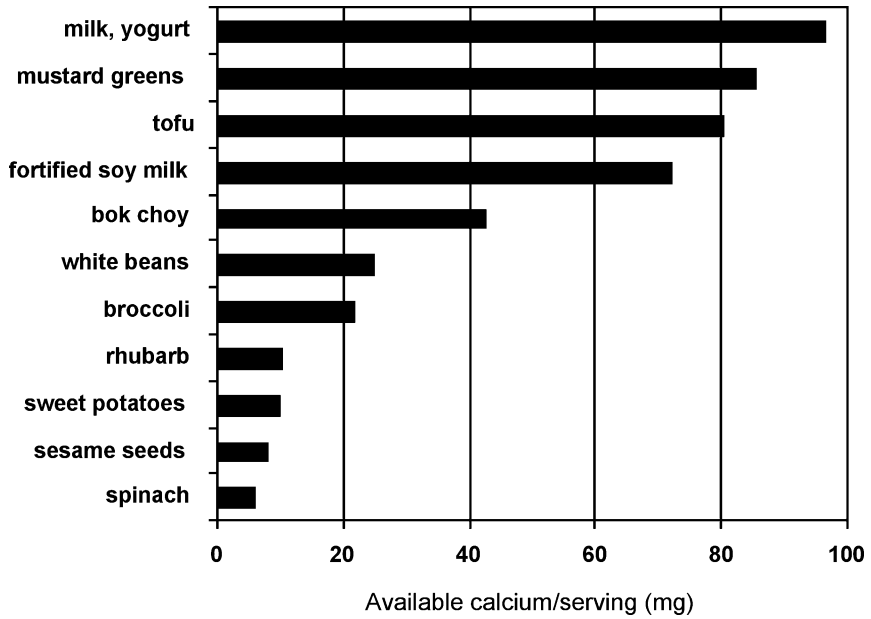

Fig. 6. Total calcium absorbed from various food sources. The value for calcium-fortified soymilk is only true if the calcium is well suspended. (Copyright Robert P. Heaney, 2004; with permission.)

3.1. Calcium Absorption Inhibitors

The most potent inhibitor to calcium absorption is oxalic acid. Oxalic acid forms an extremely insoluble salt with calcium (0.04 m*M*/L). Its presence in foods usually reduces calcium bioavailability considerably. Vegetables in the Brassica family have more calcium than other vegetables and the calcium is highly absorbable, because they do not accumulate oxalate. Spinach calcium is the least bioavailable of the calcium sources. The oxalic acid content of spinach is more than sufficient to bind all the calcium present. However, a small amount of calcium in spinach is exchangeable with an externally added isotope tracer *(27)* in contrast with the calcium in the pure salt of calcium oxalate *(28)*. Spinach as a matrix is more complex than the simple salt. Some matrices nearly neutralize the effect of the oxalate present in the food. This is the case with soybeans that have similar calcium bioavailability to milk *(29)*. Common beans are of intermediate bioavailability, and they too contain sufficient oxalate to bind all the bean calcium. Thus, for any given source, bioavailability has to be measured because it cannot be predicted.

Another inhibitor to calcium absorption is phytic acid, but it is considerably less potent than oxalic acid. Phytic acid is the storage form of phosphorus in seeds. The negative charges of the phosphate groups bind divalent cations such as calcium, as well as positively charged groups on amino acids and proteins. These phytins are poorly digested and absorbed, but calcium is bound less tightly than other cations such as zinc, thus lessening the effect. Therefore, phytic acid only appreciably affects calcium absorption when present in large amounts. High-phytate bran cereal had reduced calcium absorption, but calcium absorption from cookies and bread made from whole wheat was as high or higher than that from milk *(18)*. Phytases in yeast reduce the inhibitory effects in leavened breads and fermented products even further. A threefold increase in phytic acid reduced calcium

absorption 25% *(29)*. Fiber was once considered to reduce the bioavailability of calcium in whole-wheat bread *(30)*. However, purified fibers have little effect on calcium bioavailability and the fibers in low-oxalate vegetables do not reduce calcium absorption relative to milk. Thus, the negative calcium balance associated with high fiber diets is likely the result of the phytate associated with the fiber. High-phytate bran cereals can physically absorb great quantities of calcium and reduce absorbability in this way. The ingestion of psyllium fiber used as a laxative has no significant detrimental effect on calcium absorption *(31)*. Vegetable sources which are low in oxalate and phytate frequently have greater calcium bioavailability than milk. The reason for this is unclear. We have evaluated the effects of isolated constituents from kale, without identifying an enhancer of calcium absorption. Regardless, the concentration of calcium is so low in most of these plants that an impractical quantity would have to be consumed to meet calcium requirements, as shown by the number of servings required to replace one glass of milk in terms of absorbable calcium (*see* Table 2).

3.2. Calcium Absorption Enhancers

Although it is easier to increase the quantity of calcium absorbed simply by consuming more calcium, there is much interest in increasing calcium absorption efficiency. This is a tempting strategy given the inefficiency of calcium absorption from a typical diet. However, enhancers of calcium absorption typically would have to be present in higher concentrations than normally found in foods.

The main absorption enhancers that have been investigated for potential as additives to foods to enhance calcium absorption are selected protein products, amino acids (notably lysine), and nondigestible oligosaccharides (NDOs) *(32–34)*. Proteins such as casein phosphopeptides (CPPs) are thought to work by solubilizing calcium and thus preventing its precipitation by phosphates in the gut. The efficiency of CPPs has been modest, and in humans, a benefit was found only in those who had poor calcium absorption efficiency *(33)*. NDOs are thought to increase calcium absorption in the lower gut and to increase mucosal mass. In the lower gut, bacteria ferment the fiber, producing volatile fatty acids and lactic acid that could solubilize calcium and stimulate transcellular calcium absorption. Studies that are too short can miss the effect. because it can take more than 2 d for these adaptive changes to affect calcium absorption. The effects of fructo-oligosaccharides, especially inulin, have been mixed and possibly related to such factors as type of NDO and physiology of the host, including life stage, dietary calcium, intestinal microflora, and so on. More research is required to clarify the role of NDOs on calcium bioavailability. Complicating the picture of the effect of calcium absorption enhancers is the possibility that some putative enhancement is merely because of the presence of food in the stomach, which is known to enhance calcium absorption *(5)*. Thus, it is important not to design a test with the source ingested without food.

4. CALCIUM-FORTIFIED FOODS

Many calcium-fortified foods have been developed in an attempt to close the gap between calcium intakes and calcium recommendations. Fortification of commonly consumed foods can lead to consumption of intakes above the upper levels by some, especially men *(35)*. Fortified-food consumption by those vulnerable to low calcium

Table 2
Comparing Sources for Absorbable Calcium

Source	*Serving size*[1] *(g)*	*Calcium content*[b] *(mg/serving)*	*Estimated absorption efficiency*[c] *(%)*	*Absorbable Ca/serving*[d] *(mg)*	*Servings needed to = 1 cup milk*	*Reference*
Foods:						
Milk	240	290	32.1		1.0	12
Beans, pinto	86	44.7	26.7	11.9	8.1	13
Beans, red	172	40.5	24.4	9.9	9.7	13
Beans, white	110	113	21.8	24.7	3.9	13
Bok choy	85	79	53.8	42.5	2.3	14
Broccoli	71	35	61.3	21.5	4.5	4
Cheddar cheese	42	303	32.1	97.2	1.0	12
Cheese food	42	241	32.1	77.4	1.2	12
Chinese Cabbage Flower leaves	85	239	39.6	94.7	1.0	15
Chinese Mustard green	85	212	40.2	85.3	1.1	15
Chinese Spinach	85	347	8.36	29	3.3	15
Kale	85	61	49.3	30.1	3.2	16
Spinach	85	115	5.1	5.9	16.3	17
Sugar cookies	15	3	91.9	2.76	34.9	18
Sweet Potatoes	164	44	22.2	9.8	9.8	15
Rhubarb	120	174	8.54	10.1	9.5	15
Whole wheat bread	28	20	82.0	16.6	5.8	18
Wheat bran cereal	28	20	38.0	7.54	12.8	18
Yogurt	240	300	32.1	96.3	1.0	12
Fortified foods:						
Tofu, calcium set	126	258	31.0	80.0	1.2	19
Orange juice with Ca citrate malate	240	300	36.3	109	0.88	20
Soy milk with tricalcium phosphate	240	300	24	72	1.3	21
Bread with calcium sulfate	16.8	300	43.0	129	0.74	22

[a]Based on a one-half cup serving size (~85 g for green leafy vegetables) except for milk and fruit punch (1 c or 240 mL) and cheese (1.5 oz).

[b]Taken from refs. *10* and *11* (averaged for beans and broccoli processed in different ways) except for the Chinese vegetables which were analyzed in our laboratory.

[c]Adjusted for load using the equation for milk (fractional absorption = 0.889–0.0964 ln load *[23]*) then adjusting for the ratio of calcium absorption of the test food relative to milk tested at the same load, the absorptive index.

[d]Calculated as calcium content × fractional absorption.

Table 3
Percent Calcium in Common Salts

	%
Calcium carbonate	40
Tricalcium phosphate	38
Dicalcium phosphate, dihydrate	29
Bone meal	31
Oyster shell	28
Dolomite	22
Calcium citrate	21
Calcium citrate malate	13
Calcium lactate	13
Calcium gluconate	9
Calcium glubionate	6.5

intakes can be very helpful. However, few calcium-fortified foods have been tested for bioavailability. Some are shown in Table 2. The choice of the calcium salt used as a fortificant depends on compatibility with the food and processing considerations for texture and stability as well as cost. When calcium carbonate is heated in the presence of food acids, carbon dioxide is released, which is undesirable for many products. Anions may influence flavor. Citrate and malate anions are compatible with fruit juice. The bulk of the total salt required to fortify a food depends on the proportion of calcium in the salt (Table 3).

Most pure salts have similar calcium absorption, but the food matrix can affect absorption substantially so they must be tested. For example, calcium absorption from tricalcium phosphate-fortified soy milk was lower than that of cow's milk *(21)*, even though the pure salt is similarly absorbed to milk calcium *(26)*. This would not have been predicted from other studies using similar products i.e., calcium absorption from calcium-set tofu was not significantly different than that from milk *(19)*. Calcium as calcium sulfate in high-calcium water is also similarly absorbed *(36)*, but few waters have been tested for absorbability. When calcium citrate malate (CCM) has been used as the fortificant, absorption has been reported to be approx 5–10% higher in some studies *(20,37)* but not others *(20,38)*, nor was postprandial parathyroid hormone (PTH) suppression different between orange juice fortified with CCM and milk in elderly subjects *(39)*. Calcium absorption from $CaSO_4$-fortified bread and cereal was also found to be comparable with milk *(22)*. Calcium-fortified breakfast cereal was a good delivery vehicle for children *(40)*. Although few fortified foods have been tested for calcium bioavailability, even fewer have been tested for their benefits on bone. One randomized, controlled trial in 149 prepubertal girls, using food products fortified with 850 mg calcium from milk extracts daily for 1 yr, showed a significant gain in bone mass and bone size in six skeletal sites as well as height due with the fortified products *(41)*, compared to control foods.

5. SUPPLEMENTS

Calcium supplements are usually prescribed to prevent, or treat patients with, osteoporosis. It is considered easier to prescribe supplements than to work with a patient to meet their calcium needs through diet. Supplements vary considerably in characteristics and cost.

The ability to chew, swallow, and tolerate a supplement will influence compliance. Supplements with heavy metal contaminants should be avoided.

Most salts of calcium have similar absorbability, as shown by isotopic tracer studies, so long as the dose size is similar. Moreover, supplement calcium absorbability is comparable with that of milk (Table 4). Milk calcium, calcium citrate, and CCM have been compared with calcium carbonate by PTH suppression and found similar as well *(39,43)*. Calcium oxalate is poorly absorbed because it is extremely insoluble.

Our work with calcium oxalate demonstrated that an external calcium tracer is not exchangeable with the calcium in the salt *(28)*. Furthermore, although absorption is poor, the salt is absorbed intact, that is, without dissociation *(44)*. Small molecules like calcium oxalate and calcium carbonate can be absorbed to some extent in the lower gut without being dissociated in the presence of acid in the stomach and without requiring vitamin D-enhanced saturable absorption *(45)*.

Several calcium salts have been extensively marketed as superior sources, often based on solubility. Sometimes the evidence is based on crude methods of calcium absorption, as for coral calcium *(46)* and algal calcium (produced by heating oyster shell calcium and seaweed *[47]*). When sensitive isotopic tracer methods are used to assess calcium absorption, controversy over comparison of salts can be clarified as was done for calcium citrate. Calcium carbonate and calcium citrate salts have comparable bioavailability (Table 4). A rather new series of salts, calcium fumarate and calcium malate fumarate, are also absorbed similarly to calcium carbonate, calcium citrate, and CCM in rats *(38)*. Calcium ascorbate has unusually high absorbability, at least in the rat model *(48,49)*.

Absorbability of calcium from pharmaceutical preparations can fall short of what would be expected from studies of the pure salts. The presence of binding agents and other ingredients in the formulation can affect calcium absorption appreciably. One such supplement provided one-half of the bioavailable calcium as the pure salt *(50)*. Furthermore, the cost of supplements can vary fivefold *(43)*. Calcium carbonate supplements tend to be the least expensive supplemental source of calcium *(25)*. Supplement use is more prevalent in individuals with a higher education and higher incomes *(51)*.

The best source of calcium is food, because good health is dependent on a good diet, not adequacy of a single nutrient. Dairy products provide not only calcium, but a rich source of many nutrients and functional components. Milk and yogurt are the best and most economical way to obtain the whole package of nutrients important to bone health. Sometimes, fortified foods or supplements are important for an individual's meeting of their calcium requirements. Choices may be influenced by preference, convenience, cost, tolerability, the presence of other nutrients, and the absence of undesirable contaminants. It is important that the calcium bioavailability of the selected form of these manufactured sources of calcium be established.

6. CONCLUSIONS

Dairy products provide nearly three-fourths of dietary calcium in the Western diet. Individuals who do not consume approximately three servings of dairy products daily are likely to have inadequate calcium intakes unless they select calcium-fortified foods or supplements. They are also more likely to be deficient in other micronutrients. The various sources of calcium in the diet should be evaluated for total calcium content and bioavailability. Exogenous and endogenous factors that influence calcium absorption also influence calcium nutrition.

Table 4
Calcium Absorption From Salts

Source	*Load (mg)*	*Population*	*Absorption efficiency (%)*	*Estimated absorbable calcium (mg)*	*Normalized to milk*	*Normalized to $CaCO_3$*	*Ref.*
Calcium sulfate	250	Premenopausal women	41 ± 7	102.5			*21*
Calcium lactate	250	Premenopausal women	47 ± 8	117.5			*21*
Calcium glubionate	200	Postmenopausal women	36.8	73.6		0.75	Unpublished
Calcium glycerophosphate	300	Premenopausal women	27.1	81.3	0.868	0.712	Unpublished
Calcium oxalate	200	Premenopausal women	10.2 ± 4.0	20.4			*27*
Tricalcium phosphate	200	Premenopausal women	25.2 ± 13.0	50.4			*19*
$CaH\ PO_4$	300	Premenopausal women	24.8	74.4	0.919		Unpublished
$CaH\ PO_4 \cdot 2\ H_2O$	300	Premenopausal women	27.4	82.1	1.012		Unpublished
Calcium citrate malate	250	Premenopausal women	37.3 ± 2.0	93.3			*26*
	250	Adolescents	36.2 ± 2.7	90.5		1.37	*37*
Calcium citrate	300	Adult men, premenopausal women	37.9 ± 10.4	113.7		1.1	*42*
	1000	Adult men, premenopausal women	26.8 ± 6.9	26.8	0.975	0.89	
Calcium carbonate	200	Premenopausal women	41.2	82.6	1.117		Unpublished
	250	Premenopausal women	39 ± 7				*21*
	300	Adult men, postmenopausal women	34.2 ± 10.1	102.6			*42*
	1000	Adult men, postmenopausal women	30.1 ± 5.4	301			

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10 The Calcium Economy

Robert P. Heaney

KEY POINTS

- Calcium ion concentration in extracellular fluid (ECF [Ca^{2+}]) is the central, controlled quantity in the operation of the calcium economy.
- ECF [Ca^{2+}] is sustained by three independent control loops, involving bone resorption, renal clearance, and intestinal absorption.
- Parathyroid hormone (PTH) acts on all three effector systems to protect against hypocalcemia.
- Differences in calcium intake requirements in different ethnic groups and at different life stages are due to differences in relative responsiveness to PTH of the three effector loops.
- This system functions optimally when dietary calcium intakes are at or above currently recommended values, i.e., *both* ECF [Ca^{2+}] and bone mass are protected. At lower calcium intakes, ECF [Ca^{2+}] is sustained, but decreased calcium intake or altered calcium demands reduce bone mass.

1. CALCIUM IN THE BIOSPHERE

Calcium is the fifth most abundant element in the biosphere (after iron, aluminum, silicon, and oxygen). It is the stuff of limestone and marble, coral and pearls, seashells and eggshells, antlers and bones. Because calcium salts exhibit intermediate solubility, calcium is found both in solid form (rocks) and in solution. It was probably present in abundance in the watery environment in which life first appeared. Today, seawater contains approx10 mmol calcium per liter (approximately eight times higher than the calcium concentration in the extracellular water of higher vertebrates). Even fresh waters, if they support an abundant biota, typically contain calcium at concentrations of 1–2 mmol (in the range of vertebrate extracellular fluid [ECF] calcium levels). In most soils, calcium exists as an exchangeable cation in the soil colloids. It is taken up by plants, whose parts typically contain from 0.1 to as much as 8% calcium. Generally, calcium concentrations are highest in the leaves, lower in the stems and roots, and lowest in the seeds (a fact that has important consequences for the shift to seed-based foods at the time of the agricultural revolution).

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Edited by: C. M. Weaver and R. P. Heaney

2. CALCIUM IN THE HUMAN BODY

In land-living mammals, calcium accounts for 2–4% of gross body weight. A 60 kg adult human female typically contains approx 1000–1200 g (25–30 mol) of calcium in her body. More than 99% of that total is in the bones and teeth. Approximately 1 g (25 mmol) is in the plasma and ECF bathing the cells, and 6–8 g (150–200 mmol) in the tissues themselves (mostly sequestered in calcium storage vesicles inside of cells (*see* Chapter 3).

In the circulating blood, calcium concentration is typically 2.25–2.5 mmol (9–10 mg/dL). Approximately 40–45% of this quantity is bound to plasma proteins, approx 8–10% is complexed with ions such as citrate, and 45–50% is dissociated as free ions. In the ECF outside of the blood vessels, total calcium is on the order of 1.25 mmol (5 mg/dL). It is the ionic calcium concentration ($[Ca^{2+}]$) in the ECF which the cells see, and which is tightly regulated by the parathyroid, calcitonin (CT), and vitamin D hormonal control systems (discussed later; *see also* Chapter 11).

ECF $[Ca^{2+}]$ is one of nature's great physiological constants, extending across the vertebrate phylum (at least in healthy individuals of the species concerned). When elevations of serum calcium occur in different physiological situations (such as during egg laying in reptiles and birds), the elevation is almost always in the protein-bound fraction, not in the ionized calcium concentration.

The ECF calcium serves two major groups of functions. It is the source of the calcium that pours into the cells of many tissues at the point of their activation, thereby triggering the specific cascade that produces tissue-specific cellular responses (*see* Chapter 3). Here, ECF concentration is critically important, and clinicians have long recognized that hyper- and hypocalcemia are each associated with neuromuscular symptoms such as hypo- and hypertonia, conduction defects on electrocardiograms, and overt clinical symptoms such as constipation or muscular spasms and rigidity.

The second role of ECF calcium is that its ions constitute the multidirectional calcium "traffic," that is, calcium entering the circulation through absorption of dietary calcium or resorption of bone calcium, and calcium leaving the blood in the process of bone mineralization, or through excretory or cutaneous losses. Both sets of processes are closely integrated in many complex ways, one of the more obvious of which is the fact that the physiological apparatus regulating ECF $[Ca^{2+}]$ also affects the fluxes in and out of the ECF.

Figure 1 depicts the principal routes of entry into and exit from the ECF, and includes typical values for transfer rates in a woman approx 5 yr postmenopause. It is necessary to stress, however, that the indicated values of these transfer rates are highly interdependent. The individual processes are considered briefly in the sections that follow, but their interrelationships can be briefly summarized with some examples.

When absorptive input from the diet falls, bony resorption rises to offset the absorptive shortfall. This effect is produced by an increased secretion of parathyroid hormone (PTH). The immediate consequences are maintenance of the extracellular $[Ca^{2+}]$ and an offsetting reduction (however small) of the bony reserves of calcium. Similarly, vigorous physical exercise leads to sweat losses that can be 10–20 times the level of resting losses shown in Fig. 1 *(1)*. Also, various nutrient–nutrient interactions may alter either calcium absorption efficiency or obligatory urinary calcium losses. Sodium, (in the form of sodium chloride), for example, can increase urinary calcium by approx 1 mmol per 100 mmol

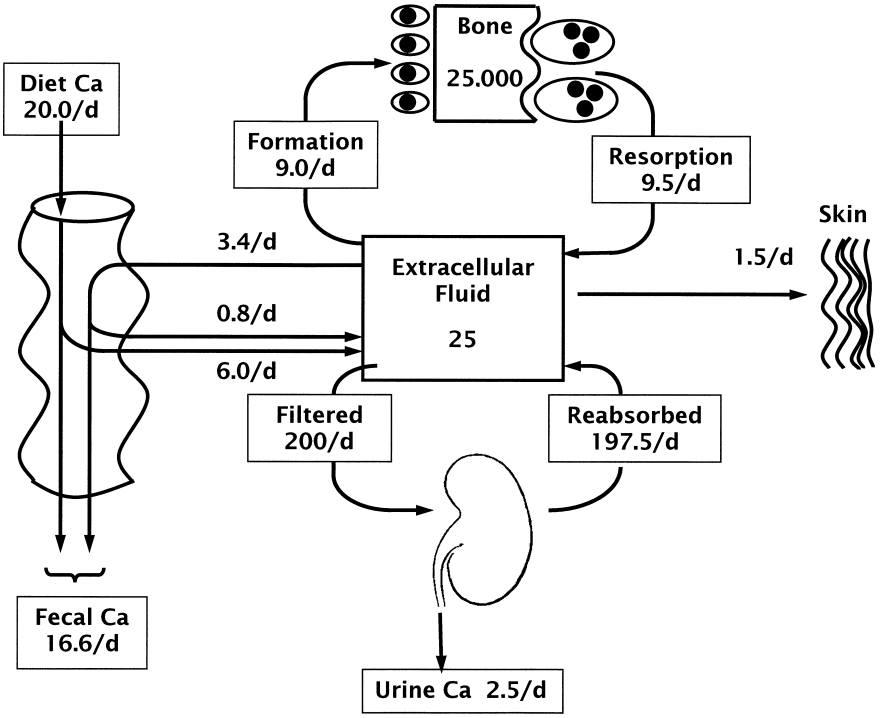

Fig. 1. Principal routes of calcium entry into and exit from the extracelluar fluid (ECF) of an adult human. The values for bone and ECF are total masses; transfer rates are given in mmol/d and represent typical values. *See also* Fig. 4 for expanded detail of endogenous calcium entry into the gut. Total body balance in this illustration is –0.5 mmol/d. (Copyright Robert P. Heaney, 1996, 2004. Used with permission.)

ingested salt *(2,3)*. These nutrient influences, together with great variability in food choices and hence, dietary calcium intake, constitute unregulated stresses on the system (i.e., they are perturbations to which the control mechanisms must respond).

In brief, the system depicted in Fig. 1 operates as an integrated whole: change in the size of one movement evokes opposite changes in one or more of the others. For most stresses, bone resorption is the factor that is regulated up or down to compensate.

The examples just cited represent influences that, if not countered would result in a lowering of ECF [Ca^{2+}]. But the opposite stress, that is, a trend toward hypercalcemia, can be equally important and/or threatening. This half of the regulatory control environment is rarely encountered in adult human physiology, largely because contemporary diets are relatively low in calcium, and hypercalcemic stresses, accordingly, uncommon. However, animals with naturally high calcium intakes, subjected to thyro-parathyroidectomy but given thyroid replacement (i.e., deprived only of PTH and CT) tend to exhibit not so much *hypo*calcemia as wildly fluctuating levels of ECF calcium—sometimes low, sometimes high—depending almost totally on absorptive inputs from the gut.

These examples are intended simply to introduce the "push–pull" character of the regulatory system and the way it responds to unregulated inputs. More detailed description of system operation follows.

3. CONTROL MECHANISMS

The concentration of calcium in the ECF is maintained in two distinct ways: (1) by a combination of adjustments to the inputs and outputs in Fig. 1, and (2) by controlling the level of the renal calcium threshold. This latter function, though very well established, is commonly underappreciated, and is at least as important as the control of inputs. A threshold, in the context of excretion, functions much like a dam at the downstream end of a pond. Inputs serve to elevate the level of the pond until that level reaches the height of the dam. Then further inputs spill out of the pond, over the dam. Because the threshold is the point at which blood calcium begins to spill into the urine, it is clear why raising that point is a first line defense against renal calcium loss. PTH, by augmenting tubular reabsorption of filtered calcium, is the principal regulator of the renal calcium threshold. The importance of the threshold in the regulation of ECF [Ca^{2+}] is clearly evidenced in the common clinical experience of the difficulty of elevating serum calcium in patients with hypoparathyroidism, even with sometimes heroic inputs of calcium into the system.

The physiological effects of PTH are complex and are diagrammed schematically in Fig. 2. These hormonal actions, in approximately the order in which they occur, can be described briefly as follows: (1) decreased renal tubular reabsorption of serum inorganic phosphate (P_i); (2) increased resorptive efficiency of osteoclasts already working on bone surfaces; (3) increased renal 1α-hydroxylation of circulating 25 hydroxyvitamin D (25[OH]D) to produce calcitriol, the chemically most active form of vitamin D; (4) increased renal tubular reabsorption of calcium (the mechanism behind elevation of the renal threshold); and (5) activation of new bone remodeling loci. These effects interact and reinforce one another in important ways, indicated by the connections between the loops of Fig. 2. For example, the reduced ECF P_i caused by the immediate fall in tubular reabsorption of phosphate is a potent stimulus to the synthesis of 1,25 dihydroxyvitamin D (1,25$[OH]_2$D), and it also increases the resorptive efficiency of osteoclasts already in place and working in bone. 1,25$(OH)_2$D directly increases intestinal absorption of both ingested calcium and the endogenous calcium contained in the digestive secretions. It also is necessary for the full expression of PTH effects in bone, particularly the maturation of cells in the myelomonocytic line that produce new osteoclasts, and ultimately for an efficient resorptive response to PTH.

The three arms of Fig. 2 make graphic the fact that the system uses three independent end-organs to regulate ECF [Ca^{2+}]—what Chapter 11 refers to as a "tri-axial system". Their actions are to reduce losses through the kidneys, to improve utilization of dietary calcium, and to draw down calcium from the bony reserves. The aggregate effect of them all, as Fig. 2 indicates, is to prevent or reverse a fall in ECF [Ca^{2+}]. Importantly, PTH secretion is inversely related to the amount of calcium made available by the aggregate effect of *all three mechanisms*, not to the response of one or the other of them.

Although hypocalcemia is a much more common risk in contemporary adults than is hypercalcemia, in infants and small children both deviations would be a physiological threat. The principal defense against hypercalcemia is release of CT by the *C* cells of the thyroid gland. CT is a peptide hormone with binding sites in the kidney, bone, and central nervous system. Absorption of calcium from an 8-oz feeding in a 6-mo-old infant dumps 150– 220 mg calcium into the ECF. This is enough, given the small size of the ECF compartment at that age (1.5–2 L), to produce near-fatal hypercalcemia if other adjustments are not made. What happens is that CT is released, in part in response to the rise

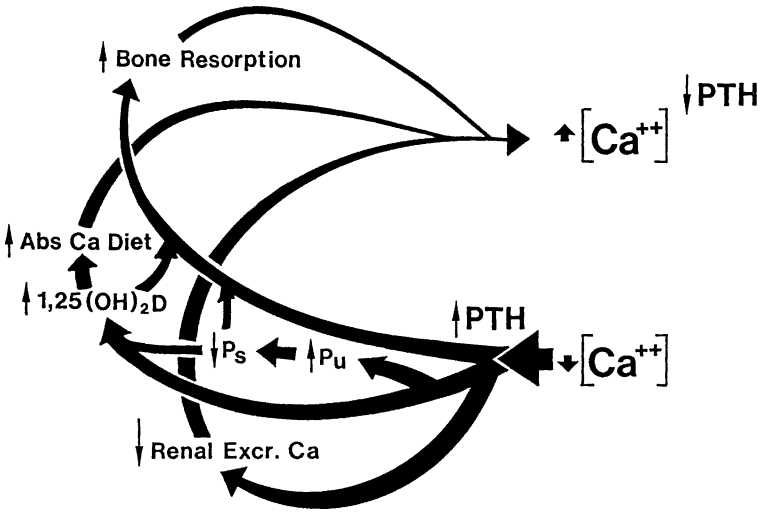

Fig. 2. Schematic depiction of the 3-arm control loop regulating extracellular fluid (ECF) [Ca^{2+}], showing specifically the response to a drop in [Ca^{2+}]. (P_s is serum inorganic phosphorus concentration and P_u is urinary phosphorus clearance.) (Adapted from Arnaud *[4]*. Copyright Robert P. Heaney, 1981. Used with permission.)

in serum calcium concentration, but even before that, in response to gut hormones signaling the digestive activity that will lead to absorption. This burst of CT slows or halts osteoclastic resorption, thus stopping bony release of calcium. Later, when absorption falls, CT levels fall also, and osteoclastic resorption resumes.

By contrast, CT has little significance in adults because calcium absorption is less efficient in adults to begin with, and the ECF is vastly larger. As a result, transient absorptive calcemia from a high calcium diet raises the ECF [Ca^{2+}] by only a few percentage points (approx 1% for each 100 mg calcium ingested at typical intakes). For this reason CT deficiency is not recognized as causing disease or dysfunction in adults consuming typical diets.

4. ENDOGENOUS FECAL CALCIUM LOSS

Calcium is contained in all of the digestive secretions, as well as in the mucosal cells themselves (which turn over approximately every 5 d). Together, these sources account for entry of endogenous calcium into the gut amounting to approx 0.05 mmol (2 mg)/kg/d, or in a typical middle-aged woman, approx 3.5 mmol (140 mg)/d *(5)*. Both because absorption efficiency for calcium is low (discussed later), and because some of the digestive juice calcium enters the lumen downstream of the sites of most active absorption, most of this endogenous calcium ends up in the feces and is generally designated "endogenous fecal calcium" (EFCa). The quantity entering the gut is not regulated to an appreciable extent by the hormones otherwise controlling the calcium economy. The principal factors known to influence that entry are phosphorus intake and mucosal mass *(6)*. Because most of the endogenous calcium entering the gut does so above the ileum, it is subject to absorption as if it were food calcium. Hence, EFCa is inversely related to absorption efficiency and directly to calcium intake. It constitutes one of the unregulated drains on

the calcium economy to which the control system must react. EFCa is measurable only by isotopic tracer methods (*see* Chapter 9), and hence cannot be assessed clinically. Nevertheless, when it is measured, it is found to account for a somewhat greater share of the variability in total body calcium balance than does actual oral calcium intake.

5. URINARY LOSS

Calcium losses in the urine are dependent on filtered load, except during infancy and adolescence. During these periods of rapid growth, at calcium intakes typically ingested, most of the absorbed calcium is diverted to bone growth and little spills into the urine.

Machinery for calcium transport, most extensively studied in intestinal epithelial cells, is also present in the nephrons of the kidney, but it is not known to what extent it is functional there (*see* Chapter 11 for details). The process is calcium load dependent, stimulated by PTH and $1,25(OH)_2D$, and has a microvillar myosin I-calmodulin complex that could serve as a calcium transporter *(7)*. Active transport occurs in the distal convoluted tubule against a concentration gradient. Renal calcium clearance is increased when PTH concentration in blood is low, thereby protecting against hypercalcemia when bone resorption is high for reasons other than homeostasis. Tubular reabsorption is determined to some extent by sodium chloride excretion. For every 100 mmol of sodium chloride excreted, approx 0.5–1.5 mmol of calcium is pulled out with it in the urine *(2,3)*.

Urine calcium rises with absorbed calcium intake, but the relationship is loose and depends strongly on the circulating level of PTH at the time. This alimentary rise is partly due to the small increase in blood calcium following absorption of ingested calcium, with a corresponding increase in the filtered load of calcium. Available data from healthy adults indicates that urinary calcium rises on dietary intake with a slope of approx +0.045, meaning that, for every 400 mg (10 mmol) rise in intake, urine calcium rises by approx 18 mg (0.45 mmol). But there is much variability around this average figure and the range of normal is accordingly very broad. Table 1 sets forth observed ranges in healthy estrogen-replete and estrogen-deprived adult women, both as absolute values and as weight-adjusted values *(8)*. The latter can be applied to men because the difference in urine calcium between the sexes is due principally to the generally greater body weight of men.

6. CUTANEOUS LOSS

Calcium is contained in all cells, and for organs such as the intestinal mucosa, which turns over approximately every 5 d, loss to the body of the component cells means loss of their calcium as well. The same is true with epidermis and skin appendages (hair and nails), all of which contain some calcium. This shedding thereby produces a steady calcium drain on the system. It is the sum total of these cell-related cutaneous calcium losses which is represented in Fig. 1 by the rough estimate of 60 mg (1.5 mmol)/d. Sweat losses have not been extensively studied, but such data as are available indicate that heavy physical exercise in a hot environment, leading to extensive sweating, can increase sweat losses to levels as high as 200–400 mg (5–10 mmol)/d. In one study of athletes, these losses were sufficient to produce a measurable decrease in bone mineral density (BMD; i.e., a detectable reduction of the nutrient calcium reserve) across a playing season, despite the relatively high dietary calcium intakes typical of varsity athletes *(1)*. A controlled trial of calcium supplementation in the same athletes showed that supplemental

Table 1
Distribution of 24-h Urinary Calcium Values in Normal Middle-Aged Women

Estrogen-replete *Percentile*	*mmol(mg)/d*	*mmol(mg)/kg/d*
97.5	6.3 (252)	0.104 (4.15)
95.0	5.4 (215)	0.093 (3.72)
90.0	4.9 (197)	0.081 (3.23)
50.0	2.9 (116)	0.046 (1.86)
10.0	1.5 (62)	0.024 (0.99)
5.0	1.3 (53)	0.021 (0.83)
2.5	1.1 (44)	0.017 (0.67)
Estrogen-deprived *Percentile*	*mmol(mg)/d*	*mmol(mg)/kg/d*
97.5	7.6 (303)	0.126 (5.05)
95.0	6.6 (264)	0.107 (4.27)
90.0	5.6 (225)	0.091 (3.66)
50.0	3.3 (134)	0.054 (2.15)
10.0	2.0 (81)	0.028 (1.12)
5.0	1.4 (55)	0.020 (0.80)
2.5	0.9 (38)	0.014 (0.56)

Reproduced from ref. *8*

calcium, above that which could be provided by diet, was able to prevent this seasonal, exercise-related bone loss. This instance probably represents an extreme situation, but it illustrates nicely the function of bone as the body's calcium nutrient reserve, and also a point, to be discussed further below, that, given relatively inefficient dietary extraction of calcium, there are limits to how much calcium the organism can get from food to offset unregulated losses.

7. INTESTINAL ABSORPTION

The pathways for calcium absorption and its regulation are discussed in Chapter 11. The relationship between calcium intake and absorption fraction is shown in Fig. 3. At lower calcium intakes, the active component contributes importantly to absorbed calcium. As calcium intakes increase, the active component becomes saturated and vitamin D-mediated synthesis of calbindin drops. Thus an increasing proportion of absorption is accounted for by passive diffusion. The figure illustrates that, across most of the intake range, the adaptive component is rather small. This partly explains the inefficiency of human ability to compensate for a fall in calcium intake.

Another key feature of Fig. 3 is the fact that absorption is substantially incomplete (fractional absorption averaging less than 0.30 at intakes in the range of recommended values). Moreover, net absorption fraction is lower still, averaging in the range of 0.10–0.15. The difference is due to the counter-movement of calcium into the gut in the form of mucosal cells and digestive secretions (*see* Subheading 4.). Figure 4 presents a worked

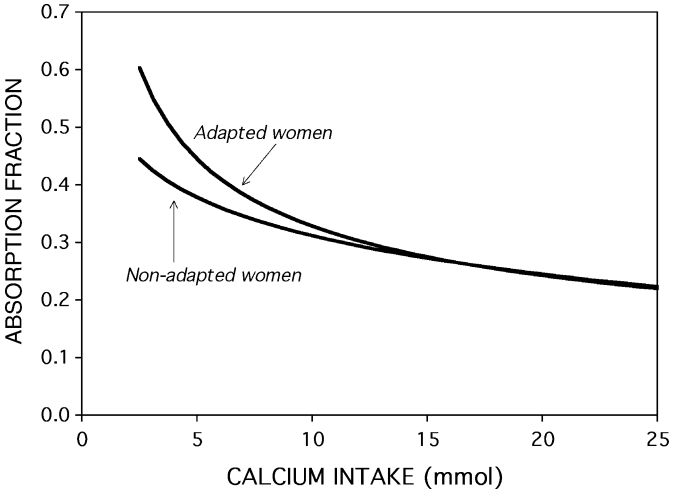

Fig. 3. Relationship between calcium intake and absorption fraction in women studied on their usual calcium intakes (adapted) and in women tested with no prior exposure to the test load (nonadapted). (Copyright Robert P. Heaney, 1999. Used with permission.)

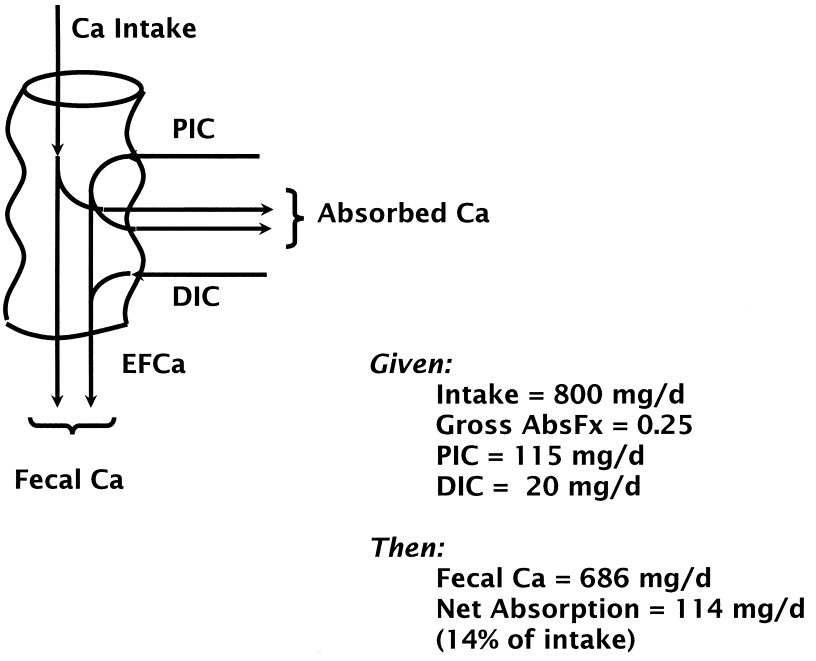

Fig. 4. Schematic depiction of the bidirectional movements of calcium into and out of the intestinal lumen. PIC, proximal intestinal calcium, i.e., calcium entering the gut effectively proximal to the principal absorption sites; and DIC, distal intestinal calcium, i.e., that calcium entering the gut distal to the principal absorption sites. (Copyright Robert P. Heaney, 2004. Used with permission.)

example in which a 25% gross extraction figure translates to 14% net absorption. The relative inefficiency of absorption of calcium is probably a reflection of the abundance of calcium in the foods available to the high primates and, presumably, to human hunter-gatherers. However, at the same time it is important to note that unabsorbed food calcium is not just wasted. As is described in Part VI of this book, luminal calcium binds with, and hence renders innocuous, potentially harmful byproducts of digestion.

Various host factors affect calcium absorption efficiency. Vitamin D status, intestinal transit time, mucosal mass, and stage of life are the best established. In infancy, absorption is dominated by paracellular diffusion. (For that reason, the vitamin D status of the mother has little effect on calcium absorption in young breast-fed infants.) Both active and passive calcium transport are increased during pregnancy and lactation. Calbindin and plasma $1,25(OH)_2D$ and PTH levels increase during pregnancy. From midlife on, absorption efficiency declines by approx 0.2 absorption percentage points per year, with an additional 2% decrease at menopause *(9)*.

It has long been recognized that calcium absorptive efficiency increases as the size of the ingested load falls. This relationship has two components: an effect of load itself and variation in vitamin D-mediated active absorption. Within individuals, absorptive efficiency generally varies approximately inversely with the logarithm of intake, but the *absolute* quantity of calcium absorbed increases nonlinearly with intake *(10,11)*. However, only 20% of the variation in calcium absorption can be accounted for by differences in intake. Individuals seem to have preset absorptive efficiencies, some high, others low.

The canonical inverse relationship between intake and absorption fraction has often been uncritically assumed to mean that the body can adapt perfectly well to reduced intake. However, extensive studies in which absorption has been measured by isotopic tracer methods show very clearly that, although fractional absorption does rise (Fig. 3), the increase is far short of what would be needed to maintain a constant mass transfer rate across the intestinal mucosa. Figure 5 illustrates this point with one such set of data. The regression line through the data in Fig. 5 is for a simple linear model, and more detailed investigations of the low intake end of the curve indicate that the rise is initially steeper, reflecting the active transport response to low intake discussed above. The slope of the line across the full range of intakes in Fig. 5 is +0.158, meaning that 15.8% of ingested calcium is absorbed, overall. If analysis is confined to intakes at the high end of the range, the slope drops to approx +0.12. This means that the body absorbs approx 12% of any additional amount of calcium that may be ingested. This value is the approximate midpoint of the range for net absorption noted above. At all intakes, the distribution of absorption values is broad, as the spread of the data in Fig. 5 demonstrates.

The relationship of absorption fraction to load size, and typical absorption values for a variety of sources, are illustrated in Fig. 6. First the figure summarizes the data from three groups of sources: milk calcium (the principal dietary source of calcium in the industrialized nations), calcium carbonate (the principal calcium salt used in calcium supplements), and finally calcium oxalate. What the figure clearly shows in this regard is that, altogether apart from the intrinsic absorbability of the calcium source, absorption varies linearly and inversely with the logarithm of the load size. Furthermore, because all of the studies summarized in Fig. 6 were acute studies, in which the subjects were not given an opportunity to habituate themselves to a particular calcium source or level of calcium intake, the relationships to load depicted are purely physical: there is no physi-

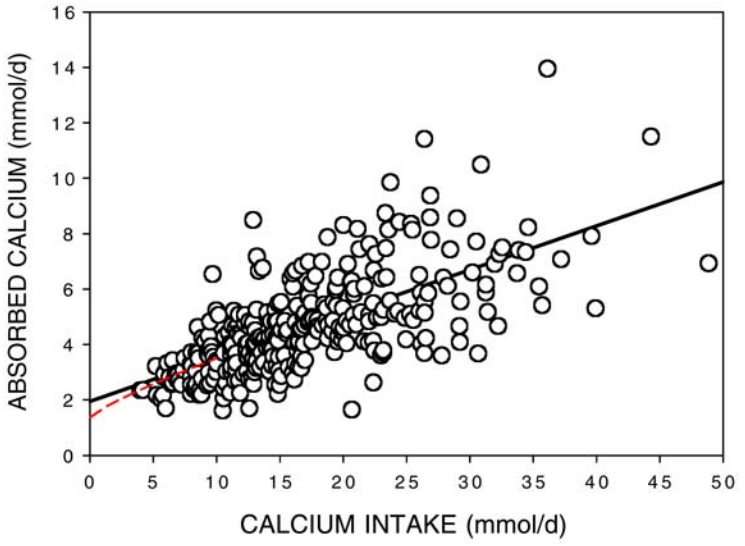

Fig. 5. Absorbed calcium plotted as a function of intake in 332 studies in middle-aged healthy women studied on their usual calcium intakes. (Copyright Robert P. Heaney, 2001. Used with permission.)

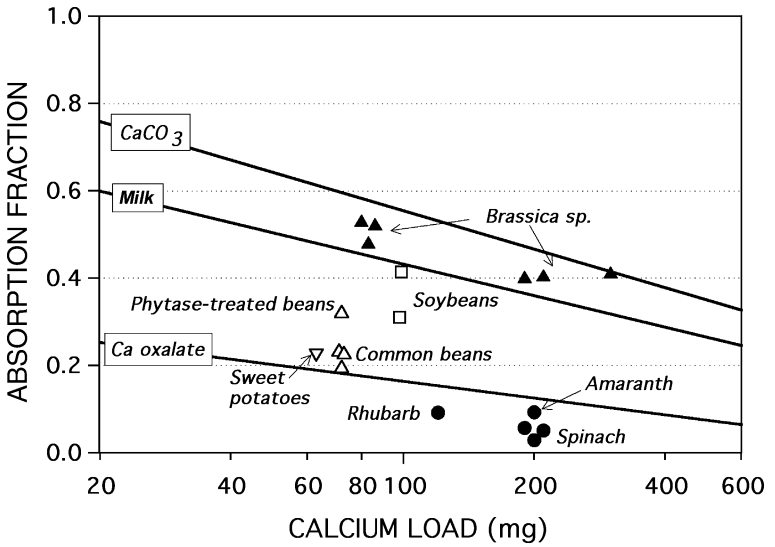

Fig. 6. Regression lines fitted to fractional absorption values at various load sizes for three families of calcium sources. Topmost is the line for plain calcium carbonate. Next is the line for milk calcium. The lowest is the line for calcium oxalate and the high oxalate vegetables (e.g., spinach and rhubarb). For all three groups there is an inverse linear relationship with the logarithm of load size (i.e., at low load sizes, a larger fraction of the load is absorbed than at high loads). Mean fractional absorption values for various other food sources are plotted for their respective intake loads. (Copyright Robert P. Heaney, 2001. Used with permission.)

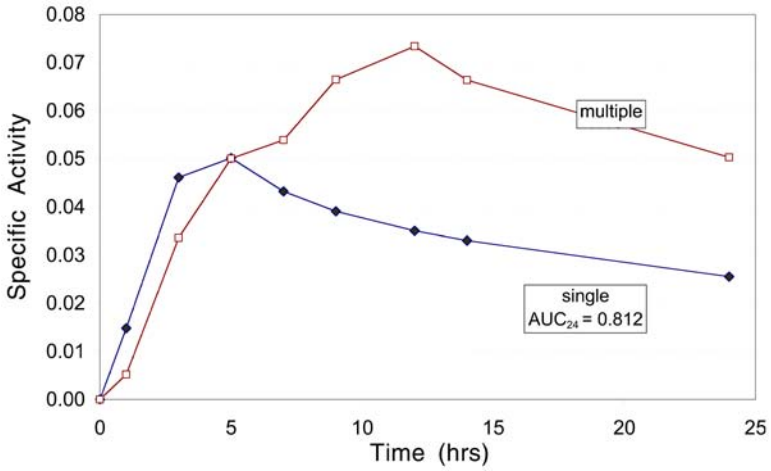

Fig. 7. Time course through 24 h for the mean specific activity values for two calcium dosing regimens. In the first (labeled "single"), 1000 mg Ca (25 mmol) was ingested as a single bolus at breakfast, and in the second (labeled "multiple"), the same total load was ingested in 17 equally spaced doses of 59 mg (1.5 mmol) each, ingested at 0.5-h intervals. (Copyright Robert P. Heaney, 2000. Used with permission.)

ological adjustment component, that is, no compensating alteration of $1,25(OH)_2D$-mediated active absorption.

There are several practical consequences of this load relationship. One is that dividing calcium intake into multiple doses over the course of a day results in much more efficient absorption than ingesting the same total quantity in a single dose. This point is illustrated in an experiment shown in Fig. 7, in which healthy individuals were given the same tracer-labeled calcium load (25 mmol), either as a single bolus at breakfast, or as 17 individual doses of 1.47 mmol at 30-min intervals, starting with the same breakfast and continuing for the next 8 h *(12)*. Figure 7 shows graphically, and pharmacokinetic calculation reveals explicitly, that the area under the curve (AUC) for the divided dose regimen was substantially higher than that for the single dose regimen. (At 24 h, AUC was approx 50% higher for the divided dose regimen, and for AUC^{∞}, the difference was nearly twofold.) A related consequence deals with the interpretation of published studies in which calcium supplements were used. Even if the aggregate daily doses were the same in two studies, when the dosing regimens are different, the effective *delivered* dose will be predictably different.

It is worth noting in passing that the primitive human diet, which would have been relatively calcium-rich in most of its constituents, would more closely have approximated the continuous dosing regimen. Hence, not only would the primitive calcium intake have been higher than we currently experience, but its pattern of ingestion would have likely delivered calcium into the body more efficiently than modern humans generally manage.

8. BONE CALCIUM TURNOVER

As the numbers in Fig. 1 indicate, the turnover of bone calcium, in the process of bone modeling and remodeling, accounts for roughly half of the total turnover of the ECF calcium in a typical healthy adult. (The proportion would be substantially higher during growth.) A single cubic centimeter of bone contains approx 400 mg (~10 mmol) calcium, equivalent to approx 40% of the total calcium in the entire ECF of an adult. Essentially all of that bone calcium is locked away in intimate association with the collagen fibers of the bone matrix, and for the most part it can be released into the blood only by physically tearing down a unit of bone through osteoclastic resorption. Similarly, calcium deposition in bone occurs as a result of another cellular activity, the osteoblastic deposition of collagen matrix, and its subsequent alteration to create crystal nuclei suitable for aggregating calcium and phosphate as hydroxyapatite.

Both processes are cell-mediated. However, with mineral deposition, the timing of the mineral entry lags behind the cell's deposition and activation of the matrix. Because hormonal control mechanisms, whether endocrine or paracrine, act only through functioning cells, it follows that mineral deposition in bone is much less *acutely* controllable than is mineral removal. Previously nucleated bone matrix creates a mineral drain, or debt, which is paid by extracting mineral from blood flowing past the new bone-forming site, and stopping osteoblastic bone formation will not stop mineralization of the last several days' accumulation of deposited matrix. By contrast, both PTH and calcitonin can act very promptly on osteoclastic resorption.

Hence, in the scheme of Fig. 1, it is the resorptive component of bone turnover which is the one most responsive to alterations of calcium movement into and out of the body. This is shown very nicely in the study by Wastney et al. *(13)* in adolescent girls, in which, across different calcium intakes, bone formation remained constant, whereas bone resorption varied inversely with calcium intake.

In the foregoing, we have emphasized transfers into and out of bone through bone remodeling. Quantitatively, this route seems by far the more important. However, there are competent bone biologists who believe physical–chemical dissolution plays an important role *(14)*. The most likely candidate for such an effect may involve the calcium carbonate of bone. Although bone mineral is commonly assumed to be hydroxyapatite, the fact is that bone contains a substantial amount of carbonate, which varies in magnitude from species to species and from one metabolic state to another. Presumably, the counter ion for the carbonate is calcium. Importantly, the carbonate content of bone appears to be substantially more labile than its phosphate content, being depleted quite rapidly under conditions of acidosis, and rising rapidly when the internal environment is alkalotic. This lability means that the carbonate is located mainly on bone surfaces, both anatomic and crystal. Calcium carbonate is more soluble than hydroxyapatite at prevailing pH and pCO_2 and may well be releasable without structural remodeling as a result of hypothesized lining cell activity. The anatomic surfaces of bone are so large that limited, *one-time* transfers of this sort could occur without leaving recognizable morphologic evidence. Thus, under conditions of acidosis, a limited amount of calcium may be available by dissolution. In brief, although calcium carbonate precipitation/dissolution may help buffer short-term oscillations in ECF $[Ca^{2+}]$, it does not have the capacity required for effective, long-term ECF $[Ca^{2+}]$ homeostasis.

9. QUANTITATIVE OPERATION OF THE SYSTEM

Although the operation of the calcium regulatory system, or any feedback loop for that matter, must first be sketched out *qualitatively* (as in Fig. 2), in the final analysis it is the *quantitative* operation of the system that will determine what ultimately happens (e.g., to the size of the calcium reserve, i.e., the mass of the skeleton). This *quantitative* working of the system for adjusting inputs and losses in response to dietary and other perturbations is often ignored. For example, it is commonly, if erroneously, assumed that, because intestinal calcium absorption efficiency varies inversely with intake, the body can fully compensate for declines in intake or increases in excretory loss. But quantitative analysis of the system (as well as data such as those assembled in Fig. 5) shows the fallacy of that assumption (discussed later). In the face of reduced intake, ECF [Ca^{2+}] tends to fall, and the prior rate of absorption of food calcium no longer suffices. The result is an increase in PTH secretion, which produces the three end-organ effects of Fig. 2, that is, more bone resorption, improved renal conservation, and increased calcium absorption efficiency. In brief, all three control loops are called upon to offset a shortfall originating in just one of them. The net effect with respect to total bone mass depends both on the relationship between the responsiveness of the three effector organs and on their capacity to provide the needed calcium *(15)*. *Sensitivity* of the effectors is genetically and hormonally determined, whereas *capacity* to respond is largely determined by unregulated factors outside the control loop, such as the calcium content of the diet and factors that influence obligatory loss.

If for some reason the response of one or the other of these effectors is blunted, PTH secretion must rise further, forcing more response from the other two effectors. Conversely, if one effector (such as bone) is highly responsive to PTH, the hormone level rises less because the needed calcium is readily supplied from the nearly limitless skeletal reserves. As a result, when the bone is more than usually responsive, less improvement in external calcium utilization ensues. Similarly, if the gut is unresponsive or the diet is so low in calcium that its capacity to yield the needed amount is exceeded, then PTH secretion rises further and bone is driven to meet the needs of the ECF [Ca^{2+}]. The three key insights here are: (1) it is ECF [Ca^{2+}] that is being regulated, not bone mass; (2) the dose–response curves for the three effector systems are independent of one another; and (3) PTH secretion is determined by the aggregate calcium output of all three end-organs, not by one or the other of them.

Examples of different patterns of effector responsiveness abound. Thus, American Blacks (and probably African Blacks as well) have a bony resorptive apparatus relatively resistant to PTH *(24–26)*. (*See* Chapter 7 for the impact of this difference on requirements.) As a result, they develop and maintain a somewhat higher bone mass than do Caucasians and Orientals, despite an often lower calcium intake. As predicted from the foregoing, African-Americans exhibit higher PTH and calcitriol levels, but lower levels of bone remodeling *(19)*. In brief, they utilize and conserve diet calcium more efficiently than Caucasians. Somewhat the opposite situation occurs in most women at normal menopause. Because estrogen acts to decrease bony responsiveness to PTH, estrogen loss at menopause increases the skeletal response to PTH. This is a part of the explanation for the increase in recommended calcium intake after menopause *(20,21)*. Obese individuals also increase their bone mass as they gain weight *(22)*, and they lose less bone at menopause *(23)*. Like blacks, they have high circulating PTH levels and (presumably) a relatively resistant bone remodeling apparatus.

10. AGE-RELATED CHANGES IN OPERATION OF THE CONTROL SYSTEM

Important changes occur both in the quantitative settings of the system with age and in the unregulated inputs. An example of the latter is the fall in calcium intake among women in the United States from early adolescence to the end of life. In National Health and Nutrition Examination Survey (NHANES)-II, median calcium intake was 793 mg (~20 mmol) in early adolescence, 550 mg (~14 mmol) in the 20s, and 474 mg (~12 mmol) at menopause (24). At the same time, absorption efficiency also falls with age. (Note: A part of this absorptive decline is due to estrogen deficiency, which both decreases renal 1α-hydroxylation of 25(OH)D and appears to have a small effect on the intestinal mucosa. A further part may be the result of a decrease in mucosal mass which, in animals, varies with food intake. Peripubertal girls absorb calcium with approx 45% greater efficiency for the same intake than do perimenopausal women *(25)*. As already noted, after age 40 yr, absorption efficiency drops by approx 0.2 absorption percentage points per year, with an added 2.0 percentage point drop across menopause *(9)*. In concrete terms, if a 40-yr-old woman absorbed a standard load at an efficiency of 30%, the same woman, at age 65 yr and deprived of estrogen, would absorb at an efficiency of 22.8%, or almost a 25% worsening in absorptive performance.

To complicate the situation further, renal calcium clearance rises at menopause *(26)*, as shown in the differences between the estrogen-replete and estrogen-deprived values for urine calcium in Table 1. This effect is seen most clearly with low calcium intakes, when urinary calcium can be as much as 36% higher than premenopause *(8)*. Vitamin D status deteriorates with age as well *(27,28)*; this decline is a function of reduced solar exposure and falls in both cutaneous vitamin D synthetic efficiency and in milk consumption. In Europe, where solar vitamin D synthesis is low for reasons of latitude and climate, and milk is generally not fortified, serum 25(OH)D concentration drops from over 100 nmol/L (40 ng/mL) in young adults to under 40 nmol/L (16 ng/mL) in individuals over age 70 yr.

Not surprisingly, serum PTH rises with age as a consequence of this aggregate of age-related changes. Twenty-four-hour integrated PTH is 70% higher in healthy 65-yr-old US women consuming diets containing 800 mg Ca per day than in third-decade women on the same diets *(29)*. That this difference is due to insufficient absorptive input is shown by the fact that the difference can be completely obliterated by increasing calcium intake *(29)*.

11. TWO EXAMPLES OF SYSTEM OPERATION

As stressed in the foregoing, it is a *quantity* of calcium that is being optimized (i.e., ECF [Ca^{2+}]); this is accomplished by the algebraic sum of various quantitative inputs and outputs. Two examples will serve to illustrate further the importance of attending to quantities. One examines in more detail the contrast in calcium handling at menarche and menopause just described, and the second describes the response of the system at any given age to a fixed increase in obligatory loss.

11.1. Menarche and Menopause

True trabecular bone density increases by approx 15% across menarche *(30)*, and approximately the same quantum of bone is lost across menopause *(31)*. Curiously, administration of estrogen to women more than 3 yr postmenopausal has generally failed

Table 2
Net Calcium Absorption at Menarche and Menopause

	Menarche	*Menopause*
Ca intake[a]	793 mg/d (19.8 mmol/d)	474 mg/d (11.8 mmol/d)
Ca absorption efficiency[b]	35.2%	30.5%
Endogenous fecal Ca[c]	67 mg/d (1.7 mmol/d)	102 mg/d (2.5 mmol/d)
Net Ca absorption	212 mg/d (53 mmol/d)	42 mg/d (10.5 mmol/d)

[a]National Health and Nutrition Examination Survey (NHANES)-II median values *(24)*.
[b]Heaney et al. *(9)*; O'Brien et al. *(25)*.
[c]Heaney et al. *(5)*.

to reproduce the pubertal increase in BMD, and in recent years it has been customary to say that, apart from whatever remodeling transient estrogen/hormone replacement therapy (ERT/HRT) may produce in postmenopausal women *(32)*, the principal effect of ERT/HRT on bone is stabilization of bone mass, rather than restoration of what had been lost. But the quantitative aspects of the age-related changes in the calcium economy, summarized in the foregoing, were not attended to as this conclusion was drawn.

Table 2 assembles published data for median calcium intake and mean data for absorption efficiency and EFCa loss, and shows very clearly how quantitative changes occurring in the 40 yr from menarche to menopause account for the rather different performance of the two age groups. In brief (and despite an intake less than recommended), a peripubertal girl is able to achieve net absorption of over 200 mg (5 mmol) calcium from the median diet of her age cohort, whereas an early menopausal woman extracts less than one-fifth as much from hers. The drop in intake amounts to approx 40%, but the fall in net absorption is 80%. As Table 2 shows, this is the resultant of lower intake, lower absorption efficiency, and higher digestive juice calcium losses. Given the level of total body obligatory losses at midlife, this absorbed quantity is simply not sufficient to support an estrogen-stimulated increase in BMD. As would be predicted from this understanding, higher calcium intakes in postmenopausal women permit estrogen to produce bony increases closer to those seen at puberty *(33)*.

11.2. Response to Augmented Losses

As already noted, it is commonly (and uncritically) considered that the absorptive apparatus is able to compensate either for a change in intake or a change in excretory loss. However, quantitative considerations make it clear that this depends entirely on the level of calcium in the diet. Thus, an individual increasing his/her salt intake by an amount equivalent to a single daily serving of a fast-food, fried chicken meal experiences an increase in urinary calcium of approx 1 mmol (40 mg)/d. Without compensating adjustments in input to the ECF, $[Ca^{2+}]$ would drop. PTH, of course, would rise, and with it, synthesis of $1,25(OH)_2D$, resulting ultimately in better extraction of calcium from the diet.

Published data allow rough estimation that a calcium drain of this magnitude produces an increase in $1,25(OH)_2D$ concentration of approx 6–7 pmol/L *(34)*, and dose–response

measurements for $1,25(OH)_2D$ indicate that this stimulus would increase calcium absorption efficiency by approx 2–3 absorption percentage points *(35)*. A 2–3% increase in extraction from a 50-mmol (2000-mg) diet yields 1–1.5 mmol (40–60 mg) of extra calcium, more than enough to offset the increased urinary loss, whereas from a 5-mmol (200-mg) diet, the same absorptive increase yields less than 0.1 mmol (4 mg). (Note: This is partly because extraction efficiency is already relatively high on low intakes, and partly because there is less calcium still unabsorbed on which the mucosa can work to extract additional calcium.) Thus, on a high-calcium diet, the body easily compensates for varying drains: both bone and ECF $[Ca^{2+}]$ are protected. But on a low-calcium diet, although the ECF $[Ca^{2+}]$ is protected, the bone is not. Why does serum $1,25(OH)_2D$ not rise more on a low-calcium diet? Simply because the 1α-hydroxylation step is responding to PTH. Bone calcium meets much (or most) of the ECF need, so $1,25(OH)_2D$ production is less than maximal. PTH secretion, as we have noted several times, is regulated by ECF $[Ca^{2+}]$, not by bone mass.

In brief, as the body adjusts to varying demands, the portion of the demand met by bone will be determined both by factors influencing bony responsiveness and by the level of diet calcium, the principal component of the system that is not regulated. However, it must also be stressed that, although an adequate calcium intake is a necessary condition for bone building and for adaptation to varying calcium demands, it is not by itself sufficient. Calcium alone will not stop estrogen-deficiency bone loss nor disuse bone loss (because neither is caused by calcium deficiency). However, recovery from immobilization or restoration of bone lost because of hormone deficiency will not be possible without an adequate supply of the raw materials needed to build bone substance.

12. CONCLUSIONS

The calcium economy consists of the traffic of calcium ions into and out of the blood, of the forces that alter that traffic, and of the control systems that regulate it. Central to the operation of the system is PTH, which stimulates calcium removal from bone, improves calcium absorption from food, and regulates loss of calcium through the kidneys. These three effects are independent of one another and their relative responsiveness to PTH differs between ethnic groups and at different life stages within individuals. Unregulated stresses to the system consist mainly of variable cutaneous losses (e.g., sweat), digestive juice losses, and obligatory urinary losses caused by interaction with other nutrients (e.g., sodium chloride). Ability to maintain constancy of both ECF $[Ca^{2+}]$ and the size of the skeletal reserve, that is, bone mass, depends on calcium intake. At intakes above the currently recommended values, both ECF $[Ca^{2+}]$ and bone are preserved. At lower intakes, bone mass may be sacrificed to sustain ECF $[Ca^{2+}]$.

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VII Appendices

APPENDIX 1: CRITERIA AND DIETARY REFERENCE INTAKE VALUES FOR CALCIUM BY LIFE STAGE GROUP

Life stage group[a]	*Criterion*[b]	*AI (mg/d)*[c]
0–6 mo	Human milk content	210
6–12 mo	Human milk + solid food	270
1–3 yr	Extrapolation of maximal calcium retention from 4 through 8 yr	500
4–8 yr	Calcium accretion/_ BMC/calcium balance	800
9–13 yr	Desirable calcium retention/ factorial/ _ BMC	1300
14–18 yr	Desirable calcium retention/ factorial/ _ BMC	1300
19–30 yr	Desirable calcium retention/ factorial	1000
31–50 yr	Calcium balance	1000
51–70 yr	Desirable calcium retention factorial/ _ BMD	1200
>70 yr	Extrapolation of desirable calcium retention from 51 to 70 yr age group/ _ BMD/fracture rate	1200
Pregnancy		
< 18 yr	Bone mineral mass	1300
19–50 yr	Bone mineral mass	1000
Lactation		
< 18 yr	Bone mineral mass	1300
19–50 yr	Bone mineral mass	1000

[a]All groups except Pregnancy and Lactation are males and females.

[b]Criteria on which the AI was based vary between life stage groups depending on the data available in the literature that were judged to be appropriate.

[c]AI, Adequate Intake. The experimentally determined estimate of nutrient intake by a defined group of healthy people. AI is used if the scientific evidence is not available to derive an EAR. For healthy infants fed human milk, AI is an estimated mean intake. Some seemingly healthy individuals may require higher calcium intakes to minimize risk of osteopenia and some individuals may be at low risk on even lower intakes. The AI is believed to cover their needs, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake. (From Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Academy Press, Washington, DC: 1997.)

APPENDIX 2: COMPARING SOURCES FOR ABSORBABLE CALCIUM

Source	*Serving size (g)*	*Calcium content (mg/serving)*	*Estimated absorption efficiency (%)*	*Food amount to equal calcium in 1 c milk*
Foods:				
Milk	240	300	32.1	1.0 c
Beans, pinto	86	44.7	26.7	4.1 c
Beans, red	172	40.5	24.4	4.8 c
Beans, white	110	113	21.8	2.0 c
Bok choy	85	79	53.8	1.2 c
Broccoli	71	35	61.3	2.3 c
Cheddar cheese	42	303	32.1	1.5 oz
Cheese food	42	241	32.1	1.8 oz
Chinese cabbage flower leaves	85	239	39.6	0.5 c
Chinese mustard green	85	212	40.2	0.6 c
Chinese spinach	85	347	8.36	1.7 c
Kale	85	61	49.3	1.6 c
Spinach	85	115	5.1	8.1 c
Sugar cookies	15	39	91.9	35 cookies
Sweet Potatoes	164	44	22.2	4.9 c
Rhubarb	120	174	8.5	4.7 c
Whole wheat bread	28	20	82.0	5.8 slices
Wheat bran cereal	28	20	38.0	12.8 oz
Yogurt	240	300	32.1	1.0 c
Fortified foods with added calcium:				
Tofu, calcium set	126	258	31.0	0.6 c
Orange juice with Ca citrate malate	240	300	36.3	0.9 c
Soy milk w/calcium phosphate	240	300	24.0	1.3 c
Bread w/calcium sulfate	17	300	43.0	1 slice

See Table 2 in Chapter 9 for a description of the values in this table and references. This table represents foods that have been intrinsically labeled during growth of the plant or animal ingredient or during preparation or processing of fortified foods.

APPENDIX 3: BOOKS WITH ADDITIONAL INFORMATION ON CALCIUM

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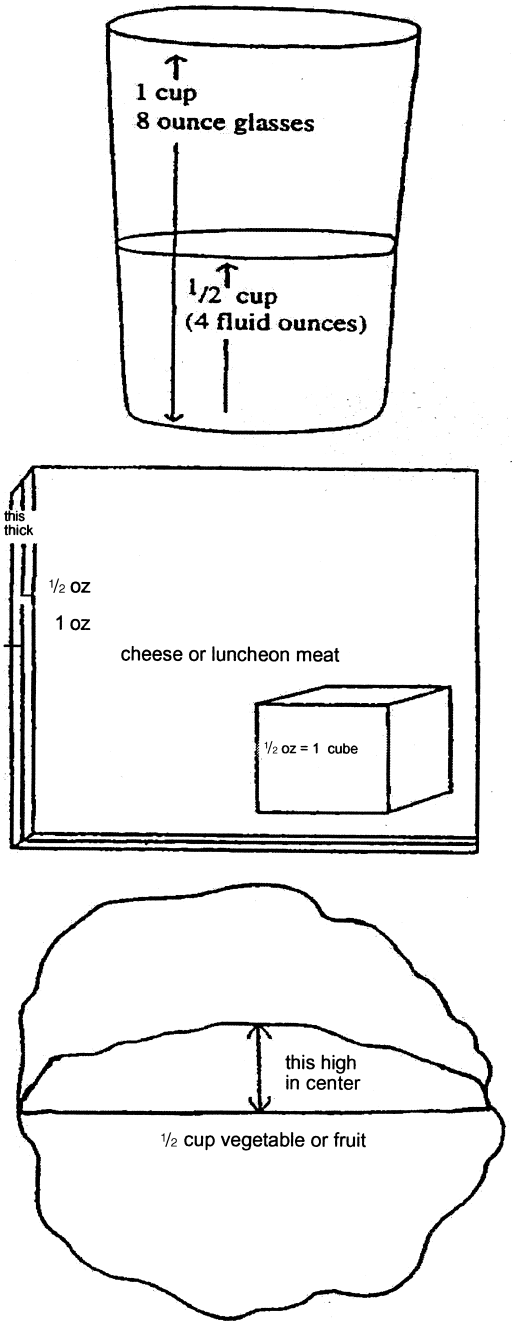
APPENDIX 4: CALCIUM CHECKLIST



I. Record the number of servings you ate on a typical day in the last week. Use the pictures to figure Serving Size.

	servings # daily	x	calcium mg
A. MILK — YOGURT- CHEESE			
cheese, 1 oz or 6 tbsp.	_____	x 200 =	_____
cottage cheese, 1/2 cup	_____	x 50 =	_____
custard, pudding, or cream pie, 1/2 cup	_____	x 150 =	_____
Ice cream, frozen yogurt, or milk shake, 1 cup	_____	x 200 =	_____
milk or cocoa, 1 cup	_____	x 300 =	_____
soy milk, 1 cup	_____	x 10 =	_____
yogurt, 1 cup	_____	x 350 =	_____
cream soups/sauces, 1 cup	_____	x 200 =	_____
macaroni and cheese, 1 cup; pizza 1/8 of 15 ; or quiche, 1/8 of 8	_____	x 250 =	_____
MILK TOTAL servings	_____	mg	_____
B. FRUITS AND C. VEGETABLES			
broccoli or cooked greens (beet/turnip greens, kale, collards), 1/2 cup	_____	x 100 =	_____
other vegetables, 1/2 cup	_____	x 30 =	_____
fruits, 1/2 cup or 1 small	_____	x 30 =	_____
F & V TOTAL servings	_____	mg	_____
D. BREADS, CEREALS, RICE, PASTA			
bread, 1 slice; or cereal, 1 oz	_____	x 20 =	_____
2" biscuit/roll, or 6" corn tortilla, or 3" muffin, cornbread, or doughnut	_____	x 40 =	_____
rice, noodles, or pasta, 1 cup	_____	x 20 =	_____
pancake, waffle, or french toast 1 serve	_____	x 100 =	_____
B & C TOTAL servings	_____	mg	_____
E. MEAT, FISH, POULTRY, DRY BEANS, NUTS			
dried beans, cooked (navy, pinto kidney), 1 cup	_____	x 50 =	_____
meat, fish, poultry, 3 oz	_____	x 10 =	_____
peanuts, 1/2 cup; 1 egg	_____	x 30 =	_____
salmon with bones, 3 oz	_____	x 150 =	_____
sardines with bones, 3 oz	_____	x 400 =	_____
3 oz shrimp; or 7-9 oysters	_____	x 100 =	_____
tofu, 2 1/2 x 2 1/2 x 1"	_____	x 100 =	_____
MEAT TOTAL servings	_____	mg	_____
F. FAT, SUGAR, ALCOHOL			
cake, 1/16 of 9" cake	_____	x 40 =	_____
beer, 12 oz	_____	x 10 =	_____
colas, 12 oz	_____	x 10 =	_____
chocolate, 1 oz	_____	x 50 =	_____
OTHER TOTAL servings	_____	mg	_____

Source: Hertzler AA, Frary RB. A dietary calcium rapid assessment method (RAM). Top Clin Nutr 9(3):76-85, 1994. Aspen Publishers, Inc.

APPENDIX 5: WEBSITES OF INTEREST

http://www.nationaldairycouncil.org/search/

The National Dairy Council website can be searched for current materials on calcium and additional links. A continually updated bibliography on certain topics including dairy and body weight can be found.

http://www.ifst.org/

Institute of Food Science & Technology (IFST) is based in the United Kingdom, with members throughout the world, with the purpose of serving the public interest in the application of science and technology for food safety and nutrition as well as furthering the profession of food science and technology. Eligibility for membership can be found at the IFST home page, an index and a search engine are available.

http://www.nysaes.cornell.edu/cifs/start.html

The Cornell Institute of Food Science at Cornell University home page provides information on graduate and undergraduate courses as well as research and extension programs. Links to related sites and newsgroups can be found.

http://www.blonz.com

Created by Ed Blonz, {sc-phd}, "The Blonz Guide" focuses on the fields of nutrition, foods, food science & health supplying links and search engines to find quality sources, news, publication and entertainment sites.

http://www.hnrc.tufts.edu/

The Jean Mayer United States Department of Agriculture (USDA) Human Nutrition Research Center on Aging (HNRC) at Tufts University. This research center is one of six mission-oriented centers aimed at studying the relationship between human nutrition and health, operated by Tufts University under the USDA. Research programs; seminar and conference information; publications; nutrition, aging, medical and science resources; and related links are available.

http://www.fao.org/

The Food and Agriculture Organization (FAO) is the largest autonomous agency within the United Nations, founded "with a mandate to raise levels of nutrition and standards of living, to improve agricultural productivity, and to better the condition of rural population," emphasizing sustainable agriculture and rural development.

http://www.eatright.org/

The American Dietetic Association is the largest group of food and nutrition professionals in the US, members are primarily registered dietitians (RDs) and dietetic technicians, registered (DTRs). Programs and services include promoting nutrition information for the public; sponsoring national events, media and marketing programs, and publications (*The American Dietetic Association*); and lobbying for federal legislation. Also available through the website are member services, nutrition resources, news, classifieds, and government affairs. Assistance in finding a dietitian, marketplace news, and links to related sites can also be found.

http://www.faseb.org

The Federation of American Societies for Experimental Biology (FASEB) is a coalition of member societies with the purpose of enhancing the profession of biomedical and life scientists, emphasizing public policy issues. FASEB offers logistical and operational support as well as sponsoring scientific conferences and publications (*The FASEB Journal*).

http://www.foodsciencecentral.com

The International Food Information Service (IFIS) is a leading information, product and service provider for professionals in food science, food technology, and nutrition. IFIS publishing offers a wide range of scientific databases, including Food Science and Technology Abstracts (FSTA). IFIS GmbH offers research, educational training, and seminars.

http://www.ift.org/

The Institute of Food Technologists (IFT) is a membership organization advancing the science and technology of food through the sharing of information; publications include *Food Technology* and *Journal of Food Science*; events include the Annual Meeting and Food Expo. Members may choose to join a specialized division of expertise (there are 23 divisions); IFT student associations and committees are also available for membership.

http://www.veris-online.org/

The VERIS Research Information Service is a nonprofit corporation, focusing on antioxidants, providing professionals with reliable sources on the role of nutrition in health. Data in VERIS publications, distributed without fee to those who qualify, is based on technical peer-reviewed journals. Quarterly written reports and newsletters, research summaries, annual abstract books, vitamin E fact book and educational programs are among the available VERIS publications and communications. Links to helpful web resources are also accessible.

http://www.osteo.org/

The National Institutes of Health Osteoporosis and Related Bone Diseases-National Resource Center (NIH ORBD-NRC) mission is to "provide patients, health professionals, and the public with an important link to resources and information on metabolic bone diseases, including osteoporosis, Paget's disease of the bone, osteogenesis imperfecta, and hyperparathyroidism. The Center is operated by the National Osteoporosis Foundation, in collaboration with The Paget Foundation and the Osteogenesis Imperfecta Foundation."

http://www.ag.uiuc.edu/~food-lab/nat/

The Nutrition Analysis Tool (NAT) is a free web based program designed to be used by anyone to analyze the nutrient content of food intake. Links to an "Energy Calculator" and "Soy Food Finder" are also available. NAT is funded by C-FAR at the University of Illinois.

http://www.calciuminfo.com

This is an online information source created, copyrighted, and maintained by GlaxoSmithKline Consumer Healthcare Research and Development. The nutritional and physiological role of calcium is presented in formats designed for healthcare professionals, consumers, and kids. References and related links, educational games for kids, calcium tutorials, and a calcium calculator are easily accessible.

http://vm.cfsan.fda.gov/

The Center for Food Safety and Applied Nutrition (CFSAN) is one of five product-oriented centers implementing the FDA's mission to regulate domestic and imported food as well as cosmetics. An overview of CFSAN activities can be found along with useful sources for researching various topics such as food biotechnology and seafood safety. Special interest areas, for example, advice for consumers, women's health, and links to other agencies are also available.

http://www.bcm.tmc.edu/cnrc/

The Children's Nutrition Research Center (CNRC) at Baylor College of Medicine is one of six USDA/ARS human nutrition research centers in the nation, assisting healthcare professionals and policy advisors to make appropriate dietary recommendations. CNRC focuses on the nutrition needs of children, from conception through adolescence, and of pregnant and nursing women. Consumer news, seminars, events, and media information are some of the sections available from this home page.

http://www.dsqi.org/

The Dietary Supplement Quality Initiative (DSQI) is designed to educate consumers on the health benefits, safety, standards and regulations, and labeling of dietary supplements. Industry news, interviews, editorials, and DSQI resources and services provide useful tools for consumers, practitioners, producers and distributors.

http://www.usda.gov

The United States Department of Agriculture (USDA) provides a broad scope of service to the nation's farmers and ranchers. In addition, the USDA ensures open markets for agricultural products, food safety, environmental protection, conservation of forests and rural land, and the research of human nutrition. Affiliated agencies, services and programs are accessible through this website.

http://www.nalusda.gov/

The National Agriculture Library (NAL), a primary resource for agriculture information, is one of four national libraries in the US and a component of the Agriculture Research Service of the US Department of Agriculture. Access to NAL's institutions and resources are available through this site.

http://www.fns.usda.gov/fns/

The Food and Nutrition Service (FNS) administers the US Department of Agriculture's (USDA) 15 food assistance programs for children and needy families with the mission to reduce hunger and food insecurity. Details of nutrition assistance programs and related links can be found.

http://www.agnic.org/

The Agriculture Network Information Center (AgNIC), established through the alliance of the National Agriculture Library (NAL) and other organizations, provides public access to agriculture-related resources.

http://www.who.int/nut/welcome.htm

The World Health Organization (WHO) has regarded nutrition to be of fundamental importance for overall health and sustainable development. The Global priority of nutritional issues, activities, mandates, resources, and research are presented in detail.

Nutritional Science Journals

Brown CM. Where to find nutritional science journals on the World Wide Web. J Nutr 1997;127:1527–1532.

http://www.crcpress.com/jour/catalog/foods.htm
Critical Reviews in Food Science and Nutrition
http://www.wiley.com/Home.html
International Journal of Eating Disorders
http://www.peakcom.com/clinnutr.org/jabs.html
Journal of Parenteral and Enteral Nutrition
http://www.lrpub.com/journals/j1013.htm
Journal of Pediatric Gastroenterology and Nutrition
http://www.elsevier.nl:80/inca/publications/store/5/2/5/0/1/3/
Journal of Nutritional Biochemistry
http://www.karger.com/journals/anm/anm jh.htm
Annals of Nutrition and Metabolism
http://www.hscsyr.edu/ñutrition/
Nutrition: The International Journal of Applied and Basic Nutritional Sciences
http://www.elsevier.nl/inca/publications/store/5/2/5/4/8/3/
Nutrition Research
http://www.humanapress.com
http://www.humanapress.com/Index.pasp

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About the Series Editor

Dr. Adrianne Bendich is Clinical Director of Calcium Research at GlaxoSmithKline Consumer Healthcare, where she is responsible for leading the innovation and medical programs in support of several leading consumer brands including TUMS and Os-Cal. Dr. Bendich has primary responsibility for the coordination of GSK's support for the Women's Health Initiative (WHI) intervention study. Prior to joining GlaxoSmithKline, Dr. Bendich was at Roche Vitamins Inc., and was involved with the groundbreaking clinical studies proving that folic acid-containing multivitamins significantly reduce major classes of birth defects. Dr. Bendich has co-authored more than 100 major clinical research studies in the area of preventive nutrition. Dr. Bendich is recognized as a leading authority on antioxidants, nutrition and bone health, immunity, and pregnancy outcomes, vitamin safety, and the cost-effectiveness of vitamin/mineral supplementation.

In addition to serving as Series Editor for Humana Press and initiating the development of the 20 currently published books in the *Nutrition and Health*™ series, Dr. Bendich is the editor of 11 books, including *Preventive Nutrition: The Comprehensive Guide for Health Professionals.* She also serves as Associate Editor for *Nutrition: The International Journal of Applied and Basic Nutritional Sciences,* and Dr. Bendich is on the Editorial Board of the *Journal of Women's Health and Gender-Based Medicine,* as well as a past member of the Board of Directors of the American College of Nutrition. Dr. Bendich also serves on the Program Advisory Committee for Helen Keller International.

Dr. Bendich was the recipient of the Roche Research Award, was a Tribute to Women and Industry Awardee, and a recipient of the Burroughs Wellcome Visiting Professorship in Basic Medical Sciences, 2000–2001. Dr. Bendich holds academic appointments as Adjunct Professor in the Department of Preventive Medicine and Community Health at UMDNJ, Institute of Nutrition, Columbia University P&S, and Adjunct Research Professor, Rutgers University, Newark Campus. She is listed in *Who's Who in American Women.*

About the Editors

Connie M. Weaver, PhD, is Distinguished Professor and Head of the Department of Foods and Nutrition at Purdue University, West Lafayette, Indiana. In 2000, she also became Director of a National Institutes of Health funded Botanical Center to study dietary supplements containing polyphenolics for age-related diseases. Her research interests include mineral bioavailability, calcium metabolism, and bone health. She was a member of the National Academy of Sciences Food and Nutrition Board Panel to develop new recommendations for requirements for calcium and related minerals. Dr. Weaver is past-President of American Society for Nutritional Sciences and is on the Board of Trustees of the International Life Sciences Institute. For her contributions in teaching, Dr. Weaver was awarded Purdue University's Outstanding Teaching Award. In 1993, she was honored with the Purdue University Health Promotion Award for Women, and in 1997, she received the Institute of Food Technologists Babcock Hart Award. In April 2003, she received the USDA A.O. Atwater Lecture Award at the annual Experimental Biology meeting. Dr. Weaver was appointed to the 2005 US Dietary Guidelines Advisory Committee. She has published more than 170 research articles. Dr. Weaver received a Bachelor of Science and Master of Science in food science and human nutrition from Oregon State University. She received a PhD in food science and human nutrition from Florida State University and holds minors in chemistry and plant physiology.

Robert P. Heaney, MD, FACP, FASNS, is John A. Creighton University Professor and Professor of Medicine, Creighton University, Omaha, Nebraska.

Dr. Heaney received his MD at Creighton and has held faculty appointments at the University of Oklahoma, at George Washington University, and at Creighton, where he served as Chairman of the Department of Internal Medicine. Dr. Heaney was Creighton's first Vice-President for Health Sciences, and since 1984 has held the all-university chair named in honor of the university's founder.

Dr. Heaney has worked for nearly 50 years in the study of osteoporosis, vitamin D, and calcium physiology. He is the author of three books and has published more than 300 original papers, chapters, monographs, and reviews in scientific and educational fields. He has received numerous honors and awards, including the Kappa Delta Award of the American Academy of Orthopaedic Surgeons and the Alumni Achievement Citation of his alma mater. In 1990 he was awarded honorary membership in The American Dietetic Association, and in 1993 he was elected Fellow of the American College of Nutrition, both in recognition of his work in delineating human calcium absorptive performance and in defining human calcium requirements. In 1994 he received the Frederic C. Bartter Award of the American Society for Bone and Mineral Research in recognition of his career in clinical research. He received three awards in 2003: France's Institut Candia awarded him their Scientific Prize for his significant contributions to raising awareness of calcium and its health benefits; he received the E.V. McCollum Award of the American Society for Clinical Nutrition in recognition of his contributions to nutritional science and medicine; and the McCollum International Lectureship of the American Society for Nutritional Sciences.