Nutrition and Health
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Orlando M. Gutiérrez
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Clinical Aspects of Natural and Added Phosphorus in Foods

Humana Press

Nutrition and Health

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Dr. Gutiérrez dedicates this text to his wife, Alecia, and his children, Owen and Evie.
Dr. Kalantar-Zadeh dedicates this text to his wife, Grace, and their children, Sara, Diana, and Hannah.
Dr. Mehrotra dedicates this text to his wife, Kushi, and his children, Kunaal and Ria.

Preface

Phosphorus is an essential micronutrient involved in a number of key biological processes. Disturbances in systemic phosphorus homeostasis have been associated with cardiovascular disease events and death, particularly among individuals with chronic kidney disease. Although the mechanisms for these associations remain incompletely understood, a considerable body of data implicate local and systemic alterations in phosphorus metabolism in the pathogenesis of cardiovascular and kidney disease. Since excess dietary phosphorus intake is common in individuals consuming Westernized diets and can lead to disturbances in phosphorus metabolism, these findings have fueled interest in the role of dietary phosphorus in the etiology of cardiovascular and kidney disease. Despite the importance of these topics, there is a relative dearth of resources that have collated the most up-to-date knowledge related to the role of dietary phosphorus in health and disease, from basic physiology to clinical application.

Recognizing this void, the International Dietary Phosphorus Consensus Conference was convened on June 26th, 2012, in Honolulu, Hawaii, USA (www.phosinfood.com) to examine phosphorus in natural and added foods and its role in diseases of the kidney, heart, bone, mineral disorders and cancer. Building on the themes from this conference, the primary purpose of this text, titled *Clinical Aspects of Natural and Added Phosphorus in Foods*, is to provide a comprehensive reference on the impact of dietary phosphorus, both naturally present and added secondarily upon food processing, in phosphorus physiology, public health, and the pathogenesis of disease. We have chosen to frame this effort with an eye on the needs of the practicing clinician, as it is in the arena of patient care that many of the key questions surrounding the role of dietary phosphorus in health outcomes is most in need of a reference text such as this. This text will also serve as a great reference for trainees at all levels (undergraduate and graduate students, nutrition students, residents, and fellows) who will find within these pages much of the basic information needed to truly appreciate the multifaceted ways in which dietary phosphorus can impact health outcomes. This text is also meant to aid individuals who want to understand policy issues related to the use of phosphorus in the food supply (whether it be direct human uses, animal or agricultural applications). For all these reasons, this text will provide an essential new tool in helping to understand the growing importance of dietary phosphorus content in health and disease.

Organization and Content

Clinical Aspects of Natural and Added Phosphorus in Foods is organized in three sections. The first section (encompassing chapters one through three) includes a basic overview of the history of phosphorus (including its somewhat confusing terminology) and the regulation of phosphorus homeostasis. The second section (encompassing chapters four through ten) focuses on specific matters related to phosphorus in the food supply, including the different types of phosphorus in the food supply, how to measure their content, their wide variety of uses both in direct human use and animal agriculture, their importance for basic nutrition and food manufacturing, and the implication of excess phosphorus content. The third section (encompassing chapters eleven through seventeen) focuses on the clinical applications of the material presented in the preceding two sections, including the importance of both phosphorus excess and phosphorus deficiency for the pathogenesis of a wide variety of disease including kidney, cardiovascular, bone, and oncologic diseases.

The chapters were designed to enhance learning of the material presented. Accordingly, each chapter starts with a brief abstract and includes keywords which can help the reader quickly decipher the salient elements of each chapter and identify the material of most interest. Readers will also find that up-to-date reference lists at the end of each chapter will serve as an indispensable resource for further in-depth study of different topics. None of these features would be available if not for the hard work of the expert authors who wrote each chapter—without their efforts, this important reference text would never have seen the light of day, and the editors are indebted to their dedication to this project from the beginning to end. At the end of the day, we believe that this collaborative effort will enhance patient care and move forward the field of phosphorus science.

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Series Editors

Dr. Adrianne Bendich, **PhD**, **FASN**, **FACN**, has served as the "Nutrition and Health" Series Editor for 20 years and has provided leadership and guidance to more than 200 editors that have developed the 70+ well-respected and highly recommended volumes in the Series.

In addition to *Clinical Aspects of Phosphorus in Foods and Preservatives: Impact on Health and Kidney Disease* edited by Orlando M. Gutiérrez, Kamyar Kalantar-Zadeh and Rajnish Mehrotra, major new editions published in 2012–2016 include:

1. *Mediterranean Diet: Impact on Health and Disease* edited by Donato F. Romagnolo, Ph.D. and Ornella Selmin, Ph.D., 2016.
2. *Nutrition Support for the Critically Ill* edited by David S. Seres, MD and Charles W. Van Way, III, MD, *2016.*
3. *Nutrition in Cystic Fibrosis: A Guide for Clinicians,* edited by Elizabeth H. Yen, M.D. and Amanda R. Leonard, MPH, RD, CDE, 2016.
4. *Preventive Nutrition: The Comprehensive Guide For Health Professionals, Fifth Edition,* edited by Adrianne Bendich, Ph.D. and Richard J. Deckelbaum, M.D., 2016.
5. *Glutamine in Clinical Nutrition,* edited by Rajkumar Rajendram, Victor R. Preedy and Vinood B. Patel, 2015.
6. *Nutrition and Bone Health, Second Edition,* edited by Michael F. Holick and Jeri W. Nieves, 2015.

7. *Branched Chain Amino Acids in Clinical Nutrition, Volume 2,* edited by Rajkumar Rajendram, Victor R. Preedy and Vinood B. Patel, 2015.
8. *Branched Chain Amino Acids in Clinical Nutrition, Volume 1,* edited by Rajkumar Rajendram, Victor R. Preedy and Vinood B. Patel, 2015.
9. *Fructose, High Fructose Corn Syrup, Sucrose and Health,* edited by James M. Rippe, 2014.
10. *Handbook of Clinical Nutrition and Aging, Third Edition,* edited by Connie Watkins Bales, Julie L. Locher and Edward Saltzman, 2014.
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14. *Handbook of Food Fortification and Health, volume I* edited by Dr. Victor R. Preedy, Dr. Rajaventhan Srirajaskanthan, Dr. Vinood B. Patel, 2013.
15. *Handbook of Food Fortification and Health, volume II* edited by Dr. Victor R. Preedy, Dr. Rajaventhan Srirajaskanthan, Dr. Vinood B. Patel, 2013.
16. *Diet Quality: An Evidence-Based Approach*, *volume I* edited by Dr. Victor R. Preedy, Dr. Lan-Ahn Hunter and Dr. Vinood B. Patel, 2013.
17. *Diet Quality: An Evidence-Based Approach*, *volume II* edited by Dr. Victor R. Preedy, Dr. Lan-Ahn Hunter and Dr. Vinood B. Patel, 2013.
18. *The Handbook of Clinical Nutrition and Stroke,* edited by Mandy L. Corrigan, MPH, RD Arlene A. Escuro, MS, RD, and Donald F. Kirby, MD, FACP, FACN, FACG, 2013.
19. *Nutrition in Infancy*, *volume I* edited by Dr. Ronald Ross Watson, Dr. George Grimble, Dr. Victor Preedy and Dr. Sherma Zibadi, 2013.
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28. *Chocolate in Health and Nutrition*, edited by Dr. Ronald Ross Watson, Dr. Victor R. Preedy, and Dr. Sherma Zibadi, 2012.
29. *Iron Physiology and Pathophysiology in Humans*, edited by Dr. Gregory J. Anderson and Dr. Gordon D. McLaren, 2012.

Earlier books included *Vitamin D, Second Edition* edited by Dr. Michael Holick; *Dietary Components and Immune Function* edited by Dr. Ronald Ross Watson, Dr. Sherma Zibadi, and Dr. Victor R. Preedy; *Bioactive Compounds and Cancer* edited by Dr. John A. Milner and Dr. Donato F. Romagnolo; *Modern Dietary Fat Intakes in Disease Promotion* edited by Dr. Fabien De Meester, Dr. Sherma Zibadi, and Dr. Ronald Ross Watson; *Iron Deficiency and Overload* edited by Dr. Shlomo Yehuda and Dr. David Mostofsky; *Nutrition Guide for Physician*s edited by Dr. Edward Wilson, Dr. George A. Bray, Dr. Norman Temple, and Dr. Mary Struble; *Nutrition and Metabolism* edited by Dr. Christos Mantzoros; and *Fluid and Electrolytes in Pediatrics* edited by Leonard Feld and Dr. Frederick Kaskel. Recent volumes include: *Handbook of Drug-Nutrient Interactions* edited by Dr. Joseph Boullata and Dr. Vincent Armenti; *Probiotics in Pediatric Medicine* edited by Dr. Sonia Michail and Dr. Philip Sherman; *Handbook of Nutrition and Pregnancy* edited by Dr. Carol Lammi-Keefe, Dr. Sarah Couch, and Dr. Elliot Philipson; *Nutrition and Rheumatic Disease* edited by Dr. Laura Coleman; *Nutrition and Kidney Disease* edited by Dr. Laura Byham-Grey, Dr. Jerrilynn Burrowes, and Dr. Glenn Chertow; *Nutrition and Health in Developing Countries* edited by Dr. Richard Semba and Dr. Martin Bloem; *Calcium in Human Health* edited by Dr. Robert Heaney and Dr. Connie Weaver; and *Nutrition and Bone Health* edited by Dr. Michael Holick and Dr. Bess Dawson-Hughes.

Dr. Bendich is President of Consultants in Consumer Healthcare LLC, and is the editor of ten books including *Preventive Nutrition: The Comprehensive Guide for Health Professionals, Fifth Edition* co-edited with Dr. Richard Deckelbaum (www.springer.com/series/7659). Dr. Bendich serves on the editorial boards of the *Journal of Nutrition in Gerontology and Geriatrics*, and *Antioxidants*, and has served as Associate Editor for the international journal *Nutrition*; served on the editorial board of the *Journal of Women's Health and Gender-Based Medicine*, and served on the Board of Directors of the American College of Nutrition.

Dr. Bendich was Director of Medical Affairs at GlaxoSmithKline (GSK) Consumer Healthcare and provided medical leadership for many well-known brands including TUMS and Os-Cal. She had primary responsibility for GSK's support for the Women's Health Initiative (WHI) intervention study. Prior to joining GSK, Dr. Bendich was at Roche Vitamins Inc. and was involved with the groundbreaking clinical studies showing that folic acid-containing multivitamins significantly reduced major classes of birth defects. She has co-authored over 100 major clinical research studies in the area of preventive nutrition. She is recognized as a leading authority on antioxidants, nutrition and immunity and pregnancy outcomes, vitamin safety, and the cost-effectiveness of vitamin/mineral supplementation.

Dr. Bendich received the Roche Research Award, a *Tribute to Women and Industry* Awardee and was a recipient of the Burroughs Wellcome Visiting Professorship in Basic Medical Sciences. She was given the Council for Responsible Nutrition (CRN) Apple Award in recognition of her many contributions to the scientific understanding of dietary supplements. In 2012, she was recognized for her contributions to the field of clinical nutrition by the American Society for Nutrition and was elected a Fellow of ASN. Dr Bendich is Adjunct Professor at Rutgers University. She is listed in Who's Who in American Women.

Connie W. Bales, **PhD**, **RD**, is a Professor of Medicine in the Division of Geriatrics, Department of Medicine, at the Duke School of Medicine and Senior Fellow in the Center for the Study of Aging and Human Development at Duke University Medical Center. She is also Associate Director for Education/Evaluation of the Geriatrics Research, Education, and Clinical Center at the Durham VA Medical Center. Dr. Bales is a well-recognized expert in the field of nutrition, chronic disease, function, and aging. Over the past two decades, her laboratory at Duke has explored many different aspects of diet and activity as determinants of health during the latter half of the adult life course. Her current research focuses primarily on the impact of protein-enhanced meals on muscle quality, function, and other health indicators during obesity reduction in older adults with functional limitations. Dr. Bales has served on NIH and USDA grant review panels and is a member of the American Society for Nutrition's Medical Nutrition Council. Dr. Bales has edited three editions of the *Handbook of Clinical Nutrition in Aging* and is Editor-in-Chief of the *Journal of Nutrition in Gerontology and Geriatrics.*

Volume Editors

Orlando M. Gutiérrez, **MD**, **MMSc** obtained his medical degree from the University of Toledo College of Medicine in 2002. He then completed internship and residency training at the Massachusetts General Hospital in Boston from 2002 to 2005, and a clinical and research fellowship in nephrology from the Brigham and Women's Hospital/Massachusetts General Hospital Joint Nephrology Training Program in 2008. He also completed a Masters in Medical Science degree in human clinical investigation from Harvard Medical School during his fellowship, graduating as the valedictorian of his class. Dr. Gutiérrez joined the faculty of the Division of Nephrology and Hypertension at the University of Miami Miller School of Medicine as an Assistant Professor of Medicine in 2008. He was then recruited to the University of Alabama at Birmingham in 2011, where he is currently an Associate Professor of Medicine in the School of Medicine. In addition, he serves as the section head for outcomes and epidemiology research in the Division of Nephrology at UAB.

Dr. Gutiérrez's research is focused on understanding pathophysiological mechanisms underlying disorders of phosphorus and vitamin D metabolism in health and in individuals with kidney disease. He has a special interest in delineating environmental and/or behavioral factors that may modulate these associations, particularly those related to poverty and nutrition. His research has been published in high-impact journals such as the *New England Journal of Medicine* and *Circulation*, and he has been an invited speaker in numerous national and international conferences.

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Prof. Kamyar Kalantar-Zadeh, (a.k.a. Kam Kalantar) studied medicine at the Universities of *Bonn* and *Nuremberg* in *Germany* (*Doctoris medicinae*, MD degree). He also received a master's degree in Public Health (*M.P.H.*) and an additional doctorate degree in Epidemiology (*Ph.D.*) from University of California, *Berkeley,* School of Public Health. Dr. Kalantar's postgraduate training includes an internship training in Nuremberg, Germany, residency training in Internal Medicine and also in Pediatrics in the *State University of New York* (SUNY) in Brooklyn and Staten Island (SUNY, 1993–1997), and a nephrology fellowship at *University of California San Francisco* (UCSF, 1997–2000).

Between 2000 and 2012, Dr. Kalantar was a full-time faculty at Harbor-UCLA including Professor of Medicine, Pediatrics and Epidemiology at *UCLA*; and medical director of a DaVita dialysis clinic in Long Beach, CA. Since 2012, he has served as a tenured Professor and Head of the Division of Nephrology and Hypertension at *University of California Irvine* (UC Irvine) School of Medicine, in Orange and Irvine, CA, and a Professor of Medicine, Pediatrics, and Public Health at UC Irvine, and attending nephrologist at UC Irvine as well as *Long Beach Veterans Affairs (VA) Hospital*, He is also an Adjunct Professor of Epidemiology at UCLA Fielding School of Public Health in Los Angeles, CA. Since his leadership position, the "UC Irvine Nephrology" has been named twice among top 50 nephrology centers by the *US News & World Report*™ out of over 1700 nephrology centers nationwide.

Dr. Kalantar-Zadeh is a board-certified physician and passed the *board certifications* and recertification in Internal Medicine (1997 and 2007) and Nephrology (1999 and 2009) and Pediatrics (1997 and 2004). He has been recognized by several prestigious top/best physician directories in the USA including TOP DOCTORS™, *BEST DOCTORS*™, and *TOP PHYSICIANS*™ Castle-Connelly. *In May 2014*, *he was ranked among top 10 experts in ESRD and Chronic Kidney Failure out of over 91,000 experts ranked throughout the world by Expertscape*™ http://expertscape.com/leaders/end-stage-renal-disease

Dr. Kalantar was appointed and served as a standing member of the *National Institutes of Health (NIH)* study section (KNOD, Kidney, Nutrition, Obesity and Diabetes 2009–2013). He has had several grants including from the NIH as the principal investigator (R01's, K24 mid-career award, R21's, R13, U01), including the PI of the *United States Renal Data System* (*USRDS*, 2014–2019), National Kidney Foundation, and American Heart Association. His research group is also supported by philanthropic grants, and Dr. Kalantar is the founder and director of Harold Simmons Center for Kidney Disease Research & Epidemiology. He serves as the President of the International Society of Renal Nutrition & Metabolism (ISRNM, www.RenalNutrition.com).

Prof. Kalantar-Zadeh has published *over 500 scientific articles*, authored many chapters, and presented over 500 abstracts and numerous grand rounds and other lectures in national and international conferences. He is an editor of nephrology and nutrition textbooks including the *Nutritional Management of Renal Disease*, 3rd edition, 2013. Dr. Kalantar is an *Associate Editor* or member of the *editorial board* of several journals including *Nephrology Dialysis*, *American Journal of Kidney Diseases*, *Journal of Cachexia, Sarcopenia & Muscle*, *Cardiorenal Medicine*, *Kidney International, Journal of American Society of Nephrology, Nature Reviews Nephrology, American Journal of Nephrology*, *Journal of Renal Nutrition*, etc.

Prof. Kalantar lectures frequently in national and international congresses and conferences on kidney disease, diabetes, and nutrition including: cardiovascular risk factors, nutritional status and assessment, dietary management of kidney disease, malnutrition and wasting disorders, cachexia, inflammation, mineral and bone disorders, dialysis treatment, hyponatremia and acid–base disorders, potassium disarrays, anemia and iron, diabetic kidney disease, obesity paradox or reverse epidemiology, racial and ethnic disparities, and quality of life and other patient-centered outcomes.

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He is a leading expert in the care of patients undergoing maintenance dialysis for the treatment of end-stage renal disease with special interest in peritoneal dialysis. His research interests include comparative effectiveness of dialysis modalities, biological determinants of peritoneal membrane function, and patient-reported outcomes in patients undergoing maintenance dialysis. His work has been supported by grants from, among others, the National Institutes of Health and Patient Centered Outcomes Research Institute. He was awarded the John Maher Award by the International Society for Peritoneal Dialysis in 2006 and has served as the Chair of the Dialysis Advisory Group of the American Society of Nephrology (2009–2015).

He has served as the Treasurer of the International Society for Peritoneal Dialysis since 2014 and the President of the North American Chapter of the International Society for Peritoneal Dialysis. He has served as the Associate Editor for the *Journal of the American Society of Nephrology, Peritoneal Dialysis International* and *NephSAP*, and section editor for *Clinical Nephrology*. He is currently a member of the editorial board for the *Kidney International*, and *Journal of Renal Nutrition*. He has served as Chair of the Education Committee and Membership Committee of the International Society for Peritoneal Dialysis and a member of the Council of the Society in the past.

Dr. Mehrotra completed his medical schooling at the All India Institute of Medical Sciences in New Delhi, India, underwent residency training at the All India Institute of Medical Sciences, New Delhi, and Medical College of Pennsylvania, Philadelphia, and fellowship training at the University of Missouri-Columbia. He obtained a Master's of Science degree in Clinical Research at the University of California, Los Angeles.

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Part I

Basic Phosphorus Physiology

The Story of Phosphorus

1

Orlando M. Gutiérrez

Key Points

- Phosphorus, phosphate and phosphorous are often used interchangeably but have different meanings and so should be used according to the context intended.
- When in doubt, phosphorus is the best term to use.
- The mass introduction of phosphorus for industrial and agricultural uses has occurred fairly recently in human history.

Introduction

Few elements could boast as colorful and entertaining a history as that of phosphorus. First discovered by an amateur alchemist, it was initially considered merely a novelty, relegated to entertaining royal courtesans on account of its luminescent properties. However, once chemists learned how to harness its flammable properties, it became an ideal ingredient for match manufacturing and, later, military ordnance. Arguably, phosphorus's most enduring legacy came after it was shown to be an essential element in natural fertilizers, resulting in its widespread incorporation in agriculture. Today, phosphorus has become nearly indispensable in supporting the global food supply. Among the most intriguing aspects of this story is how recently it has unfolded in human history, relatively speaking—indeed, it has only

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been in the past ~150 years that the manufacture and use of phosphorus for agricultural purposes has become widespread. As such, much of what we understand about phosphorus is fluid, and continues to evolve to this day. This text is in many ways a compendium of what we have learned about phosphorus from the time of its initial discovery in the seventeenth century to the modern era, with a particular focus on its clinical applications. Before launching into these topics, however, it is instructive to review the history of this intriguing element.

Terminology: So Many Names but One Meaning

The word phosphorus derives from the ancient Greek words φως (phôs) meaning "light," and φορυς (phorus) meaning "bearer." [1] The story behind this etymology is recounted in detail in section "The Story of Phosphorus: From Serendipity to Novelty Item" below. In the current literature, phosphorus is one of three spellings commonly used for this element, the other two being phosphorous and phosphate. These spellings are often used interchangeably, which in clinical practice is normally of little consequence, but scientifically does not do justice to the important differences between each term. The most important distinction to be made in this regard is between the use of phosphorus vs. phosphate, which are both nouns. Phosphorous, in contrast, is the adjectival form of phosphorus, and thus refers or relates to containing phosphorus—e.g., "phosphorous soil" would refer to soil that contains phosphorus [1].

The distinction between phosphorus and phosphate is relatively straight forward. Phosphorus generally refers to the phosphorus atom (P), with a molar mass of 30.97 g/mol [2]. Since phosphorus is highly reactive, it rarely exists by itself in nature, but instead is normally present in ionic form, usually complexed with four oxygen atoms in the form of phosphate (P_i) with a total molar mass of 94.9 g/mol [2]. In biological systems, P_i is found in organic or inorganic forms, complexed with various organic esters, mono- or divalent cations such as calcium, magnesium, or sodium, or as free (nonesterified) forms such has $H_2PO_4^{1-}$ or HPO_4^{2-} [1]. In nature, phosphorus exists as several different allotropes, the most common being white, red, and black forms each of which have distinct chemical properties [3].

All procedures used to measure phosphorus in clinical laboratories actually are measuring Pi—in the United States, these values have been traditionally expressed in milligrams per deciliter of P instead of P_i, in part because the valence of Pi changes depending on the pH of the medium being sampled [1]. Thus, a serum phosphorus concentration of 4 mg/dL actually equates to the serum P concentration and not the serum Pi concentration.

The appropriate use of P or Pi in many ways depends on the context in which phosphorus is being used. For example, when referring to systemic phosphorus metabolism broadly, Pi would be most appropriate since phosphate ions are the actual factors participating in metabolic processes [1]. When in doubt, however, P is probably the best term to use since each Pi molecule contains one atom of phosphorus by definition. For this reason, "phosphorus" is the term used to describe the

element in this chapter and throughout this text. In clinical practice, these distinctions are often of little consequence. Nonetheless, it is important to be aware of the differences in the terminology, so as not to become confused when encountering the different forms of this word in the scientific literature.

The Story of Phosphorus: From Serendipity to Novelty Item

Hennig Brandt (sometimes spelled as Brand) is widely credited for being the first person to discover phosphorus [4]. Brandt was a former soldier and glass-maker-turned-alchemist who lived in Hamburg in what is now modern-day Germany [5]. Like all good alchemists of his day, Brandt's singular obsession was discovering the philosopher's stone, an unknown substance that could facilitate the chemical conversion of base metals into silver or gold. His favorite medium of experimentation was urine, and he reportedly collected gallons of urine from himself, his family and his friends to support his experiments [5]. According to most accounts, he first stumbled upon phosphorus in or around 1669 while heating and processing urine samples in order to isolate substances that might serve as precursors to the philosopher's stone [4, 5]. One such experiment accidentally resulted in the generation of a shiny liquid that spontaneously emitted a pale-green light for many hours. What particularly fascinated Brandt was that the new substance gave off light without producing any heat. Believing that he had finally discovered the philosopher's stone, Brandt attempted to use his amateur chemist skills to convert this new substance into gold, only to find that his efforts were met in vain [5]. Nonetheless, Brandt shared his discovery with several colleagues and friends who marveled at the luminescent properties of this new substance, and word quickly spread about the new discovery. This ultimately led to his recognition as the first person to discover phosphorus. Further, he holds the distinction of being the first person to discover a new element in recorded history (that is to say, the time and circumstances of when he made the discovery are known unlike other elements such as silver and gold). The importance of this discovery has been memorialized by a painting entitled *The Alchymist* by Joseph Wright (Fig. 1.1), which is currently displayed in the Derby Museum and Art Gallery in Derby, England.

A key person who became interested in learning more about Brandt's discovery was Kunckel von Löwenstern, an alchemist who focused on luminescent materials [6]. After observing sample stock in Brandt's lab, Kunckel informed his fellow alchemist Johann Daniel Kraft about the discovery, who promptly visited Brandt himself. After being unable to convince Brandt to divulge his method for producing phosphorus, Kraft had the presence of mind to purchase all available stock that Brandt had with the intention of displaying phosphorus as a novelty item in various royal courts in Germany, the Netherlands, and England (including its colony in America) [5]. Kraft and Kunckel were later able to determine how to isolate phosphorus from urine, a secret later revealed to Gottfried Wilhelm Leibniz, who wrote a history of the discovery of phosphorus which revealed much of the details outlined above [7]. Eventually phosphorus came to the attention of Robert Boyle in England,

Fig. 1.1 *The Alchymist* by Joseph Wright of Derby (1771)

a serious chemist and one of the greatest scientists of this time [8]. Boyle went on to prepare phosphorus through different and more efficient methods, allowing him to study its properties much more thoroughly than had been done before. After Boyle's death, one of his assistants by the name of Ambrose Godfrey Hancwitz was able to develop a process to manufacture phosphorus for commercial use [5]. Further refinements by his own students and other scientists in the eighteenth century opened up the production of phosphorus on an industrial scale, paving the way for phosphorus to be isolated and used for a number of purposes. Antoine Lavoisier was the first to recognize phosphorus as a distinct chemical element in the late eighteenth century, cementing its place in chemistry.

From Novelty to Utility: Early Uses of Phosphorus

Among the earliest uses of phosphorus was as a medicinal agent [5]. While various medications in the modern era contain phosphorus, the limited understanding of phosphorus metabolism in the eighteenth and nineteenth centuries led to

phosphorus being used for a variety of ailments with little effect. It wasn't until the importance of phosphorus for bone and dental health was fully appreciated in the twentieth century that its use in these arenas became a fruitful application.

Due to its relatively low efficacy as a therapeutic agent, the first major commercial use of phosphorus was in the manufacturing of matches [5]. Apart from its luminescent qualities, one of the earliest appreciated qualities of phosphorus was its flammability. While initially regarded as a nuisance (in fact, an early account suggests that Kunckel was badly burned when mishandling a sample of freshly made phosphorus [5]), entrepreneurs eventually determined how to incorporate phosphorus unto small wooden splints to facilitate the production of matches. The first patents for phosphorus matches were filed in Europe in the 1830s and led the way for the widespread manufacturing of matches in factories located in Europe and the United States [5]. This continued into the twentieth century, only to be replaced by more efficient (and less dangerous) methods to mass-produce matches that exist today. Nonetheless, the utility of phosphorus for producing controlled flames did not escape the attention of individuals charged with producing incendiary products for military use.

The utilization of phosphorus for military applications was first made possible in the late nineteenth century when large-scale production of phosphorus was developed [5]. Among the earliest recorded uses of phosphorus in battle were in World War I, in which it was used as a smokescreen to conceal troop movements. It wasn't until World War II when the use of phosphorus in ordnance was widely adapted to increase the destructive capability of bombs dropped in enemy territory, and also as a critical element in the production of the nerve gas VX [5]. Phosphorus continues to be used for military purposes to this day, but the development of more lethal and efficient methods for wiping out entire populations (e.g., nuclear weapons) has lessened the need for phosphorus to be used primarily for these purposes. Instead, the realization that phosphorus is critical for agricultural purposes was the biggest driver of the exponential increase in the production of phosphorus following World War II (Fig. 1.2) [9].

Several historical developments in the recycling of phosphorus played a key role in the utilization of phosphorus for agriculture in the modern era. Even prior to its discovery in seventeenth century Germany, humans indirectly utilized phosphorus for increasing the fecundity of soil by gathering human and animal excreta and applying it to fields used for raising crops [3]. This setup constituted a relatively stable, self-sustaining phosphorus cycle in which humans and animals consumed phosphorus in foods and then returned much of that phosphorus into the fields through their excreta. This arrangement served communities well when the distance between living quarters and agricultural fields was relatively short, allowing phosphorus from human and animal wastes to be transported back to their fields of origin relatively efficiently.

The mass mobilization of individuals into urban settings during the Industrial Revolution of the eighteenth century touched off major public health dilemmas, not the least of which was how to safely and efficiently remove human waste. This ultimately led to the development of modern sanitation systems that involved the

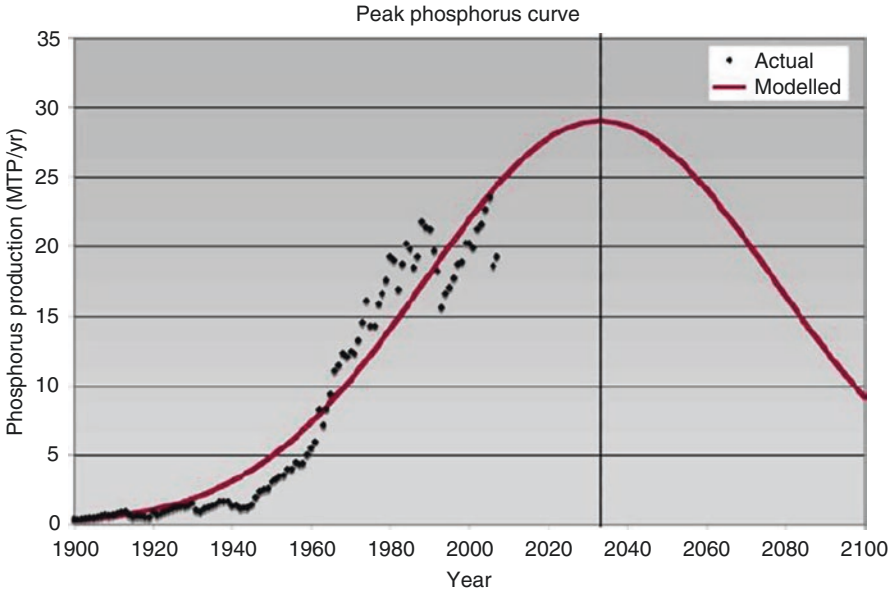

Fig. 1.2 Actual and projected phosphorus production over time (Cordell et al. [9])

removal of wastes using water-based as opposed to land-based disposal. As a result, the self-contained phosphorus cycle of the pre-Industrial Revolution was breached as phosphorus was no longer returned to the soil but instead was disposed of in water-based sewers systems.

Today, by far the highest use of phosphorus for commercial purposes is in agriculture. Once individuals realized the key importance of the phosphorus in the life-cycle of plants, its use as a fertilizer exploded in the twentieth century as part of the so-called Green Revolution [3, 9]. This development was enabled by the discovery that it was the phosphorus and nitrogen components of decaying materials that provided the most potent fertilizing effects of animal and human waste products [3]. This in turn led to the use of concentrated forms of phosphorus in the form of animal excreta (predominantly guano from bat or bird droppings) and then later phosphorus rock deposits that could be extracted from the ground. The discovery that application of phosphorus rock as a fertilizer markedly increased crop yields led to exponential increases in the mining of phosphorus, fueling the massive augmentation of crop yields characteristic of the Green Revolution. This has served an important purpose in increasing crop yields in developing nations with rapidly expanding populations. However, when coupled with the development of water-based disposal systems for human and animal excreta, it has also resulted in a virtual one-way system in which phosphorus is mined from the ground and then eventually lost in the sewers with very little recycled back into the soil [3, 9]. This has led to repeated warnings that phosphorus may soon become a limited resource, potentially threatening the ability to produce farm yields necessary to feed rapidly expanding

populations in both the developed and developing worlds [3, 9, 10]. Now seen as a possibly rare commodity, efforts to moderate the use of phosphorus in the food supply (whether in agriculture or as a food additive) will need to be married with methods to recycle used phosphorus primarily from human and animal waste to ensure that a scarcity of phosphorus does not threaten food supplies in the future.

Conclusion

The importance of phosphorus for all biological systems was emphasized by the late Isaac Asimov who wrote, "Life can multiply until all phosphorus has gone and then there is an inexorable halt which nothing can prevent." [11] Initially discovered by accident and utilized as a novelty for display in the royal courts of seventeenth and eighteenth century Europe, phosphorus has come to the forefront of modern life as a key engine driving the production of food and the safety of food processing. What is particularly important is that this process has occurred over the past 150 years, a relative "blip" in the history of human civilization. Thus, while our understanding of the myriad ways in which phosphorus impacts biological systems has grown exponentially since its early discovery, there remains much to learn. This is particularly the case in human food supplies, which have witnessed the widespread and growing use of phosphorus as additives only over the past century [3]. The following chapters in this text will aim to delineate the importance of these developments by focusing on the biology of phosphorus, its uses and misuses, and what we still need to learn to fully appreciate the importance of this remarkable element in the modern era.

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Overview of Phosphorus Homeostasis

2

Keith Hruska

Key Points

- The regulation of phosphorus is under tight control through numerous integrated physiological systems.
- The gastrointestinal tract and the kidney are the key organs involved in phosphorus handling from dietary sources.
- Transcellular phosphorus movement represents an important facet of overall systemic phosphorus balance.

Introduction

Phosphorus is a common anion ubiquitously distributed throughout the body as phosphates. Approximately 80–85 % of the phosphorus is present in the skeleton. The rest is widely distributed in the form of organic phosphate compounds that play fundamental roles in several aspects of cellular metabolism. The energy required for many cellular reactions including biosynthesis derives from hydrolysis of adenosine triphosphate (ATP). Organic phosphates are important components of cell membrane phospholipids. In the extracellular fluid (ECF), phosphorus is present predominantly in the inorganic form (HPO_4^-, or HPO_4^{-2}). The physiologic concentration of serum phosphorus ranges from 2.5 to 4.5 mg/dL (0.9–1.45 mmol/L) in adults [1]. In serum, phosphorus exists mainly as the free anions or as complexes. Only a small

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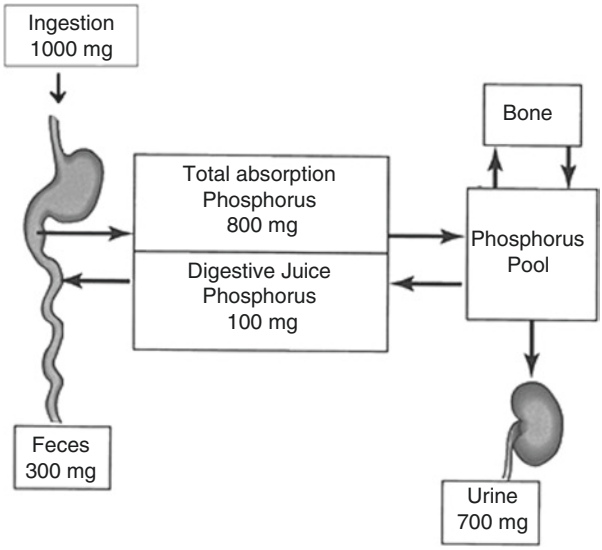

Fig. 2.1 Summary of phosphorus metabolism. Humans ingest approximately 1 g of phosphorus daily, of which 300 mg is excreted in the stool and 700 mg in the urine. The gastrointestinal tract, bone and kidney are the major organs involved in phosphorus homeostasis

fraction (less than 15 %) is protein bound [2, 3]. There is a diurnal variation in serum phosphorus of 0.6–1.0 mg/dL, with the nadir occurring between 8 am and 11 am.

Phosphorus Balance: Gastrointestinal Absorption

Approximately 1 g of phosphorus is ingested daily in an average diet in the United States. About 300 mg is excreted in the stool, and 700 mg is absorbed (Fig. 2.1). Most of the phosphorus is absorbed in the duodenum and jejunum with minimal absorption occurring in the ileum [4]. Phosphorus transport in proximal segments of the small intestine appears to involve both passive (paracellular) and active (transcellular) components and to be under the influence of vitamin D. The paracellular pathway is a diffusion-driven nonsaturable process in which phosphate transport increases with increasing phosphate in the diet. Thus, with increasing dietary phosphate intake, phosphate absorption increases linearly. The transcellular movement of phosphorus from the intestinal lumen to the blood requires: (a) transport across the luminal brush-border membrane of the intestine; (b) transport through the cytoplasm; and (c) transport across the basolateral plasma membrane of the epithelium. The rate-limiting step and the main driving force of absorption is the luminal membrane step [1].

Intestinal Epithelial Luminal Membrane Transport

The mechanism of transport across the intestinal brush border epithelial membrane involves a sodium–phosphate (NaPi) cotransport system, NaPiIIb (Npt2b/SLC34A2) [5]. The NaPi cotransporters are a secondary active form of ion transport using the energy of the Na gradient from outside to inside the cell to move phosphate ion uphill against an electrochemical gradient (Fig. 2.2).

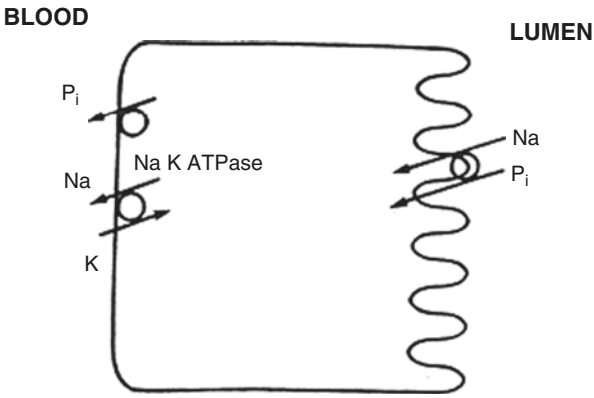

Fig. 2.2 The apical membrane sodium-inorganic phosphate (Pi) cotransport proteins utiltize the electrochemical driving force for sodium to move Pi into the cell. The electrochemical sodium gradient is maintained by active sodium extrusion across the basolateral membrane through the action of Na^+-K^+-ATPase

The intestinal NaPiIIb transporter is upregulated by a low phosphate diet and 1,25-dihydroxyvitamin D3 [6, 7]. Although low phosphate diets upregulate 1,25-dihydroxyvitamin D3, studies in vitamin D–receptor (VDR), null mice indicate that the intestinal NaPi cotransport adaptation to a low phosphate diet occurs independent of vitamin D [7].

Intestinal NaPi cotransport activity and NaPiIIb protein is also regulated by several other factors including the aging process, glucocorticoids, and epidermal growth factor (EGF) that decrease intestinal NaPi transport, and estrogen and metabolic acidosis that increase intestinal NaPi transport.

Studies of phosphorus accumulation by rat intestinal brush-border vesicles have demonstrated that it is affected by the transmembrane potential, indicating that like the renal cotransporter, NaPi2a, the intestinal NaP-2b, is electrogenic [5]. The $K_m(P_i)$ of NaPi2b is approximately 50 μm, similar to the renal transport protein. In contrast to the renal NaPi2a isoform, the intestinal NaPi2b cotransporter is less dependent on the pH level.

Transcellular Movement of Phosphorus

The second component of transcellular intestinal phosphorus transport involves the movement of phosphorus from the luminal to the basolateral membrane. Although little is known about the cellular events that mediate this transcellular process, evidence suggests a role for the microtubular microfilament system of intestinal cells [4]. Microfilaments in the cell may be important in conveying phosphorus from the brush-border membrane to the basolateral membrane and may be involved in the extrusion of phosphorus at the basolateral membrane from the epithelial cell.

Phosphate Exit at Basolateral Membrane

Little is known about the mechanisms of phosphorus extrusion at the basolateral membrane of intestinal epithelial cells. The electrochemical gradient for phosphorus favors movement from the intracellular to the extracellular compartment because

the interior of the cell is electrically negative compared with the basolateral external surface. Therefore, the presumption has been that the exit of phosphorus across the basolateral membrane represents a mode of passive transport [8].

Renal Excretion of Phosphorus, Reabsorption

Most of the inorganic phosphorus in serum (90–95 %) is ultrafilterable at the level of the glomerulus. At physiologic levels of serum phosphorus, approximately 7 g of phosphorus is filtered daily by the kidney, of which 80–90 % is reabsorbed by the renal tubules and the remainder is excreted in the urine (approximately 700 mg on a 1-g phosphorus diet) equal to intestinal absorption [9]. As a result, adults are generally in balance between phosphorus intake and excretion (Fig. 2.1). Micropuncture studies have demonstrated that 60–70 % of the filtered phosphorus is reabsorbed in the proximal tubule. However, there is also evidence that a significant amount of filtered phosphorus is reabsorbed in distal segments of the nephron [10]. When serum phosphorus levels increase and the filtered load of phosphorus increases, the capacity to reabsorb phosphorus also increases. However, a maximum rate of transport (Tm) for phosphorus reabsorption is obtained usually at serum phosphorus concentrations of 6 mg/dL. There is a direct correlation between Tm phosphorus values and glomerular filtration rate (GFR) even when the GFR is varied over a broad range. Micropuncture studies suggest two different mechanisms responsible for phosphorus reabsorption in the proximal tubule. In the first third of the proximal tubule, in which only 10–15 % of the filtered sodium and fluid is reabsorbed, the ratio of tubular fluid (TF) phosphorus to plasma ultrafilterable (UF) phosphorus falls to values of approximately 0.6. This indicates that the first third of the proximal tubule accounts for approximately 50 % of the total amount of phosphorus reabsorbed in this segment of the nephron. In the last two-thirds of the proximal tubule, the reabsorption of phosphorus parallels the movement of salt and water. In the remaining 70 % of the pars convoluta, the TF: UF phosphorous ratio remains at a value of 0.6–0.7, whereas fluid reabsorption increases to approximately 60–70 % of the filtered load. Thus, in the last two-thirds of proximal tubule, the TF:UF phosphorus reabsorption ratio is directly proportional to sodium and fluid reabsorption. A significant amount of phosphorus, perhaps on the order of 20–30 %, is reabsorbed beyond the portion of the proximal tubule that is accessible to micropuncture. There is little phosphorus transport within the loop of Henle, with most transport distal to micropuncture accessibility occurring in the distal convoluted tubule. In this location, Pastoriza-Munoz et al. [10] found that approximately 15 % of filtered phosphorus is reabsorbed under baseline conditions in animals subjected to parathyroidectomy, but that the value falls to about 6 % after administration of large doses of PTH. The collecting duct is a potential site for distal nephron reabsorption of phosphorus [11–13]. Transport in this nephron segment may explain the discrepancy between the amount of phosphorus delivered to the late distal tubule in micropuncture studies and the considerably smaller amount of phosphorus that appears in the final urine of the same kidney. Phosphorus transport in the cortical

collecting tubule is independent of regulation by PTH. This is in agreement with the absence of PTH-dependent adenylate cyclase in the cortical collecting tubule [11].

Comparison of Superficial and Deep Nephron Transport

The contribution of superficial nephrons and deep nephrons of the kidney to phosphorus homeostasis differs. Nephron heterogeneity in phosphorus handling has been evaluated under a number of conditions by puncture of the papillary tip and the superficial early distal tubule, with the recorded fractional delivery representing deep and superficial nephron function, respectively. Microinjection of phosphorus tracer into thin ascending and descending limbs of loops of Henle reveals that only 80 % of phosphorus was recovered in the urine, whereas 88–100 % of phosphorus was recovered when the tracer was injected into the late superficial distal tubule. It was concluded that a significant amount of phosphorus must be reabsorbed by juxtamedullary distal tubules or by segments connecting the juxtamedullary distal tubules to the collecting ducts to account for the discrepancy between the results of superficial nephron injection and juxtamedullary nephron injections. These data support an increased reabsorptive capacity for phosphorus in deep as opposed to superficial nephrons and increased responsiveness to body Pi requirements [14, 15].

Cellular Mechanisms of Phosphate Reabsorption in the Kidney

The apical membrane of renal tubular cells is the initial barrier across which phosphorus and other solutes present in the tubular fluid must pass to be transported into the peritubular capillary network. Because the electrical charge of the cell interior is negative to the exterior, and phosphorus concentrations are higher in the cytosol, phosphorus must move against an electrochemical gradient into the cell interior, whereas at the antiluminal membrane, the transport of phosphorus into the peritubular capillary is favored by the high intracellular phosphorus concentration and the electronegativity of the cell interior. Studies with apical membrane vesicles have demonstrated cotransport of Na^+ with phosphate across the brush-border membrane, whereas the transport of phosphorus across the basolateral membrane is independent of that of Na^+ [16]. The apical membrane Na^+-phosphate cotransport proteins (NaPi2a and NaPi2c) energize the uphill transport of phosphate across the brush-border membrane (BBM) by the movement of Na^+ down its electrochemical gradient. The latter gradient is established and maintained by active extrusion of Na^+ across the basolateral cell membrane into the peritubular capillary through the action of Na^+-K^+-ATPase (Fig. 2.2) [17].

There are three families of NaPi cotransport proteins in the proximal tubule (types I, II, and III) [18–23]. The DNA clones encode 80–95-kd proteins that reconstitute Na^+-dependent concentrative, or "uphill," transport of phosphate [18, 21, 24]. The type I cotransporter, NaPi1 (Npt1/SLC17), is expressed predominantly in the renal proximal tubule, and it accounts for about 13 % of the known NaPi

cotransporter mRNA in the mouse kidney [25]. NaPi1 is not regulated by dietary P_i, and studies in NaPi1-cRNA–injected oocytes revealed that it functions not only as a NaPi cotransporter but also as a chloride and organic anion channel [26].

The type II cotransporter NaPi2/SLC34 proteins are similar between several species including humans [19, 20, 22]. In addition to NaPi2a (Npt2a/SLC34A1), the predominant isoform in the renal proximal tubule, another isoform NaPi2c (Npt2c/SLC34A3) is important as mutations in its coding cause hereditary hypophosphatemic rickets with hypercalciuria (HHRH) along with proximal tubulopathy without metabolic acidosis [27–30]. Nephron localization of Npt2 proteins has been limited to the proximal tubule of superficial and deep nephrons (greatest in the latter, concordant with physiologic studies) [31–33]. Immunolocalization studies in renal epithelial cells demonstrated apical membrane and subapical membrane vesicle staining [31–33] for NaPi2c in S1 while NaPi2a is present along the entire proximal tubule, suggesting that a functional pool of transporters is available for insertion into or retrieval from the brush-border membrane itself. This is a major mechanism of P_i transport regulation in response to acute changes in phosphorus, PTH, MEPE, and FGF23 levels [19, 20, 22, 34, 35]. The NaPi2 family is upregulated at message and protein levels by chronic feeding of low-P_i diets [36, 37] and regulated at message and protein levels by PTH and FGF23 [36–38].

The type III NaPi cotransporters SLC20 were originally identified as retroviral receptors for gibbon ape leukemia virus (Glvr1), Pit1, and rat amphotropic virus (Ram1), Pit2 [39]. They are ubiquitously expressed, and they comprise about 1 % of the known NaPi cotransporter mRNAs in the mouse kidney [25]. Their levels and activity adapt to dietary phosphate changes. They were viewed as "housekeeping" NaPi cotransporters due to their ubiquitous expression serving cellular needs for Pi in many processes including ATP generation, but recent studies have found Pit2 on the apical membrane of the PCT and it participates in regulation of phosphate transport, although not importantly in basal conditions. More recently, the possibility that they represent "Pi sensors" stems from experimental data from osteocyte and vascular smooth muscle cells where cellular responsiveness to extracellular Pi concentrations have been blocked by siRNA or genetic deletion of Pit1 and Pit2.

Studies of phosphorus exit across the basolateral membrane suggest that it is accompanied by the net transfer of a negative charge and occurs down a favorable electrochemical gradient via sodium-independent mechanisms [40].

Proteins That Interact with the Type IIa Na/Pi Cotransporter Protein

Several proteins with PDZ domains have been identified that interact with the NaPiIIa protein and are localized in the BBM or the subapical compartment (Fig. 2.3). PDZ domains are modular protein interaction domains that often occur in scaffolding proteins and bind in a sequence-specific fashion to the C-terminal peptide sequence or at times the internal peptide sequences of target proteins. These domains of approximately 90 amino acids are known by an acronym of the first three PDZ-containing proteins identified including the postsynaptic protein PSD-95/SAP90, the Drosophila septate junction protein Discs-large, and the tight junction protein ZO-1 [41–44].

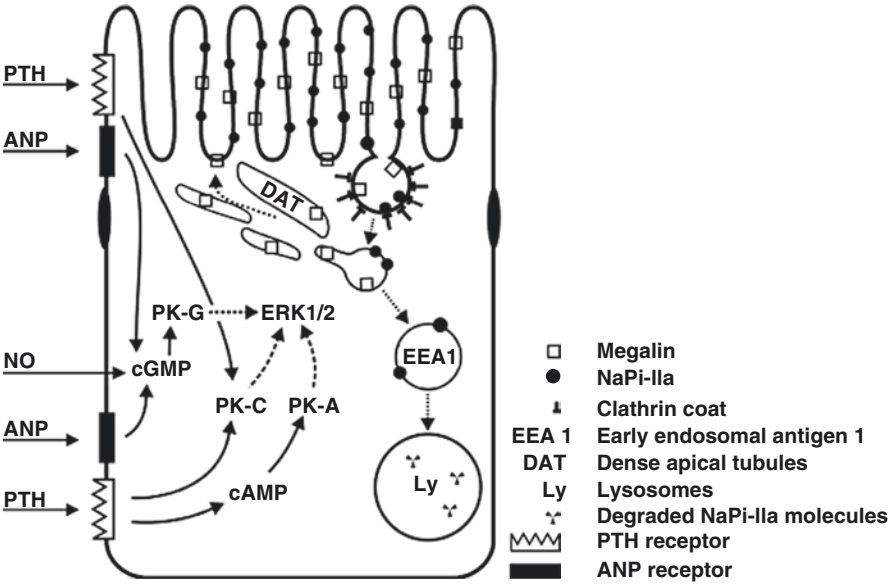

Fig. 2.3 Mechanisms of NaPi-IIa traffic in the apical membranes of proximal tubular cells. Microvillar NaPi-IIa is retrieved in magalin comtaining clathrin coated vesicles. NaPi-IIa moves from the vesicles into an endosomal pool marked by EEA-1, whereas megalin is recycled through dense apical tubules back to the microvilli. The endosomal NaPi-IIa is targeted for lysosomal degradation. The processes of NaPi-IIa retrieval and lysosomal degradation is stimulated by several factors (parathyroid hormone, fibroblast growth factor 23, atrial natriuretic peptide, nitric oxide) whose mechanisms of signal transduction (PK-A, PK-C, PK-G) merge in the activation of ERK 1/2 that modulates the process (From [34] with permission)

PDZ domain containing proteins including NHERF-1, NHERF-2, PDZK1, CAL, and ZO-1 play an important role in: (a) the regulation of the expression and activity of renal proximal tubular BBM transport proteins including NHE-3 [44–48] and NaPi IIa [34, 49], basolateral membrane transport proteins including Na-K-ATPase [49] and Na-HCO_3 cotransporter (NBC) [48]; (b) the regulation of the expression and activity of cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated chloride channel and channel regulator [50–54]; (c) parathyroid hormone 1 receptor signaling [55] and endocytic sorting of the β2-adrenergic receptor [56] and platelet-derived growth factor receptor (PDGFR) [57, 58]; (d) epithelial cell polarity and formation of tight junctions [59, 60]; and (e) maintaining the integrity of the glomerular barrier to proteins through interactions with podocalyxin, negatively charged sialoprotein expressed on the surface of podocytes, the glomerular visceral epithelial cells [61–66].

In addition to their interaction with membrane proteins and receptors, the PDZ domain-containing proteins also interact with the F-actin cytoskeleton through their interactions with the ezrin-radixin-moesin (ERM) proteins (Fig. 2.3) [67–69]. ERM proteins are typically located peripherally in the membrane and link the cytoplasmic tails of membrane proteins and receptors to the cortical actin cytoskeleton. The ERM proteins play an important role in the formation of microvilli, cell-cell junctions, and membrane ruffles, and also participate in signal transduction pathways. The ERM proteins contain an F-actin binding site within their carboxy-terminal 30

residues. In addition, the ERM proteins have FERM (four-point one, ezrin, radixin, moesin) domains, which are generally located at or near the amino terminal, and act as multifunctional protein and lipid binding sites. The FERM domain of ezrin interacts strongly with NHERF-1 and NHERF-2. NHERF-1 and NHERF-2 have 2 PDZ domains and have a carboxy-terminal sequence of 30 amino acids that bind ezrin.

Using the molecular approach (yeast two-hybrid) several proteins with PDZ domains that interact with the C terminus of NaPi 2a have been identified including: (i) NHERF-1/EBP50, (ii) NHERF-2/E3KARP, (iii) PDZK1/NaPi-Cap1, (iv) PDZK2/NaPi-Cap2, and (v) CAL, a CFTR-associated ligand [70–78].

Different studies suggest that apical expression of NaPi2a depends on the presence of NHERF-1. Expression of NaPi2a was reduced upon introduction of dominant-negative form of NHERF-1 in OK cells [62, 72, 73, 75]. The in vivo importance of the NaPi2a interaction with NHERF-1 was also shown in a study where targeted disruption of the mouse NHERF-1 gene was associated with decreased BBM expression and increased intracellular localization of NaPi2a resulting in decreased renal phosphate reabsorption and renal phosphate wasting [79]. On the other hand, targeted disruption of NHERF-1 did not modulate the BBM expression and localization of NHE3; however there was impaired regulation of NHE3 in response to PKA [79].

In contrast to NHERF-1, targeted disruption of the PDZK1 gene failed to modulate the BBM expression of NaPi2a [80, 81]. NHERF-1, therefore, plays a critical and unique role in the renal proximal tubular apical membrane targeting of NaPi2a protein and maintenance of phosphate homeostasis.

Recent studies have identified at least three additional proteins that may be important in the regulation of NaPi2a targeting and trafficking: (i) the peroxisomal protein PEX 19, a farnesylated protein that confers PTH responsiveness to NaPi2a [82], (ii) the calcium binding protein Vilip-3, a myristoylated protein that may be important in calcium dependent regulation of NaPi2a [83], and (iii) MAP 17 which may be important for apical expression of PDZK1 [77].

Regulation of Homeostasis by Factors That Affect the Urinary Excretion of Phosphorus

Of the multiplicity of factors that regulate phosphate transport in the kidney, the most important are dietary phosphate, PTH and FG23.

Alterations in Dietary Phosphorus Intake

The mechanism by which the kidney modulates phosphorus excretion when dietary phosphorus is reduced or increased continues to be intriguing. Micropuncture studies [84] suggested that the entire nephron participates in the reduction of phosphorus excretion during dietary phosphorus deprivation. It has been shown that isolated perfused tubules obtained from rabbits that were fed a normal or low-phosphorus

diet differ in their capacity to reabsorb phosphate. In normal animals, the proximal convoluted tubule (PCT) is capable of reabsorbing 7.2 ± 0.8 pmol/mL per minute, whereas tubules obtained from phosphorus-deprived animals reabsorb 11.1 ± 1.3 pmol/mL per minute. Conversely, animals that are fed a high phosphorus diet show reduced phosphorus reabsorption when the proximal tubules are perfused in vitro (2.7 ± 2.6 pmol/mL per minute).

The effect of reduced dietary phosphorus to stimulate renal phosphorus transport is intrinsic to the renal tubular epithelium and occurs at the BBM Na^+-phosphate cotransporter. The adaptation to phosphate supply by NaPi2a and NaPi2c is biphasic [70, 85, 86]. Incubation of cells in a low-phosphate medium results in a twofold increase in Na^+-independent phosphate cotransport. The first phase of adaptation is observed rapidly (within 10 min) and is characterized by an increase in the V_{max} of the transporter. This initial phase is independent of new protein synthesis [36, 87, 88]. A slower phase resulting in a doubling of the phosphate transport rate, also through an increase in V_{max}, occurs over several hours and is inhibited by blocking new protein synthesis [89]. The adaptation to acute Pi deprivation occurs independent of de novo transcription and protein synthesis and is mediated by apical insertion of cytoplasmic NaPi2a transporters by a microtubule dependent mechanism [37] (Fig. 2.3). Secondly, through gene transcription and increased NaPi protein synthesis, additional units are produced and inserted into the brush border.

Effects of PTH on Phosphorus Reabsorption by the Kidney

Parathyroidectomy decreases urinary phosphorus excretion, whereas PTH administration increases phosphorus excretion [90, 91]. Micropuncture studies indicate that PTH inhibits phosphorus transport in the proximal tubule [92] and in segments of the nephron located beyond the proximal tubule [10]. TF: UF phosphorus ratio reaches a value of 0.6 by the S_2 segment of the proximal tubule, and once achieved, this equilibrium ratio is maintained along the accessible portion of the proximal tubule. Within 6–24 h after parathyroidectomy, the proximal TF: UF phosphorus ratio falls to a value of 0.2–0.4, indicating an increase in phosphorus reabsorption [84, 93, 94]. TF phosphorus falls progressively with continuous fluid absorption along the length of the tubule, so by the end of the proximal tubule, the reabsorption of phosphorus is 70–85 % of the filtered load, resulting in decreased phosphorus delivery to distal segments of the nephron. In the nonphosphorus-loaded, acutely parathyroidectomized animal, virtually all the distal load of phosphorus is reabsorbed by the distal nephron, reducing urinary phosphorus excretion to very low levels [92, 95]. In the phosphorus-loaded animal, the distal reabsorption of phosphorus increases until saturation is approached and urinary phosphorus excretion begins to rise. Acute administration of PTH to phosphorus-loaded parathyroidectomized dogs sharply lowers the distal reabsorption.

Administration of PTH in vivo results in decreased rates of Na^+-dependent phosphorus transport in brush-border membrane vesicles isolated from the kidneys of treated rats [96, 97]. Intravenous infusion of dibutyryl cyclic adenosine

monophosphate (cAMP) also decreased Na^+-dependent phosphorus uptake in isolated brush-border vesicles, but neither PTH nor dibutyryl cAMP decreased phosphate transport when added directly to membrane vesicles [96]. PTH stimulates two signaling pathways in proximal tubule cells: adenylate cyclase and phospholipase C (PLC), resulting in activation of protein kinase A (PKA) and protein kinase C (PKC) [98]. The first pathway, activation of the adenylate cyclase, differs from that of PKC. Studies in OK cells show that PKA activation by PTH decreases the expression of NaPi2a cotransporter due to internalization and degradation of the transporter. Binding of PTH to its receptor leads to activation of PLC with the subsequent hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 generation leads to the release of intracellular calcium stores. DAG activates PKC [98]. In addition to its direct effect on NaPi2a, PTH inhibits NaPi transport indirectly by inhibiting the Na^+-K^+-ATPase by decreasing the favorable Na^+ gradient for Pi entry into the cell [99]. The activation of PKA and PKC signaling pathways by PTH also activates mitogen activated protein (MAP) kinase (MAPK) or extracellular receptor kinase (ERK1/2) which also induces inhibition of NaPi transport [100, 101].

Measurement of in vivo renal reabsorption of phosphorus and calculations of kinetic parameters of Na^+-dependent phosphorus transport in membrane vesicles isolated from the renal brush-border membranes of normal dogs, parathyroidectomized dogs, dogs fed a low-phosphorus diet, and dogs receiving human growth hormone [97, 102] revealed greater baseline values for absolute tubular reabsorption of phosphorus in the latter three groups compared with normal dogs. Na^+-dependent phosphate transport in brush-border membrane vesicles isolated from kidneys of these dogs was significantly increased compared with transport in brush-border vesicles from kidneys of normal dogs. Administration of PTH decreased significantly the apparent V_{max} for Na^+-dependent phosphorus transport in brush-border membrane vesicles isolated from kidneys of each of the four groups of dogs. The apparent K_m (intrinsic binding affinity) for Na^+-dependent phosphorus transport was not significantly changed by experimental maneuvers. Absolute tubular reabsorption of phosphorus measured in vivo was decreased by administration of PTH in each group of dogs with the exception of the dogs fed a low-phosphorus diet [97, 102]. Thus, alterations in phosphorus reabsorption measured in vivo were paralleled by alterations in Na^+-dependent phosphorus transport in isolated membrane vesicles, and the administration of PTH in vivo resulted in altered transport characteristics of the isolated brush-border membranes.

Parathyroidectomy of rats causes a twofold to threefold increase in the NaPi IIa protein content of brush-border membrane vesicles [103]. Immunocytochemistry reveals the increase in protein exclusively in apical brush-border membranes of proximal tubules. PTH treatment of parathyroidectomized rats for 2 h decreased protein levels and decreased the abundance of NaPi2a-specific messenger RNA (mRNA) by 31 % [103]. Parathyroidectomy did not affect NaPi2a mRNA levels. The effects of PTH indicate that PTH regulation of NaPi2a is determined by changes in the expression of NaPi2a protein in the renal brush-border membranes [103].

PTH decreases the NaPi2a protein content of the apical membrane by an endocytic retrieval pathway which is megalin dependent [104] (Fig. 2.3). In megalin intact mice or rats, following treatment with PTH NaPi IIa is internalized via clathrin-coated pits, and NaPi IIa is then delivered to early endosomes and eventually to the lysosomes where the protein is degraded. At the present time unlike some of the other proximal tubular transport proteins or receptors, there is no evidence that the NaPi2a protein is present at the recycling endosomes.

Fibroblast Growth Factor 23 (FGF23)

Through studies of familial hypophosphatemia and tumor induced osteomalacia, a new hormone operating in a systems biology network regulating phosphate homeostasis between the skeleton and the kidney was discovered, fibroblast growth factor 23 (FGF23) [105, 106]. FGF23 is secreted by skeletal osteocytes in response to changes in calcitriol and serum phosphorus [107]. The physiology is that the osteocyte monitors deposition of phosphorus into the skeletal reservoir and the saturation of the exchangeable phosphorus pool. When Pi levels increase within the pool due to decreased exit into the skeleton or due to increased plasma phosphorus, osteocytes secrete FGF23, which acts on the renal proximal tubule to decrease reabsorption and increase phosphorus excretion [108, 109].

FGF23 actions at the proximal tubule (PCT) have not been studied as extensively as the actions of PTH described above. Studies indicate that FGF23 acts to decrease expression of NaPi2a and NaPi2c in the PCT [109, 110]. The actions of FGF23 on the PCT are mediated through binding to a FGF receptor (predominantly FGFR1(IIIc)) and a coreceptor, Klotho [111]. Signal transduction is stimulated through phosphorylation of extracellular signal-regulated kinase (ERK) and the immediate early response gene, early growth response-1 (Erg-1), a zinc finger transcription factor [111, 112]. A matter of current uncertainty is related to Klotho expression which is mainly in the renal distal tubule whereas its signaling function is in the PCT. Recent studies have shown that it is expressed in the PCT, but a paracrine role for a cleavage product comprising the extracellular domain of klotho remains unclear.

Besides inhibiting PCT renal Pi transport, FGF23 signaling inhibits PCT CYP27B1, the 25-OH cholecalciferol 1α hydroxylase, and activates 24-OH hydroxylase (CYP24R1) resulting in decreased production and increased catabolism of calcitriol [106]. In addition, activation of 24-OH hydroxylase results in the high prevalence of vitamin D deficiency associated with elevations of FGF23 levels, especially in chronic kidney disease.

Other Regulatory Factors

Space does not permit discussion of the other regulatory factors of phosphate homeostasis shown in Table 2.1 in this chapter.

Table 2.1 Causes of hypophosphatemia

I. Increased excretion of phosphorus in the urine
A. Primary hyperparathyroidism
B. Secondary hyperparathyroidism
C. Renal tubular defects (Fanconi's)
D. Diuretic phase of acute tubular necrosis
E. Postobstructive diuresis
F. Extracellular fluid volume expansion
G. Familial
1. X-linked hypophosphatemia
2. ADHR
3. Oncogenic hypophosphatemic osteomalacia
4. McCune-Albright syndrome/fibrous dysplasia
5. Mutations in NaPiIIa
6. Hereditary hypophosphatemic rickets with hypercalciuria (HHRH)
H. Posttransplant hypophosphatemia
II. Decrease in gastrointestinal absorption of phosphorus
A. Abnormalities of Vitamin D metabolism
1. Vitamin D–dependent rickets
2. Familial
(a) Vitamin D–dependent rickets
(b) X-linked hypophosphatemia
B. Malabsorption
C. Malnutrition-starvation
III. Miscellaneous causes/translocation of phosphorus
A. Diabetes mellitus: during treatment for ketoacidosis
B. Severe respiratory alkalosis
C. Recovery phase of malnutrition
D. Alcohol withdrawal
E. Toxic shock syndrome
F. Leukemia, lymphoma
G. Severe burns

Phosphate Homeostasis in Chronic Kidney Disease

Loss of nephron function due to kidney disease or kidney injury changes the filtered load of phosphorus, and acutely decreases Pi excretion. Adaptation to mild chronic reductions in glomerular filtration, stage 2 chronic kidney disease (CKD), restores Pi excretion to normal and reduces the fraction of filtered Pi reabsorbed. The serum Pi is not changed, and the restoration of homeostasis is due to increased activity of PTH and FGF23 on the PCT. In stage 2 CKD, elevations of FGF23 in the presence of normal PTH and serum Pi can be found, with elevations of PTH but normal serum Pi with more severe reductions in GFR. The mechanism of increased

FGF23 in the presence of normal serum Pi is unclear, but it may be due to the increased Pi concentration of the tubular fluid delivered out of the proximal tubule, and production of cleaved circulating klotho activating FGF23 secretion. This mechanism was discovered in a patient with a mutation in klotho resulting in increased cleavage of the transmembrane αklotho. Thus, cleaved, cut, or circulating klotho, cklotho, is a new hormone regulating Pi homeostasis, while the parent αklotho protein is multifunctional including serving as the coreceptor for FGF23.

As CKD progresses, stage 3, decreased αklotho expression in the tubules produces FGF23 resistance, and despite progressive increases in FGF23 and PTH levels hyperphosphatemia develops due to decreased Pi excretion. Hyperphosphatemia sets up a vicious cycle of increasing FGF23 and PTH secretion that is ineffective leading to the severe complications of the chronic kidney disease – mineral bone disorder that will be discussed in subsequent chapters.

Conclusions

The regulation of phosphorus is under tight control through a variety of local and systemic factors that modulate phosphorus balance in the body physiological systems. This chapter has reviewed the key factors involved in this regulation and the consequences of disruptions in these systems that can lead to chronic disease via disturbances in phosphorus balance.

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Hormonal Regulation of Phosphorus Homeostasis: Parathyroid Hormone, Fibroblast Growth Factor 23, and Klotho

3

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Key Points

- Fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), and vitamin D ensure homeostatic balance of phosphorus in the human body.
- Klotho is a key cofactor that allows FGF23 to bind to its cognate receptor with high affinity and effect downstream signaling that regulates phosphorus and vitamin D metabolism.
- Dysregulation of FGF23, PTH, and/or klotho is associated with vascular and skeletal anomalies due to altered phosphate turnover.

Introduction

The optimal balance of phosphate level is biologically important, as inorganic phosphorus performs many essential cellular functions within the body. Phosphate is a component of nucleic acids (DNA and RNA), and it is also an important component in the structure of phospholipids in cell membranes. Phosphate also plays roles in cell signaling (through phosphorylation), in energy metabolism (as ATP), and in

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bone mineralization (as hydroxyapatite). Recent studies have provided evidence of endocrine regulation of systemic phosphate homeostasis; such regulation of phosphate depends on a delicate balance among circulating factors like active form of vitamin D, parathyroid hormone (PTH), bone-derived fibroblast growth factor 23 (FGF23), and kidney-derived klotho. Dysregulation of these factors can induce phosphorus imbalances which can affect the functionality of almost every human system, eventually leading to an increase in morbidity and mortality.

In Vivo Phosphate Turnover

Renal function is essential to preserve physiologic water, electrolyte, and mineral ion balance. In most chronic renal diseases, impaired renal function perturbs the physiologic water, electrolyte, and mineral ion levels, including phosphate homeostasis. Phosphate is widely distributed in the body and is an important factor in bone formation. In addition, phosphate is also involved in cell signaling, energy metabolism, nucleic acid synthesis, and the maintenance of acid-base balance through affecting urinary buffering system.

The coordinated interactions of small intestine, bone, parathyroid gland, and kidney maintain physiologic phosphate balance; structural and functional impairments in any of these organs can lead to abnormal phosphate balance (Table 3.1) [3–6]. Dietary

Table 3.1 Causes of serum phosphate imbalance [1, 2]

Acidosis (respiratory or lactic acidosis, diabetic ketoacidosis)
Alkalosis
Cortical hyperostosis
Drugs (amphotericin B, bisphosphonate, etc.)
Glucocorticoid deficiency
Growth hormone impairment (acromegaly)
Heat impairment (hyperthermia or hypothermia)
Hemolysis
Infections
Intestinal impairment (bowel infarction)
Magnesium deficiency
Milk-alkali syndrome
Parathyroid hormone impairment (pseudo/hypoparathyroidism)
Phosphate-containing laxatives or enemas
Renal impairment
Rhabdomyolysis
Sarcoidosis
Trauma (burns, crush injuries, etc.)
Tumoral calcinosis
Tumors (leukemia, lymphoma, bone tumors)
Vitamin D intoxication

phosphate is usually absorbed from the intestine and taken up by the cells that need it, and the remaining amount is mostly excreted out of body through urine. Transepithelial phosphate transport in the intestine (by enterocytes) and in the kidney (by proximal epithelial cells) is primarily mediated by the sodium phosphate (Na/Pi) cotransporter family (Na/Pi-2a, Na/Pi-2b, and Na/Pi-2c) that are expressed in the apical membranes. More than 80 % of the filtrated phosphate in the kidney is reabsorbed in the proximal tubules, mostly with the help of Na/Pi-2a and Na/Pi-2c. Various endocrine factors, including PTH, active vitamin D metabolites, and FGF23 can directly or indirectly control Na/Pi activities to influence systemic phosphate balance (Fig. 3.1) [7–10].

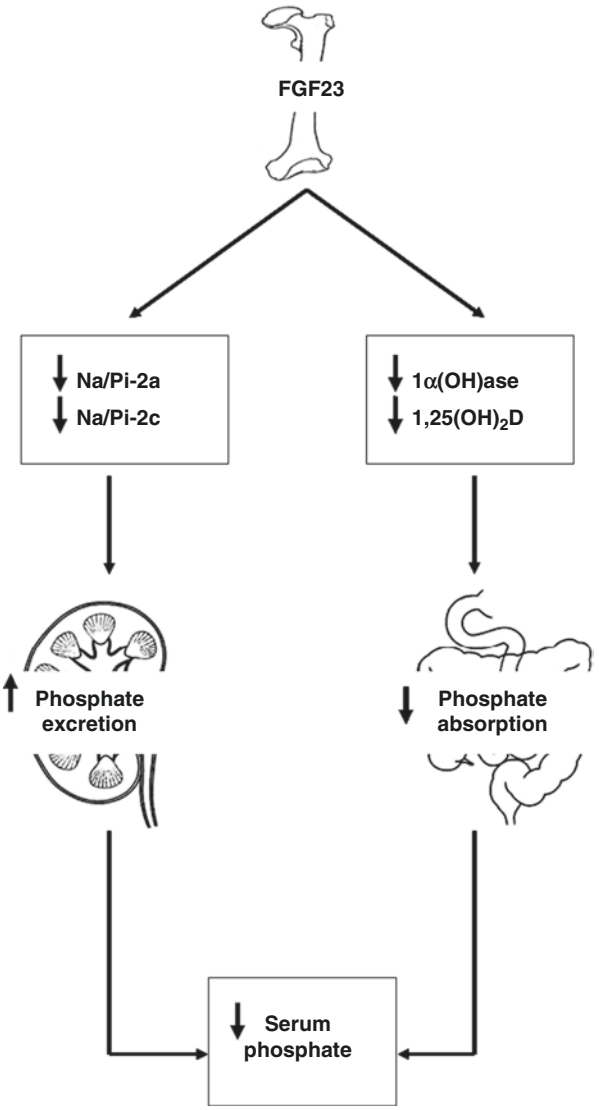

Fig. 3.1 FGF23 produced in the bone can suppress Na/Pi-2a and Na/Pi-2c cotransporters to increase the renal excretion of phosphate. Similarly, FGF23 can also suppress renal expression of 1α(OH)ase to reduce production of $1,25(OH)_2D$ to decrease intestinal phosphate absorption, resulting in reduced serum levels of phosphate [1, 2]

Among these factors, PTH is one of the most potent regulators of phosphate metabolism. PTH can suppress the reabsorption of phosphate in the proximal tubules by reducing Na/Pi-2a and Na/Pi-2c activities. Such PTH-mediated suppression of Na/Pi-2a and Na/Pi-2c is achieved by internalization of Na/Pi proteins from the luminal side of the proximal tubular epithelial cells [11–13]. PTH can also mobilize phosphate from the bone into the bloodstream, possibly by enhancing osteoclastic bone resorption. Moreover, PTH can increase the production of 1,25- dihydroxyvitamin D3 [1,25$(OH)_2$D] by inducing the renal expression of 1α-hydroxylase [1α(OH)ase] to influence intestinal phosphate absorption. In addition to PTH and vitamin D, numerous hormones can also affect renal phosphate handling. Growth hormone, insulin, and thyroid hormone can all increase phosphate reabsorption, while calcitonin, glucocorticoids, and atrial natriuretic factor can decrease phosphate reabsorption, primarily by influencing Na/Pi-2a activities [14, 15].

Cellular, intracellular, transcellular, and pericellular mineral ion transports are complex processes achieved by both active and passive translocation. Phosphate transport across renal proximal tubular epithelial cells is mostly driven by a high extracellular sodium gradient, which is thought to be maintained by the membrane-associated Na,K-ATPase. It has been claimed that the transmembrane protein klotho can influence Na,K-ATPase activity to increase the Na+ gradient and drive transepithelial calcium transport in the choroid plexus and kidney. Until very recently, changes in serum phosphate levels were thought to be a secondary process related to calcium transport and balance. A major breakthrough in understanding the active regulation of phosphate homeostasis was achieved by the identification of the novel phosphatonin FGF23 [16, 17].

Fibroblast Growth Factor 23

FGF23 is a ~30 kDa protein that is proteolytically processed to smaller N-terminal (~18 kDa) and C-terminal (~12 kDa) fragments. The receptor-binding domain of FGF23 is present in the N-terminus. FGF23 is able to suppress Na/Pi-2a and Na/Pi-2c cotransporters either directly or through influencing PTH activity to induce urinary phosphate excretion [18–24]. Transgenic mice overexpressing FGF23 have hypophosphatemia due to the suppression of the renal Na/Pi cotransporters, as well as reduced serum 1,25$(OH)_2$D levels and skeletal mineral deposition defects in the form of rickets/osteomalacia [25–27]. Of relevance, FGF23 can also influence systemic vitamin D activity by suppressing the renal expression of 1-α(OH)ase to decrease the production of 1,25$(OH)_2$D. In addition, FGF23 can reduce 1,25$(OH)_2$D activity by increasing the synthesis of the catabolic enzyme 24-hydroxylase [28–31].

Vitamin D-resistant rickets/osteomalacia in patients with X-linked hypophosphatemia (XLH) is caused by inactivating mutations in the *PHEX* gene (a phosphate-regulating gene that is homologous to endopeptidases on the X-chromosome) that, in turn, increase serum levels of FGF23 [32–36]. In the case of autosomal-dominant

hypophosphatemic rickets (ADHR), gain-of-function mutations of the FGF23 gene are associated with excessive urinary phosphate wasting, causing rickets in the bones [32, 37, 38]. Patients with ADHR have mutations in the *FGF23* gene that are located within three nucleotides between residues 176 and 179 in the pro-protein convertase cleavage site, and thus prevent the proteolytic cleavage of the FGF23 protein. The net effect of such change is phosphate wasting in the affected patients due to enhanced FGF23 activity. In some patients with epidermal nevus syndrome (ENS), which is reported to be caused by activating mutations of FGFR3, increased serum levels of FGF23 are found to be associated with renal phosphate wasting [39, 40].

Similarly, increased production of FGF23 by tumor cells in patients with tumor-induced osteomalacia (TIO) can induce excessive renal phosphate wasting and mineralization defects in the bone [41–46]. These clinical symptoms can be reversed by surgical removal of the FGF23-producing tumor. A pathological role for FGF23 has also been suggested in McCune-Albright syndrome, in which FGF23 is believed to cause hypophosphatemia [47]. Furthermore, in some patients with osteoglophonic dysplasia (OGD), increased serum levels of FGF23 can cause hypophosphatemia; OGD is an autosomal-dominant disorder characterized by non-ossifying bone lesions with abnormal mineral ion balance, including hypophosphatemia. Heterozygous missense mutations in FGFR1 lead to constitutive receptor activation in OGD and induce disease pathology [48].

Recently, hypophosphatemia in patients with autosomal recessive hypophosphatemic rickets/osteomalacia (ARHR) has been attributed to high serum FGF23 levels [49–52]. It is worth mentioning that mutations in the dentin matrix protein-1 (DMP- 1) gene have been reported in patients with ARHR, but the mechanism by which mutation in DMP-1 can lead to increased FGF23 production is not yet clear. In a related experimental study, increased production of FGF23 was detected in *Dmp-1* knockout mice; such increased levels of FGF23 is thought to induce hypophosphatemia in these mutant mice, as genetic deletion of fgf23 in *Dmp-1* knockout mice resulted in hyperphosphatemia, a comparable phenotype as seen in FGF23 single knockout mice [52, 53].

In contrast to the diseases associated with increased production and bioactivity of FGF23, there are human diseases that can be caused by reduced FGF23 activity (Table 3.2). For instance, patients with familial tumoral calcinosis (FTC) usually develop hyperphosphatemia and ectopic calcification due to loss-of-function mutations in the FGF23 gene [54–59]. Similarly, mutations in the GALNT3 gene, which encodes the glycosyl transferase ppGaNTase-T3 (UDP-N-acetyl-a-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase-3), have also been identified in patients with FTC. In these patients, serum levels of intact FGF23 are reduced, while levels of the processed C-terminal FGF23 fragment are increased, suggesting an accelerated proteolytic degeneration of full-length bioactive FGF23 protein. Similarly, recently developed GALNT3 knockout mice showed impaired secretion of intact FGF23, despite increased expression of FGF23 in the bone, indicating an important in vivo role of GALNT3 in the processing and secretion of FGF23 [60]. Together, these observations imply that mutations in the GALNT3 gene can impair

Table 3.2 A list of several human diseases with abnormal phosphate balance due to dysregulation of FGF23

Diseases associated with increased FGF23 activity	*Cause*
ADHR	*FGF23* mutation
ARHR	*DMP1* mutation
ENS	*FGFR3* mutation
McCune-Albright syndrome	*GNAS1* mutation
OGD	*FGFR1* mutation
TIO	FGF23-producing tumor
XLH	*PHEX* mutation
Diseases associated with decreased FGF23 activity	
FTC	*GALNT3* mutation
FTC	*FGF23* mutation
FTC	*KLOTHO* mutation

Please note that the serum levels of both C-terminal and intact FGF23 are high in FTC caused by a klotho mutation, while serum levels of C-terminal are high, the levels of intact FGF23 are low to normal in FTC caused by GLANT3 or FGF23 mutations [1, 2]
ADHR autosomal-dominant hypophosphatemic rickets, *ARHR* autosomal recessive hypophosphatemic rickets/osteomalacia, *DMP1* dentin matrix protein 1, *ENS* Epidermal nevus syndrome, *FTC* familial tumoral calcinosis, *GNAS1* guanine nucleotide-binding protein alpha-stimulating activity polypeptide 1, *GALNT3* UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-3, *OGD* osteoglophonic dysplasia, *PHEX* phosphate-regulating gene with homology to endopeptidases on the X-chromosome, *TIO* tumor-induced osteomalacia, *XLH* X-linked hypophosphatemia

O-glycosylation of the FGF23 protein in patients with FTC, thereby increasing the susceptibility of the FGF23 protein to proteolytic inactivation. Study of these human genetic disorders and knockout mice models that influence the functionality of FGF23 have substantially increased our understanding of how systemic phosphate homeostasis is regulated. However, the exact role and mechanisms by which FGF23 regulation is perturbed in acquired human diseases should be investigated further, particularly in patients with renal diseases.

FGF23 and Chronic Kidney Disease

Patients with advanced stages of chronic kidney diseases (CKD) have elevated serum levels of FGF23. Despite increased serum levels of FGF23, CKD patients develop hyperphosphatemia. It is not clear why the serum level of FGF23 is high in patients with CKD, but there are several possibilities, including decreased renal clearance of FGF23 and increased production of FGF23 to counteract hyperphosphatemia. This second possibility is supported by both human and experimental studies that have shown that a high dietary phosphorus load can increase serum levels of FGF23 [61]. Moreover, calcitriol therapy in patients with CKD may also contribute to increased serum levels of FGF23. Saito and colleagues have reported that both phosphorus and 1,25-dihydroxyvitamin D independently promote an increase in circulating FGF23 levels [62]. Patients with CKD tend to have a low

levels of $1,25(OH)_2D$ and secondary hyperparathyroidism. Whether increased levels of FGF23 can influence this dysregulation is a complex issue and deserves additional study. Since FGF23 can suppress vitamin D activity, the increased levels of FGF23 in patients with CKD may reduce vitamin D activity and eventually facilitate the development of compensatory secondary hyperparathyroidism.

The endocrine functions of PTH help in the maintenance of phosphate balance by promoting renal phosphate excretion. It may also reduce urinary calcium excretion and stimulate the renal production of active vitamin D metabolites. Nevertheless, even though serum PTH levels are high in patients with CKD, PTH fails to reduce serum phosphate levels in patients with this chronic illness. Increased production of PTH to counteract hyperphosphatemia could significantly contribute to the development of secondary hyperparathyroidism [63]. Of particular interest, elevated serum FGF23 levels are suggested to be an important predictor of secondary hyperparathyroidism in patients undergoing dialysis treatment [64].

Hyperphosphatemia is an important determinant of mortality in patients with CKD, irrespective of other associated biochemical changes. However, serum phosphate levels can be influenced by numerous factors including diet, the use of phosphate lowering drugs, or abnormal skeletal conditions. Serum phosphate levels, therefore, can at times be misleading in risk assessment, particularly when serum levels remain within the normal range. Recent studies have suggested that under normophosphatemic conditions, serum levels of FGF23 may be a better biomarker than serum phosphate levels for risk assessment in patients with CKD [19, 65].

A number of studies have pointed to an association between increased serum levels of FGF23 and increased mortality in patients with CKD, particularly in patients undergoing hemodialysis [66]. The cause of high mortality in CKD patients with higher serum levels of FGF23 is not clear, but recent studies have found a correlation between elevated serum FGF23 and an increased rate of left ventricular hypertrophy [67–70]. Although these association studies are suggestive, they do not provide enough mechanistic evidence to prove that FGF23 has a direct role in affecting cardiovascular structural components to influence cardiac functions and eventual mortality. The available (genetically altered) animal models may be able to show a direct effect of FGF23 on cardiovascular structure and function more convincingly. Klotho knockout mice have extremely high serum levels of FGF23 compared to control mice, and a shortened lifespan due to early sudden death. The sudden death in klotho knockout mice is linked to cardiac dysfunction of the sinoatrial node [71]. Determining whether high serum levels of FGF23 contribute to the cardiac dysfunction and early mortality of klotho knockout mice may help us understand the pathologic role of elevated serum levels of FGF23 in patients with CKD.

Klotho and Its Structure

Klotho is a type 1 membrane protein, with a single transmembrane domain near its C-terminus that is believed to anchor the protein to the membrane [72] (Fig. 3.2). Once the short transmembrane domain is removed, the remaining fragment

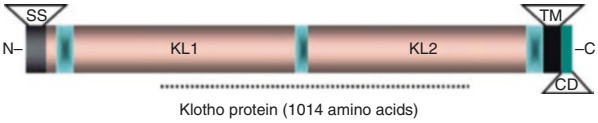

Fig. 3.2 Schematic diagram of the α-klotho protein structure, which is composed of 1014 amino acids and possesses a putative signal sequence (*SS*) at its N-terminus and a putative transmembrane domain (*TM*) with a short cytoplasmic domain (*CD*) at the C-terminus. The extracellular domain of the klotho protein consists of two internal repeats (*KL1 and KL2*) that share sequence homology with β-glucosidase (adapted from reference [1])

(the "secreted form") can be released into the circulatory system. The mouse klotho gene has 5 exons and 4 introns and is located on chromosome 13q12. The transcript of mouse klotho is about 5.2 kb. Interestingly, the promoter region lacks a TATA-box and contains four potential binding sites for Sp1-transcription factor. A splice site in the third exon of the klotho gene can be alternatively spliced to generate two transcripts encoding the transmembrane and secreted forms of the klotho protein. The full-length klotho (transmembrane form) transcript encodes a protein of 1014 amino acids with a molecular weight of 130 kDa. Alternative mRNA splicing generates a truncated klotho protein (the secreted form) that encodes a protein of 550 amino acids with a molecular weight of approximately 65–70 kDa [73–75]. The full-length mouse klotho cDNA and its protein have around 93 % and 80 % homology to those of rat and human, respectively. The transmembrane form of the mouse klotho protein possesses a putative signal sequence at its N-terminus, a putative transmembrane domain, and a short cytoplasmic domain at the C-terminus. The extracellular domain of the klotho protein consists of two internal repeats of about 550 amino acids (KL1 and KL2) that share sequence homology with beta-glucosidase. Between two internal repeats (KL1 and KL2), there is a short stretch of basic amino acids (Lys-Lys-Arg-Lys) that forms a possible site for proteolytic cleavage, similar to the polybasic proteolytic processing site. This short stretch of basic amino acids is present in the rat, human, and mouse klotho protein. The secreted form of mouse klotho only contains the N-terminal half of klotho, including its extracellular domain (KL1) [72].

FGF23-Klotho Signaling Pathways

Klotho expression has been detected in the distal convoluted tubules of the kidney, the parathyroid gland, and the epithelium of the choroid plexus in the brain [72]. The klotho knockout mice exhibit increased renal expression of Na/Pi-2a and Na/Pi-2c protein with concomitant hyperphosphatemia and develop physical, biochemical, and morphological phenotypes identical to those of FGF23 knockout mice [76]. The identical phenotypes of these two separate knockout lines eventually led to the identification of klotho as an essential cofactor in FGF23 signaling pathways [76].

In general, most FGFs bind to FGF receptors on the cell surface and activate downstream signaling events to exert diverse biological functions. FGF23 is a

member of the FGF19 subfamily, which also contains FGF19 and FGF21. FGF23 has been shown to bind to multiple FGF receptors, including FGFR1c, FGFR3c, and FGFR4 [77–80]. Further research has suggested that the klotho protein can bind to multiple FGF receptors, and that the klotho-FGF receptor complex binds to FGF23 with much higher affinity than either the FGF receptor or klotho alone. The binding of the klotho-FGF complex can then activate downstream signaling events, as demonstrated by the activation of early growth response element-1 (Egr-1) and the phosphorylation of FGF receptor substrate-2a, extracellular signal-regulated kinase (ERK), p38, Jun N-terminal kinase (JNK), and AKT [80, 81]. It is worth noting that these signaling phosphoproteins were detected only when cells were treated with both FGF23 and klotho together, and not in cells treated with FGF23 without klotho. These results, along with earlier observations, clearly suggest that the FGF23–FGF receptor interaction and subsequent signaling activities require klotho as a cofactor.

In response to elevated serum phosphate levels, FGF23 is produced in the bone and exerts endocrine effects on the kidney in coordination with klotho protein, which is mostly expressed in the distal tubular epithelial cells to promote renal phosphate excretion. The phosphate lowering action of FGF23 is partly mediated through the reduced expression of Na/Pi-2a and 1α(OH)ase in the proximal tubular epithelial cells. Despite the fact that klotho is mostly present in the distal tubular epithelial cells, how FGF23-mediated phosphate metabolism takes place in the proximal tubules is an intense area of research. In a recent study, presence of low level klotho has been reported in proximal tubules [82], and formed the basis for in-depth study of FGF23- klotho interactions in FGF23-mediated phosphate metabolism.

Klotho and Systemic *FGF23* Function

Transgenic mice overexpressing human or mouse FGF23 develop hypophosphatemia due to severe urinary phosphate wasting, while FGF23 knockout mice develop hyperphosphatemia due to increased renal uptake of filtrated phosphate. A genetic restoration of the systemic actions of human FGF23 in FGF23 knockout mice reversed this hyperphosphatemia to hypophosphatemia and prevented associated complications, including ectopic calcification [83]. Recent studies have clearly demonstrated the in vivo importance of klotho in FGF23-mediated regulation of phosphate homeostasis. For instance, serum phosphate levels were significantly reduced following an injection of bioactive FGF23 in wild-type or $FGF23^{-/-}$ mice; since wild-type and $FGF23^{-/-}$ mice both have endogenous klotho, the exogenous FGF23 is able to influence systemic phosphate homeostasis. In contrast, the injection of bioactive FGF23 protein into either $klotho^{-/-}$ mice or $FGF23^{-/-}$/$klotho^{-/-}$ double knockout mice does not produce any obvious changes in the serum levels of phosphate [76], implying that klotho is essential for the FGF23-mediated regulation of phosphate homeostasis. This essential in vivo role of klotho has recently been demonstrated further in a genetically engineered hypophosphatemic (Hyp) mouse model [84].

Hyp mice possess a mutation that inactivates PHEX, a phosphate-regulating gene that is homologous to the endopeptidases of the X-chromosome. This mutation is associated with severe hypophosphatemia due to excessive urinary phosphate wasting caused by increased serum accumulation of FGF23. In vivo genetic manipulation studies have shown that the inactivation of klotho in Hyp mice resulted in hyperphosphatemia, not hypophosphatemia, even though Hyp/klotho$^{-/-}$ double mutant mice have significantly elevated serum levels of FGF23 [84]. The opposing phenotypes of *Hyp* and Hyp/klotho$^{-/-}$ mice suggest that the disruption of klotho-mediated pathways abrogates the hypophosphatemic phenotype normally caused by the increased serum levels of FGF23. Furthermore, genetic inactivation of klotho in FGF23 transgenic mice resulted in a phenotype consistent with klotho deficiency, again emphasizing the in vivo importance of klotho in FGF23 function. Similarly, a homozygous loss-of-function mutation in the Klotho gene causes tumoral calcinosis, severe hyperphosphatemia, and ectopic calcification despite high serum levels of FGF23 in the affected patient [85]. Together, these human and mouse genetic studies provide compelling evidence that klotho is essential in the FGF23-mediated regulation of systemic phosphate homeostasis in vivo.

Nevertheless, under pathological conditions where the concentration of FGF23 is extremely high, FGF23 may exert nonspecific effects without klotho, as FGF23 can bind to FGF receptors with low affinity in the absence of klotho [80]. Several in vitro studies also support the possibility of such off-target responses. Additional studies will explain whether effects of FGF23 on klotho nonexpressing or low expressing tissues, including bone, blood vessels are off-target responses or not. For example, FGF23 was shown to exhibit weak proliferative effects on a murine bone marrow-derived pro-B cell line that overexpresses FGFRs but does not express klotho. Experimental explanation is needed to know if extremely high serum levels of FGF23 can lead to ectopic activation of FGF receptors and thus induce cardiac morbidity in patients with CKD. In this scenario, patients with CKD might benefit from therapy to lower FGF23. However, a better understanding of the exact role of elevated serum levels of FGF23 in CKD patients is needed before any therapeutic strategy can be proposed. For example, it is not clear whether increased FGF23 levels are a protective response (in early stages) or a detrimental side effect (in later stages) in CKD patients. Thus, any therapeutic designs will need to be assessed carefully. Moreover, vitamin D deficiency has been linked to increased mortality in advanced CKD patients; since FGF23 can suppress the production of active vitamin D metabolites, it is possible that any detrimental effect of FGF23 on the mortality of CKD patients may be influenced by reduced vitamin D activity.

As discussed above, the generation of Hyp/klotho$^{-/-}$ double mutant mice has clearly demonstrated that the FGF23-mediated hypophosphatemia in Hyp mice is klotho dependent. These genetic studies have provided in vivo evidence suggesting that klotho may be a potential therapeutic tool to manipulate FGF23 function, and that direct manipulation of klotho may prove a novel therapeutic strategy for FGF23-related hypophosphatemic diseases. The clinical application of a controlled reduction of FGF23 might be of therapeutic benefit for patients with excessive urinary phosphate wasting diseases, including ADHR, ARHR, and XLH. The current

treatments for these genetic hypophosphatemic diseases are mostly palliative, such as oral phosphate replacement. Also, the prolonged use of these therapies can cause complications, notably secondary hyperparathyroidism. Finally, in contrast to anti-FGF23 therapy, providing exogenous bioactive FGF23 protein might help restore phosphate balance and delay associated complications, such as the ectopic calcifications in patients with FTC that are usually caused by reduced FGF23 activity. In a recent study, exogenous FGF23 treatment was shown to delay the progression of experimental nephritis-induced renal failure. However, this treatment also aggravated renal osteodystrophy due to reduced levels of $1,25(OH)_2D$, demonstrating one potential limitation of FGF23 therapy [86]. Renal osteodystrophy is often described as a CKD-mineral and bone disorder (CKD-MBD). Of particular interest, $1,25(OH)_2D$ can exert opposing effects on serum phosphate levels: $1,25(OH)_2D$ can induce both FGF23 and klotho to increase urinary excretion of phosphate and lower serum phosphate levels, but can also facilitate increased intestinal absorption of phosphate to increase serum phosphate levels.

Concluding Remarks

The regulation of systemic phosphate homeostasis appears to be strictly controlled by a limited number of factors, as demonstrated by the opposing phenotypes of FGF23 transgenic and knockout mice, their similarities with klotho-mutant mice, and—more importantly, the corresponding clinical phenotype in hereditary diseases caused by *FGF23* or klotho mutations in humans (Table 3.1) [1, 2, 21, 27, 32, 76, 87–92]. The overlapping phenotypes and lack of redundancy suggest that a limited number of essential factors form the biological network that actively regulates phosphate homeostasis. Our understanding of the essential in vivo endocrine role of FGF23 in maintaining systemic phosphate homeostasis has laid the foundation for future work to determine the therapeutic benefit of manipulating the FGF23-klotho network in patients with excessive urinary phosphate wasting diseases. In addition, serum FGF23 measurements may have both diagnostic and prognostic significance in these patients, and may be used to determine the underlying causes of diseases associated with abnormal mineral ion metabolism; for instance, serum FGF23 levels can aid in the diagnosis of tumor-induced osteomalacia (TIO). Studies suggest that the pretreatment serum level of FGF23 can be a good predictor of vitamin D therapy efficacy in dialysis patients, and is also believed to be a useful predictor for the development of refractory hyperparathyroidism [63].

Conclusions

Recent studies have provided compelling evidence of the in vivo importance of klotho in FGF23-mediated regulation of systemic phosphate homeostasis. Translating this research to new therapies for patients suffering from the complications of abnormal mineral ion metabolism will be a challenging, and yet clinically rewarding effort.

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Part II

Phosphorus in Food

Phosphorus in the Modern Food Supply: Underestimation of Exposure

4

Mona S. Calvo and Jaime Uribarri

Key Points

- Modern food processing techniques have introduced phosphate additives to provide a number of different needed functions that improve processed foods in a variety of ways.
- Phosphate additives add to the already high phosphorus consumption in the majority of adults and may disrupt the delicate balance between calcium and phosphorus intake.
- Mandatory labeling of total phosphorus content on the Nutrition Facts Panel of processed foods may be necessary to inform individuals of the true phosphorus quantity in different foods.

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Introduction

Over the last century, technical improvements in food processing leading to the development of our modern food supply enabled the eradication of many serious food borne illnesses and significant reduction in life-threatening nutritional diseases, including pellagra, goiter, and rickets. Through the use of modern processing techniques, food scientists have clearly improved the safety, nutritional quality, palatability, and ease of preparation of the foods we enjoy today. However, in the twenty-first century, we now realize that some of these technological improvements in food processing may contribute to serious public health problems facing many people [13, 15]. This chapter focuses on one of these processing techniques—the widespread use of phosphate additives and how this technology has unwittingly increased phosphorus exposure in individuals who must restrict their intake in order to maintain renal function and possibly prevent other serious diseases. The main indication accepted by nephrologists for serum phosphate control is the prevention of secondary hyperparathyroidism and therefore, bone disease; and consideration is now also being given to the emerging evidence linking either serum phosphorus or phosphate intake to cardiovascular disease [62]. However, although in theory these food processing changes should create greater health risks, it is not clear if the resulting increase in phosphorus exposure affects the incidence and progression of chronic kidney disease (CKD) and/or cardiovascular disease (CVD) and merits further study [9]. There is a further possibility that increased exposure to a highly bioavailable form of phosphorus, which occurs with the unlimited use of phosphate additives, may increase the risk of CVD and osteoporosis in the general healthy population [15, 16, 20, 50, 52, 61, 65].

Phosphorus is naturally present in most foods and the modern addition of phosphate-containing food additives during processing has increased the content of phosphorus in the general food supply [17, 26, 70]. The evidence presented in this chapter supports the need for mandatory labeling of the phosphorus content of even minimally processed foods to help the greater than 20 million Americans (one in ten adults) with some level of CKD to assure normal phosphate balance [45]. It is an overwhelming task to estimate daily exposure to total phosphorus intake without clear product labeling of the phosphorus content of processed foods, inclusive of the specific additives used and the accurate capture of the quantity of phosphate added, as well as the natural phosphorus content of the food. This information is not only critical to the health and well being of CKD patients, but also essential to regulatory scientists who, given this label information, could evaluate the safety and health risks of these changes in phosphorus exposure for the general population [15].

Phosphorus Content of Food

Phosphorus is found in almost all foods consumed in the United States, both raw and processed. Table 4.1 presents the various food categories in descending order of their percent contribution to phosphorus intake estimated from 24 h recall data from the recently completed National Health and Nutrition Education Survey (NHANES

Table 4.1 Contribution of different food categories to daily phosphorus intake

Food category	% Contribution
Milk and dairy	20.9
Mixed dishes–grain-based	10.1
Breads, rolls, tortillas	5.8
Quick breads, bread products, sweet bakery products	5.2
Poultry	5.1
Pizza	4.8
Vegetables	4.8
Mixed dishes–meat, poultry, seafood	4.5
Meats	4.2
Cured meats and poultry	4.4
Plant-based protein foods	3.7
Cereals	3.2
Eggs	2.8
Seafood	2.5
All above categories	82

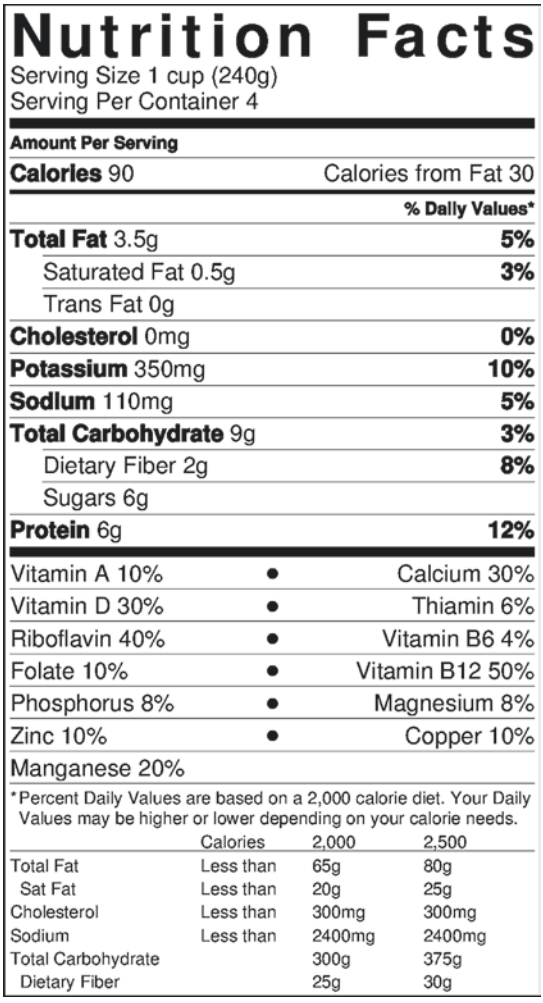
Data source: Alanna Moshfegh, 2013 *What We Eat in America*, NHANES 2009–2010. Included only food categories that make up 82% of the total daily dietary phosphorus intake

2009–2010). The foods contributing the greatest amount of phosphorus to daily intake are milk and dairy, followed by meat and poultry contributions. CKD patients are educated as to which food categories they must limit in order to keep their phosphorus intake within 800–900 mg/day [44, 63]. The contributions to phosphorus intake shown here largely reflect the natural phosphorus content of the food and may or may not include contributions from phosphate additives.

Information contained on a product's Nutrition Facts Panel is the only option available for consumers to determine the true phosphorus content of a processed food. However, current regulations do not require manufacturers to list phosphorus content on the Nutrition Facts Panel; consequently, the phosphorus content of foods may appear in one of four different ways as shown in Fig. 4.1a–d—labels for different milk products. The manufacturer of the milk shown in Fig. 4.1a voluntarily listed the phosphorus content as a percent of the daily value (%DV) which is 1000 mg for both phosphorus and calcium. On the other hand, the producer of the milk product shown in Fig. 4.1b, c chose not to list phosphorus content in the Nutrition Facts Panel, but when the product contains phosphate additives, as in Fig. 4.1c, these ingredients must be listed in the ingredients list on the milk label. The milk product whose label is shown in Fig. 4.1d is fortified with calcium through the addition of calcium phosphate and the manufacturer has opted to label the phosphorus content and is required to list the added calcium phosphate in the ingredients list. The confusion CKD patients face when trying to control their phosphorus intake is not surprising with all these different ways of listing or not listing phosphorus content of foods. Calcium, iron, and sodium content are required to be listed on the label and this labeling has been shown to effectively guide consumers to healthy food choices.

Presentation of the phosphorus content as the %DV is another source of confusion for people with CKD. To manage their disease, they know to limit their intake of phosphorus to less than 900 mg/day; however, many find it difficult to convert the %DV to mg/regulatory serving size and often confuse this label guideline developed by the Food and Drug Administration (FDA) with the Recommended Daily Allowance (RDA) of 700 mg/day of phosphorus for an adult [27]. Because the DV (1000 mg P/day) is often confused with the RDA of 700 mg/day, many CKD patients reading the Nutrition Facts Label can underestimate their phosphorus intake by up

a



Nutrition Facts

Serving Size 1 cup (240g)
Serving Per Container 4

Amount Per Serving	
Calories 90	Calories from Fat 30
	% Daily Values*
Total Fat 3.5g	**5%**
Saturated Fat 0.5g	**3%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Potassium 350mg	**10%**
Sodium 110mg	**5%**
Total Carbohydrate 9g	**3%**
Dietary Fiber 2g	**8%**
Sugars 6g	
Protein 6g	**12%**

Vitamin A 10%	•	Calcium 30%
Vitamin D 30%	•	Thiamin 6%
Riboflavin 40%	•	Vitamin B6 4%
Folate 10%	•	Vitamin B12 50%
Phosphorus 8%	•	Magnesium 8%
Zinc 10%	•	Copper 10%
Manganese 20%		

*Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2400mg	2400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Soy Milk

INGREDIENTS:
Filtered Water, Organic Whole Soybeans, Organic Evaporated Cane Juice, Natural Flavors, Calcium Carbonate, Sea Salt, Sodium Citrate, Potassium Citrate, Carrageenan, Vitamin A Palmitate, Ergocalciferol (Vitamin D_2), DL-Alpha Tocopherol Acetate (Vitamin E) Riboflavin (Vitamin B_2), Cyanocobalamin (Vitamin B_{12}), Zinc Sulfate.

Fig. 4.1 (**a**–**d**) are representative Nutrition Facts Labels from milk products currently in the market place. These examples illustrate the different ways that the phosphorus content of processed foods may or may not appear on the product label

b

Nutrition Facts

Serving Size 1 cup (240g)
Serving Per Container 4

Amount Per Serving	
Calories 130	Calories from Fat 45
	% Daily Values*
Total Fat 5g	**8%**
Saturated Fat 3g	**15%**
Trans Fat 0g	
Cholesterol 20mg	**7%**
Sodium 130mg	**5%**
Total Carbohydrate 12g	**4%**
Dietary Fiber 0g	**0%**
Sugars 12g	
Protein 8g	**16%**

Vitamin A 10%	•	Vitamin C 2%
Calcium 30%	•	Vitamin D 25%
Riboflavin 25%		

*Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2400mg	2400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

2% Reduced Fat Milk

INGREDIENTS: Grade A UHT Reduced Fat Milk, Vitamin A Palmitate and vitamin D3

Fig. 4.1 (continued)

c

Nutrition Facts

Serving Size 1 cup (240g)
Serving Per Container 4

Amount Per Serving	
Calories 50	Calories from Fat 40
	% Daily Values*
Total Fat 4.5g	**7%**
Saturated Fat 4g	**20%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Potassium 65mg	**2%**
Sodium 65mg	**3%**
Total Carbohydrate 2g	**1%**
Dietary Fiber 1g	**4%**
Sugars 0g	
Protein 0g	**0%**

Vitamin A 10%	•	Calcium 10%
Vitamin D 30%	•	Folate 6%
Vitamin B12 50%	•	Magnesium 8%
Selenium 8%		

*Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2400mg	2400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Coconut Milk

INGREDIENTS:
Organic Coconut milk (Water, Organic Coconut Cream), Natural Flavors, Vanilla Extract, Calcium Phosphate, Magnesium Phosphate, Kosher Sea Salt, Carrageenan, Guar Gum, REB A (Stevia Extract), Monk Fruit, Vitamin A Palmitate, Vitamin D2, L-Selenomethionine (Selenium), Zinc Oxide, Folic Acid, Vitamin B12

Fig. 4.1 (continued)

d

Nutrition Facts

Serving Size 1 cups (240g)
Serving Per Container 8

Amount Per Serving

Calories 110	Calories from Fat 20
	% Daily Values*
Total Fat 2.5g	**4%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 15mg	**5%**
Potassium 410mg	**12%**
Sodium 130mg	**5%**
Total Carbohydrate 13g	**4%**
Dietary Fiber 0g	**0%**
Sugars 12g	
Protein 8g	**16%**

Vitamin A 10%	•	Calcium 50%
Vitamin D 25%	•	Vitamin B12 15%
Phosphorus 35%		

*Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2400mg	2400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

1% Low Fat Milk
Calcium Enriched

INGREDIENTS:
Low Fat Milk, Tribasic Calcium Phosphate (Calcium ingredient not in regular milk) Carrageenan, Guar Gum, Lactase Enzyme, Vitamin A Palmitate and Vitamin D_3

Fig. 4.1 (continued)

to 300 mg/per serving, thinking that the phosphorus content is a given percent of 700 mg rather than 1000 mg. Such underestimations of their daily exposure to phosphorus can have negative health effects depending on their stage of CKD.

Milk and dairy products are the food category contributing the greatest amount of total phosphorus daily intake in the healthy population as shown in Table 4.1. While milk and dairy products are high in natural phosphorus bound to milk proteins, milk, and dairy products can also contain a wide variety of phosphate additives whose specific functions are shown in Table 4.2. The contribution to total phosphorus intake by these additives in milk and dairy products is not usually captured in the nutrient content databases; and thus, dairy contributions may be even higher than shown in Table 4.1.

Table 4.2 Examples of phosphate additive use in dairy foods

Dairy products	Function	Ingredient
Cheese dips and sauces	Emulsifying agent	Disodium phosphate, trisodium phosphate, sodium hexametophosphate
Cheese powders	Protein stabilization	Dipotassium phosphate, sodium tripolyphosphate, sodium hexametaphosphate
Cheese slices	Emulsifying action	Disodium phosphate, trisodium phosphate, tetrasodium pyrophosphate
Cheese starter cultures	Inhibit bacteriophage	Disodium phosphate, dipotassium phosphate
Cottage cheese	Direct set by acidification	Phosphoric acid
Grated cheese	Protein stabilization	Dipotassium phosphate, sodium tripolyphosphate, sodium hexametaphosphate
Ice cream (hard, soft, imitation frozen dessert)	Improve dairy protein foaming property	Sodium hexametaphosphate, tetrasodium pyrophosphate
Imitation cheese	Emulsifying action	Disodium phosphate, trisodium phosphate, sodium hexametaphosphate
Instant pudding (no-bake cheese cake)	Protein coagulant	Monocalcium phosphate, disodium phosphate, tetrasodium phosphate
Nondairy creamer	Buffer for smooth mixing into coffee	Dipotassium phosphate
Reduced sodium dairy	Potassium enrichment	Dipotassium phosphate, tripotassium phosphate, tetrapotassium pyrophosphate, potassium tripolyphosphate
Whey processing	Protein stabilization	Disodium phosphate, dipotassium phosphate, sodium hexametaphosphate
Whipped topping	Improve dairy protein foaming property	Sodium hexametaphosphate, tetrasodium pyrophosphate

Data adapted in part from online presentation "Phosphate Use in Foods" presented by the International Food Additive Council, last accessed October 23, 2013 at: http://www.foodadditives.org/phosphates/phosphates_used_in_food.html

Evidence of Excess Dietary Phosphorus Intake Relative to Requirements

In 1997, the Institute of Medicine of the US National Academy of Sciences [27] established guidelines for the daily intake requirements for phosphorus for both genders over all ages—the Estimated Average Requirement or EAR. For both genders, the EAR ranges from 380 to 580 mg/day with a high of 1055 mg/day required for 9–18 year olds who are actively growing bone. With the exception of growing teens, the "usual" median phosphorus intake estimated in the NHANES 2005–2006 survey shown in Fig. 4.2, exceeds this requirement guideline [42]. In the consecutive waves of this nationally representative survey since NHANES 2001–2002, mean and median intakes of phosphorus remained relatively unchanged and always in far excess of phosphorus requirements (EAR). Not only

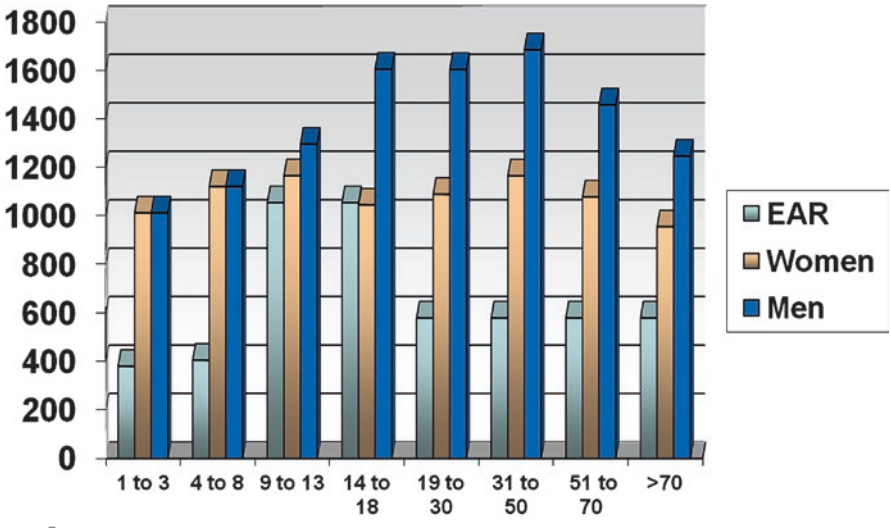


Fig. 4.2 NHANES 2005–2006 usual intake of phosphorus relative to EARs (*Source*: USDA, Agricultural Research Service, Belstville Human nutrition Research Center, Food Surveys Research Group http://www.ars.usda.gov/ba/bhnrc/fsrg. EAR, Estimated Average Requirement. Reproduced with permission from Calvo and Uribarri AJCN 2013)

the estimated intakes exceed phosphorus need, many believe they underestimate actual phosphorus intakes, particularly in individuals consuming more restaurant, fast, and highly processed convenience foods [15, 16]. This underestimate is presumably due to the failure to account for the use of phosphate additives in food processing.

The phosphorus content of the US food supply increases continuously as food producers find new and effective ways to improve their products through the use of phosphate additives [18]. Studies shown in Table 4.3 support an observed 25–30 % underestimation in existing nutrient content databases which likely obscure relationships between dietary phosphorus intake and disease biomarkers or outcomes [7, 49, 57, 58, 60]. A 30 % underestimation of an average 1400 mg phosphorus intake translates to 420 mg per day from phosphate additives alone, which is very close to the values determined more than 40 years ago [24]. Nonetheless, 420 mg is considerably more than the additive contribution of 300 mg P/day determined by the FDA in 1975 and later confirmed in 1997 by the International Food Additive Council (IFAC) using their "United States Disappearance of Food Grade Phosphates 15 Year Summary and Related Data" [69]. To our knowledge, IFAC has not publically released any current estimations of cumulative phosphate additive use based on food use disappearance data submitted by industry producers. These early IFAC estimates were made in 1980–1994 before the increase in marketing of enhanced beef, pork, poultry, and other meats [43, 57, 58] or the increased use of phosphate modified starches in frozen foods [13], which cannot be identified in the food ingredients list since they lack the critical term—phosphate. Furthermore, the

Table 4.3 Evidence supporting underestimation of phosphorus intake from food

Study	Approach and method of phosphorus intake estimation	% Underestimation of phosphorus intake
Oenning et al. [49]	Duplicate meals, direct chemical analyses vs. Nutritionist II and III software and hand calculation	25–30 %
Sullivan et al. [60]	Direct chemical analyses vs. ESHA Food Processor SQL version 9.8 software of Midwestern grocery market chicken products ($n = 38$)	~34 % Mean = 84 mg underestimated P/100 g chicken
Sherman and Mehta [57, 58]	Direct chemical analyses of enhanced meat products vs. natural meat and poultry products expressed as mg P/g protein ratio	28 % higher P/protein ratio in enhanced meat/poultry products compared to additive-free meat/poultry
Benini et al. [7]	Direct chemical analyses of 60 foods, 30 containing declared phosphorus additives and 30 similar foods without additives	Additive containing products had nearly 70 % higher phosphorus content contributing an average >100 mg P/100 g protein

changes in the eating patterns of certain individuals over the last two decades, particularly in lower socioeconomic populations, involve greater consumption of fast food and restaurant foods, which have been shown to contain more phosphate additives than fresh foods or food prepared from scratch [16, 25].

There is concern that some individuals may be consuming dietary phosphorus at levels that approach the Upper Level of Safe Intake (UL = 4000 mg/day) set by the Institute of Medicine [27]. Figure 4.3a, b used existing NHANES intake data to hypothetically estimate how close to the UL individuals consuming at the 95th percentile of intake would come if these intakes were corrected for the presumed 30 % contribution from phosphate additive use. Men consume more total phosphorus than women, and there are health concerns for those in the 95th percentile intake, which represents 5 % of the general male population, whose intake approaches the UL of 4000 mg/day when these hidden sources of phosphorus are taken into account [15]. Recent studies, however, suggest that there may be gender differences in susceptibility to disease risk with healthy women, but not men, in the highest quartile of phosphorus intake showing greater incidence of left ventricular hypertrophy, a hallmark of cardiovascular disease and mortality risk [71]. The Upper Level of Safe Intake for phosphorus of 4000 mg is exceedingly high and needs to be reassessed and differences in potential health consequences due to gender or age need to be addressed.

Accurate estimates of total phosphorus intake and the growing use of phosphate additives reflecting the increased phosphorus content of the US food supply can only be captured in the nutrient content databases when foods are reanalyzed for their current nutrient composition [18]. Recent direct chemical analyses of a few specific food products that included frozen chicken, pizza, and processed meats, for which the use of phosphate additives has increased over the past decade, has shown a mean increase of 90 mg for men and 64 mg of phosphorus for women in the last NHANES survey (2009–2010) [3, 13, 18]. Requiring the phosphorus content to be included in

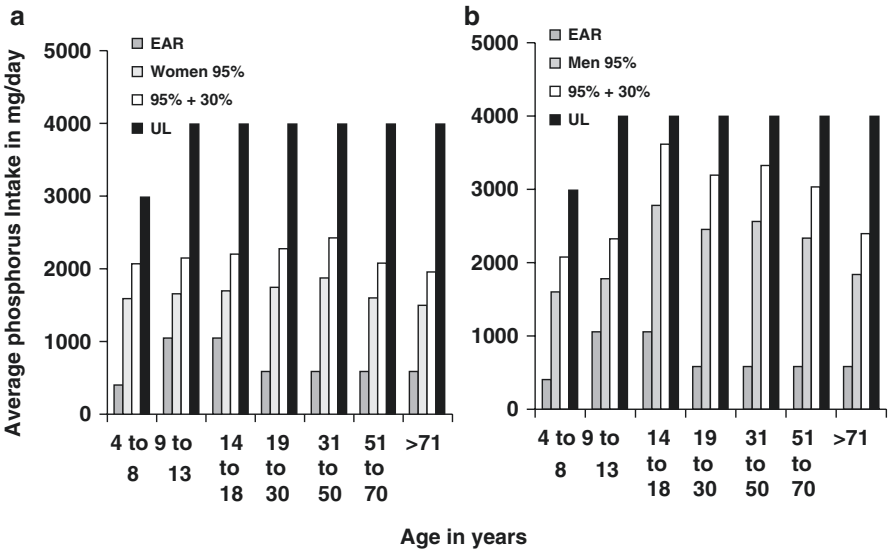


Fig. 4.3 NHANES 2005–2006 usual 95th percentile intakes plus 30 % additive contribution compared with the 1997 Dietary Reference Intake guidelines for the EAR and UL in women (**a**) and men (**b**) (*Source*: USDA, Agricultural Research Service, Beltsville Human Nutrition Research Center, Food Surveys Research Group; http://www.ars.usda.gov/ba/bhnrc/fsrg. EAR, Estimated Average Requirement; UL, Tolerable Upper Intake Level. Reproduced with permission from Calvo and Uribarri AJCN 2013)

the labeling of processed food would facilitate accurate estimates of phosphorus intake and support accurate determinations of the relationship between intake and chronic disease, in addition to making it easier for CKD patients to limit their intakes. It would also allow estimates of exposure that could be used to determine the safety and health risk of the cumulative use of these phosphate additives in foods. In past safety evaluations, phosphate additives were tested separately, mostly in rodent models, but to our knowledge, no studies have looked at the effects of consuming more than one phosphate additive as regularly occurs in the modern food supply [69]. Of the 38 poultry products examined by Sullivan et al. [60], only 3 contained no additives, 19 contained more than one, and 10 contained three or more phosphate additives—further evidence of the growing cumulative use of phosphate additives.

Importance of the Type and Physiologic Effects of Phosphorus in Food

In nature, highly reactive phosphorus is always bound to oxygen and another element or compound. The substance or element that phosphate is bound to defines the type of phosphorus in the food supply, and in turn, significantly influences its physiologic effect. The two types of phosphorus in the food supply are described as either

natural (organic) or added (inorganic). Naturally occurring phosphorus in the foods we eat is largely covalently bound to protein, lipids, and other cell components, which make it less bioavailable and more slowly absorbed. In contrast, phosphate salts, which can also occur naturally but are more commonly added during processing, will readily dissociate in the acid environment of the stomach and are rapidly and efficiently absorbed. Thus, there is a large degree of difference in how these two types of dietary phosphorus might influence disease risk in CKD patients and in the healthy general population [15, 16, 22].

Natural or Organic Phosphorus

New understanding about the nature and physiologic effects of organic phosphorus has influenced our thinking about past dietary guidance given to CKD patients. Such past dietary guidelines may have misguided CKD patients with respect to healthy food consumption, including whole-grain products, legumes, and nuts, which they were advised to avoid because of their high natural phosphorus content. These high phosphorus plant-based foods contain phosphorus largely in the form of phytic acid (Fig. 4.4). Phytate is abundant in the seed coat of widely consumed grains and nuts, such as wheat, peanuts, soy, and legumes and serves as the plant storage form of phosphorus needed in order to germinate. Unless pretreated in processing with an enzyme that cleaves the covalently bound phosphorus (phytase), the phosphorus is not easily liberated or bioavailable. Theoretically, less phosphorus is absorbed; thus, reducing the phosphorus burden on the failing kidneys. Although plausible, this theory has not been tested with specific food sources in CKD patients and should also consider the potential exposure to phytase activity that may occur in the preparation of food. Natural phytase enzymes are limited in the human gastrointestinal system, but leavening of whole-grain breads with yeast or fermentation will provide a source of phytase that improves the bioavailability of phosphorus from whole wheat and whole-grain products [56].

Further research is needed to determine the bioavailability of phosphorus from natural healthy foods that are shown through analyses to be rich in phytate

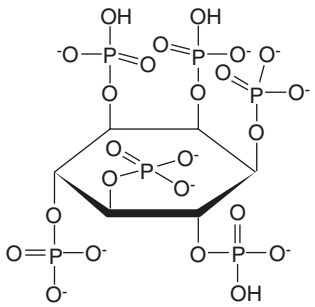


Fig. 4.4 The structure of phytic acid, the phosphorus storage form in plant seeds

phosphorus, such as nuts, nut butters, dried beans, and lentils, but are not usually exposed to phytase in processing [6]. Phytate content of beans and lentils can be reduced by soaking and discarding the water prior to boiling [19]; prolonged boiling will also reduce the phosphorus content of other foods [67]. Breeding cereal crops for reduced content of phosphorus in grains is another potentially very important approach to lowering phosphorus content of plant-based foods for CKD patients [53].

Despite the need for high quality dietary protein, past dietary guidance given to CKD patients encouraged avoidance of these high protein foods based solely on their high phosphorus content. Protein-rich foods are naturally high in organic phosphorus but the rate of absorption and bioavailability of phosphorus when consumed as whole foods varies with the protein source—animal or plant. In general, phosphorus absorption from organic sources ranges from 40 to 60 %, with that from animal sources more completely and efficiently absorbed than from plants [18]. Phytate-rich food sources, such as whole-grain food, in the absence of phytase, have the lowest bioavailability and therefore may not present as high a dietary phosphorus load as its actual phosphorus content would indicate. Few studies have empirically tested the bioavailability of phosphorus from various whole food sources and their influence on serum phosphorus, 24 h urine excretion, and regulatory hormones, including parathyroid hormone (PTH), fibroblast growth factor-23 (FGF-23), and the active form of vitamin D, calcitriol—all factors that can be disrupted with phosphorus loading [15]. These differences in bioavailability and hormone response have been explored only in acute response studies in young adults [31], in 4-week feeding studies using foods available in grocery stores with high content of phosphate additives [12] and in studies using in vitro digestibility of phosphorus from whole foods from animals and plants [28, 32, 33, 34]. Research is needed to determine the bioavailability of phosphorus from food sources which have been considered forbidden to CKD patients in the past, but with confirmed health benefits, such as those associated with nut consumption [6] or the lower incidence of hypertension and metabolic syndrome associated with dairy and dairy products [1, 2, 8].

Moe and coworkers [41] used a crossover study design in CKD patients to compare the effects of meat and vegetarian diets with similar content of protein and phosphorus. They found significantly lower serum and urine phosphorus levels in CKD patients during the week of the vegetarian diet compared to the levels during the meat diet. An earlier study in a large population of CKD patients showed no association between phosphorus intake from plant-based foods and serum phosphorus or PTH concentrations and only a weak association with lower serum FGF-23 and higher serum bicarbonate levels [55]. Other population approaches attempting to determine effects of phosphate intakes from food additives in a free-living population experience similar difficulties and weak associations with adverse health changes such as increased carotid intimal media thickness [29]. The latter studies are examples of the problems researchers face in trying to determine total phosphorus intake from food rich in phosphate additives in the absence of phosphorus labeling and the use of diet history questionnaires that have not been validated for phosphorus.

In the absence of labeling information, direct chemical analyses confirming the phosphorus content in processed foods, as done earlier [12, 49], is currently the only accurate way to determine phosphorus exposure from processed foods. Moe et al. [41] meticulously assembled the meals fed for both the vegetarian- and meat-protein arms of their study from information in the nutrient content databases, but they also confirmed the phosphorus content of each by wet ashing and spectrophotometric analyses. The authors found the analyzed phosphorus content of the vegetarian-based proteins to be 33 % overestimated by the database used to determine nutrient content, while the analyzed phosphorus content of the meat-protein sources were consistent with the database. These are important findings suggesting that if the protein quality of plant-based protein sources are maintained, then a vegetarian diet approach may be effective in maintaining lower serum phosphorus in CKD patients.

Added or Inorganic Phosphorus

The majority of phosphate additives are mineral salts of ortho-, pyro-, and polyphosphates as shown in Fig. 4.5. Contrary to older studies examining the effects of phosphate additives on gastrointestinal absorption of calcium, recent findings indicate that there is no significant decrease with either ortho- or polyphosphates [34]. In the most recent review of the toxicity of inorganic phosphates, these additives were grouped into one of four different classes of phosphates based on their similar toxicity data and chemistry: monovalent salts, divalent salts, ammonium salts, and aluminum salts [69]. The authors of this review of the toxicology of inorganic phosphate additives, which the US FDA considers GRAS or "Generally Recognized as Safe," used this classification scheme to select one representative compound to assess the toxicity of others in the same class [69]. Their overall conclusions were that humans are unlikely to experience adverse effects if the daily phosphorus

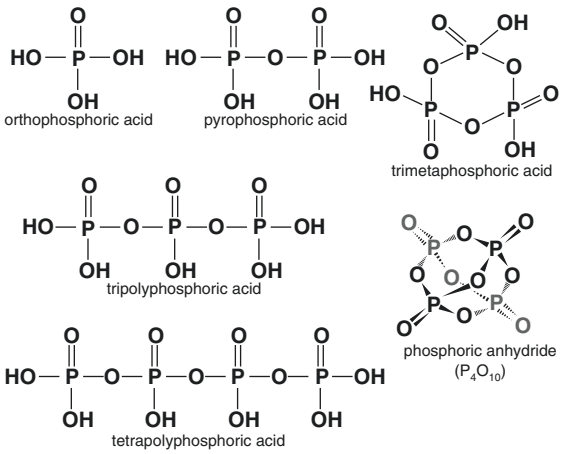


Fig. 4.5 The structure of various chemical forms (ortho-, pyro-, polyphosphates) of the phosphate moieties of phosphate salts used in food processing

consumption remains below 70 mg/kg/day, which translates to 4900 mg phosphorus/day for an average 70 kg man. However, none of the toxicity studies in this 2001 literature review examined the sensitive biomarkers and hormonal changes that have been shown to be associated with disrupted phosphorus homeostasis and changes in vascular endothelial function with consumption of foods high in phosphate additives [15] or phosphate salt loading that have been demonstrated in both healthy humans and rodents [5, 10, 11, 59]. These older studies were using twentieth-century toxicological endpoints to examine the safety of substances which have recently been shown to cause subtle physiologic changes which in time may have fatal adverse effects [66]. We join others in calling for modern and improved approaches to the assessment of the safety of chemicals used in the US food supply, specifically the cumulative use of phosphates [39].

We are not aware of any studies that examined more than one phosphate additive, which is the way the general population exposure typically occurs. The FDA has reported that the average per capita daily intake of phosphorus due to inorganic phosphates added to food was approximately 300 mg in 1975 [69]. The authors also reported an estimated daily 300 mg/person inorganic phosphate intake determined from the "United States Disappearance of Food Grade Phosphates 15 Year Summary and Related Data" conducted by the International Food Additive Council (IFAC) for the period covering 1980–1994. Many consider these intake estimates low and out-of-date. These are inconsistent with other lines of evidence indicating 25–30 % of total phosphorus intake may be derived from phosphate additives [17]. Accurate estimates of current intake will require mandatory labeling of the phosphorus content of processed food and reanalyses of many of the new food product formulations for the nutrient databases, which will give renal dieticians and others access to this important information.

While over exposure to phosphorus may be a problem, we do not want to lose sight of the many ways that phosphate food additives contribute to the overall quality, safety, nutritive value, taste appeal, convenience, and economy of foods [37, 38]. In the United States, the FDA is responsible for protecting the public health by assuring the safety of over 1000 food additives in our nation's food supply and consideration needs to be given to these established procedures [39, 48, 54]. Several resources are available explaining how different types of food and color additives are reviewed for safety of their intended use and their regulation by the FDA and international organizations [40, 47, 54]. The International Food Additive Council (IFAC), a trade association for companies producing food additives and ingredients, is another source for information specific to phosphates (www.foodadditives.org). With respect to global assessment of the safety of food additives, the European counterpart to the US FDA is the European Food Safety Authority (EFSA). EFSA is considered the keystone of the risk assessment by the European Union concerning the safety of food additives in the food supply. It is responsible for providing independent scientific advice and clear communication on existing and emerging risks. For further information concerning all the phosphate-containing food ingredients in the food supply in the United States and European Union, their approved intended uses (functions), toxicological and chemical information, and specific regulatory citations, interested readers should explore the searchable databases presented in Table 4.4.

Table 4.4 Searchable online databases containing regulatory and safety information on the global use of food additives

Database [Web URL]	Data content description and capabilities
Everything added to food in the United States [http://vines.narod.ru/food_additive_database.html]	Inventory of regulation numbers in Title 21 of the US Code of Federal Regulations, chemical and toxicological information on about 3000 substances directly added to foods. Database is maintained by the US FDA, Center for Food Safety and Applied Nutrition (CFSAN) under an ongoing program, the Priority-based Assessment of Food Additives (PAFA), from which selected examples are presented in Table 4.5
US GRAS Notice Inventory Introduction and pending and closed US FDA GRAS notifications, GRAS notice records including complete submissions, and FDA responses [http://www.accessdata.fda.gov/scripts/fcnNavigation.cfm?rpt=graslisting&displayAll=false&page=2]	Detailed information of the Generally Regarded as Safe or GRAS notice submissions filed since 1998, intended use of ingredients and filing history For list of commonly used phosphate GRAS ingredients, see Table 4.7
Codex General Standard for Food Additives (GSFA) Online Database [http://www.codexalimentarius.net/gsfaonline/index.html?print=true]	Provisions for food additives adopted by the Codex Alimentarius Commission
Joint FAO/WHO Expert Committee on Food Additives (JECFA) Combined Compendium of Food Additives Specifications: Database of Food Additives [http://www.fao.org/foodsafety.quality/scientific.advice/jecfa/jecfa-additives/en/index.html]	The JECFA Committee is a group of independent scientific experts who provide advice for the Codex Alimentarius Commission, which is responsible for development of food standards, guidelines, and related text such as codes of practice under the JECFA Food Standards Programme tasked to protect the health of consumers, ensure fair trade practice, and promote international coordination of all food standards work. Database contains searchable information on food additives
International Food Additives Database [http://www.foodadditivedatabase.com]	A Codex-aligned database intended as an initial reference for exporters that provides a single source of information on global food additive ingredients. It can be used for different international markets and allows comparison of maximum use levels among different countries. Users can search by additive name or technical function or by market for 800 food additives across 60 markets
FoodEssential: Beyond the Label Proprietary Ingredients Database and Processing System Video demo: [http://youtu.be/WevMVZmBZIk]	FoodEssential is a proprietary ingredient database and processing system that makes food label data accessible and easily analyzable. The database specializes in aggregating food label data sources and processing of the raw label information to assign additive, allergen, ingredient, and nutrient properties to each product. Through indexing of the relational property database, all products can be searched and compared for by any variable at the enterprise (retail store) level. They have developed a proprietary ingredient database and processing system, which extracts individual ingredients listed on the label and attaches all the additive and allergen properties. The database provides the subscriber with the ability to search, query, and compare an updated product label base of over 500,000 products (US and Australian). For examples of use, see Table 4.8

Table 4.5 illustrates examples of information on phosphate food additive that can be found in the Priority-based Assessment of Food Additive (PAFA) database maintained by the FDA. These examples illustrate how more than one phosphate additive can be used in a product and the many approved functional (intended) uses each of the additives may have. These examples underscore the need to test the cumulative safety of these additives. With more household members entering the workforce, our society will become more reliant on the use of processed convenience foods that are preprepared or quick to prepare; and therefore foods containing more than one phosphate additive as shown in Table 4.6 have substituted the typical home-prepared foods [16].

The Select Committee on GRAS Substances (SCOGS) compiled a database that allows access to opinions and conclusions from the 115 reports they published between 1972–1980 on the safety of over 370 GRAS food substances. The review of ingredients of GRAS was conducted in response to a 1969 presidential mandate. The SCOGS database allows users to search for the SCOGS opinion and conclusion on the approximately 48 phosphate-containing GRAS ingredients shown in Table 4.6 and includes the United States Code of Federal Regulations (21 CFR) citation for those GRAS food substances that have been codified in the CFR [23]. Many of the SCOGS reports reviewed more than one GRAS substance and each substance was evaluated and assigned its own individual type of conclusion on safety, and assigned a numerical score. Forty-one of the 48 phosphate GRAS ingredients shown in Table 4.7 were awarded safety scores of 1, which corresponds to the following: *There is no evidence in the available information on [substance] that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or might reasonably be expected in the future*.

Scientific experts outside the FDA arrived at the conclusions reported by the SCOGS nearly 40 years ago and their findings conveyed a lack of any concern to public health from phosphate additives used at that time. Twenty-four of these phosphate GRAS products share the same report number, 32, where the SCOGS Committee recognized potential safety risks to the general public with the use of these phosphates in food, citing three concerns: (1) phosphate ingredients have multiple characteristics and many functions in foods; (2) phosphates have a close metabolic relationship with calcium and vitamin D (two nutrients whose intake is recognized as critically limited in the general population), and (3) they have variable levels of consumption in foods and beverages among different segments of the general population [46]. The reports also emphasized the need for better data on the calcium and phosphate intake and the Ca:P ratio in the American diet, recognizing that the Ca:P mass ratio between 2:1 and 1:1 is optimal. The reports stated that there was no need for concern at current (1975) levels of use and phosphorus intake, but warned about future changes, establishing what may be considered as a "threshold of action" to initiate GRAS reassessment of the safety of phosphate ingredients [46]: "*The possibility that unreasonable increases in the usage of these phosphates in common foods would significantly lower the Ca:P ratio and increase the total phosphorus intake for some segments of the population, must be considered in assessing the probability of a health hazard existing because of the ingestion of excessive levels of phosphorus.*"

Table 4.5 Examples for select phosphorus-containing food additives as presented in the Priority-based Assessment of Food Additives (PAFA) database maintained by the US Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN)

Type of information on toxicology status in PAFA[a,b]	PAFA No[c]	Main term for substance[d]	Chemical abstract registry number[e]	US CFR No[f]	FDA approved function[g]
ASP[a]	815	Calcium phosphate, tribasic	007758-87-4	169.179 137.105 182.1217 182.8217 175.300	Anticaking Flour bleaching GRAS multipurpose GRAS nutrient Coatings indirect
ASP	2548	Phosphoric acid	007664-38-2	182.1073 175.300 177.2260 178.1010 73.85 73.275 133.123 133.124 133.129 133.169 133.173 133.178 133.179 163.110 163.111 163.112 178.3520	GRAS multipurpose Coatings indirect Filters resin-bonded Filters resin-bonded Assist caramelization Color enhancement Acidifying agent cheese Acidifying agent cheese Curd formed cottage cheese Acidifying-processed cheese Acidifying-processed cheese Acidifying-soft cheese Acidifying-processed cheese Neutralizing agent–cocoa Neutralizing agent-cocoa Neutralizing agent-cocoa Processing aid

ASP	2602	Potassium phosphate, tribasic	007778-53-2	175.105 176.170 176.180 73.85	Adhesive/coating Adjuvant paper manuf. Indirect food contact paper Assist in caramelization
EAF[b]	3220	Sodium hexa-metaphosphate	010124-56-8	173.310 182.90 182.6760	Boiler water additive GRAS migrating sub GRAS sequestrant
ASP	2764	Sodium phosphate, dibasic	007558-79-4	182.1778 135.110 182.6290 182.6778 173.310 139.110 150.141 133.169 150.161 133.179 133.173 137.305 182.8778 73.85 181.29 175.210 175.300	GRAS multipurpose Lactose reduction GRAS sequestrant GRAS sequestrant Boiler water additive Macaroni drying agent Jellying agent Emulsifying agent Jelly acidifying agent Emulsifying proc. cheese Emulsifying proc. cheese Farina enrichment GRAS nutrients Assist in caramelization Stabilizer Coating agent Coating agent
ASP	2779	Sodium tri-polyphosphate	007758-29-4	172.892 182.90 173.31 182.6810 182.1810 74.302	Food starch modified GRAS migrating-sub Boiler water additive GRAS sequestrant GRAS multipurpose Citrus red #2 color add.

(continued)

Table 4.5 (continued)

Type of information on toxicology status in PAFA[a,b]	PAFA No[c]	Main term for substance[d]	Chemical abstract registry number[e]	US CFR No[f]	FDA approved function[g]
ASP	1418 1421 1422 1423 1428 1431	Starch food modified: Acetylated distarch phosphate Distarch phosphate from Phosphorus oxy-chloride Distarch phosphate from Sodium trimeta-phosphate Hydroxy-propyl distarch phosphate Phosphated distarch phosphate Starch phosphate	068130-14-3 977088-75-7 977088-74-6 053124-00-8 977043-58-5 011120-02-8	175.105 172.892	Adhesive/coatings Food starch modified

[a]ASP indicates fully up-to-date toxicology information has been sought
[b]EAF indicates that there is reported use of the substance, but it has not yet been assigned for toxicology literature search
[c]PAFA database number of the Food Additive Safety Profile volume containing information on substance
[d]Name of the substance as recognized by CFSAN
[e]Chemical Abstract (CAS) registry number for the substance or a numerical code assigned by CFSAN to those substances that do not have a CAS registry number (888nnnnnn or 977nnnnnn-series)
[f]Regulation numbers in Title 21 of the US Code of Federal Regulations (CFR) where chemicals appear
[g]FDA-approved function or intended use of the substance

Table 4.6 Examples of convenience food contributions to total phosphate intake in a typical day's meals

Meal	Phosphate ingredient/additive
Breakfast	
Blueberry pancakes, frozen	Sodium aluminum phosphate, monocalcium phosphate
Home-style syrup	Sodium hexametaphosphate, modified corn starch
Bacon (low sodium)	Sodium phosphate
Calcium-fortified orange juice	Calcium phosphate
Lunch	
Grilled cheese sandwich	Sodium phosphate, calcium phosphate
Tomato soup	Monopotassium phosphate
Pringles potato chips, salt/vinegar	Tricalcium phosphate
Cottage cheese and fruit	Calcium phosphate
Cola	Phosphoric acid
Dinner	
Panko-breaded tilapia fillet, frozen	Food starch modified, sodium acid pyrophosphate
Ore-Ida crispers French fries	Sodium acid pyrophosphate, disodium dihydrogen pyrophosphate
Birdseye broccoli and cheese sauce, frozen	Sodium phosphate, disodium phosphate
Garden salad with Hidden Valley ranch dressing	Phosphoric acid, disodium phosphate, modified food starch
Refrigerator buttermilk reduced-fat biscuits	Sodium acid pyrophosphate, sodium aluminum phosphate
Jello instant lemon pudding	Modified corn starch, disodium phosphate, tetrasodium pyrophsophate
Nabisco Ginger Snap cookies	Calcium phosphate
Diet coke	Phosphoric acid
Snack	
Cheese Nachos (Tostitos brand salsa con queso)	Sodium phosphate, sodium hexametaphosphate

Table 4.7 Alphabetical list of commonly used phosphate containing GRAS[a] substances and their SCOGS[b] safety score

	GRAS ingredient[a]	Score[c]	Report #[d]	CFR[e]
1	Acetylated distarch phosphate	2	115	–
2	Ammonium phosphate dibasic	1	32	184.1141
3	Ammonium phosphate dibasic	1	34	184.1141
4	Ammonium phosphate monobasic	1	34	181.1141a
5	Calcium glycerophosphate	1	74	–
6	Calcium hexametaphosphate	1	32	–
7	Calcium hyphosphate	1	73	–
8	Calcium phosphate dibasic	1	32	–
9	Calcium phosphate monobasic	1	32	–

(continued)

Table 4.7 (continued)

	GRAS ingredient[a]	Score[c]	Report #[d]	CFR[e]
10	Calcium phosphate tribasic	1	32	–
11	Calcium phytate	1	45	586.6219
12	Calcium pyrophosphate	1	32	182.8223
13	Dibasic magnesium phosphate	1	60	184.1434
14	Ferric phosphate	2	35	184.1301
15	Ferric pyrophosphate	5	35	–
16	Ferric sodium pyrophosphate	5	35	–
17	Hydropropyl distarch phosphate	3	115	–
18	Manganese glycerophosphate	1	74	–
19	Manganese glycerophsophate-pkg	1	74	–
20	Manganous hypophosphite	1	73	–
21	Monostarch phosphate	2	115	–
22	Phosphoric acid	1	32	182.1073
23	Potassium glycerophosphate	1	74	
24	Potassium hypophosphite	1	73	–
25	Potassium phosphate dibasic	1	32	–
26	Potassium phosphate monobasic	1	32	–
27	Potassium phosphate tribasic	1	32	–
28	Potassium polymetaphosphate	1	32	–
29	Potassium pyrophosphate	1	32	–
30	Potassium tripolyphosphate	1	32	–
31	Riboflavin 5′-phosphate	1	114	–
32	Sodium acid pyrophosphate	1	32	182.087
33	Sodium aluminum phosphate, acidic	1	43	182.1781
34	Sodium aluminum phosphate, basic	1	43	182.1781
35	Sodium ferricytropyrophosphate	5	35	–
36	Sodium hexametaphosphate	1	32	–
37	Sodium hypophosphite	1	73	184.176
38	Sodium metaphosphate	1	32	182.6769
39	Sodium phosphate dibasic	1	32	–
40	Sodium phosphate monobasic	1	32	–
41	Sodium phosphate tribasic	1	32	–
42	Sodium phosphoaluminate-pkg	1	43	–
43	Sodium pyrophosphate	1	32	182.6760
44	Sodium tetrametaphosphate	1	32	–
45	Sodium tetraphosphate	1	32	–
46	Sodium trimetaphosphate	1	32	–

Table 4.7 (continued)

	GRAS ingredient[a]	Score[c]	Report #[d]	CFR[e]
47	Sodium tripolyphosphate	1	32	182.1810
48	Tribasic magnesium phosphate	1	60	184.1434

[a]GRAS = Generally Recognized As Safe; this term is used to refer to a food substance that is not subject to premarket review and approval by FDA because it is generally recognized, by qualified experts, to be safe under the intended conditions of use
[b]SCOGS = Select Committee on GRAS Substances
[c]Score represents the SCOGS Committee conclusion as to the safety of the GRAS ingredient. For example, a score of one (1) indicates no safety concerns under approved conditions of use
[d]Report number in the fourth column represents the number of the report that contains details of the safety studies that formed the basis of the opinion made by the committee [46]
[e]CFR = Code of Federal Regulations. The number refers to the citation in Title 21 of the US Code of Federal Regulations if the substance is subject to a regulation

Safety of Current Phosphorus Intakes Relative to Calcium Intakes

Following the warnings of the SCOGS Committee, some experts believe it is time to reassess the current status of the distortion in the Ca:P intake ratio [14, 18, 35]. The median Ca:P intake ratio for persons with lower calcium intakes ranged between 0.4 and 0.6 in the 1989–1991 Continuing Surveys of Food Intakes by Individuals conducted by the USDA; however at the time, this finding raised little public health concern [13, 14]. In Fig. 4.6, current individual Ca:P intake ratios estimated from dietary intakes determined in NHANES 2009–2010 show the percentage of the population at potential risk of excess phosphorus intake and adverse health outcomes. Although phosphorus intakes are underestimated in this recent survey of national food consumption, it is clear from Fig. 4.6 that a significant segment of the US population (25%) consume excessive phosphorus relative to calcium with typical Ca:P intake ratios of 0.6 or lower, which some consider a threshold for regulatory action. The use of phosphorus containing food additives will increase with the greater consumption of processed foods, while recent evidence show calcium intake from foods is declining (Fig. 4.7), further distorting the balance between calcium and phosphorus in the modern food supply [68]. Kemi et al. [35] found significantly higher serum parathyroid hormone levels in young adult women whose Ca:P intake ratios ranged from 0.5 to 0.6, despite their adequate calcium intakes. This raises the important regulatory question: Is this sufficient evidence of disease risk to merit reassessment of the cumulative use of the phosphate-containing GRAS ingredients?

Until recently, it has been very difficult to establish how many food and beverage products in the US market place contain phosphorus ingredients in order to determine what constitutes an unreasonable increase in usage. To this end, we evaluated

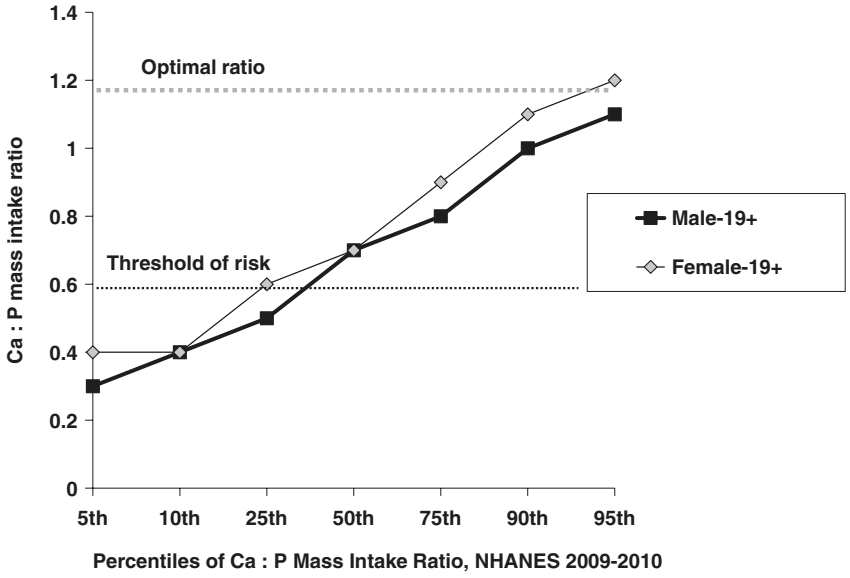


Fig. 4.6 Daily individual calcium to phosphorus mass intake ratios for men ($n = 2880$) and women ($n = 3038$) 19 years of age and older for selected percentiles of intake from day-1 dietary intake estimates of NHANES, 2009–2010 (Reproduced with permission from [18])

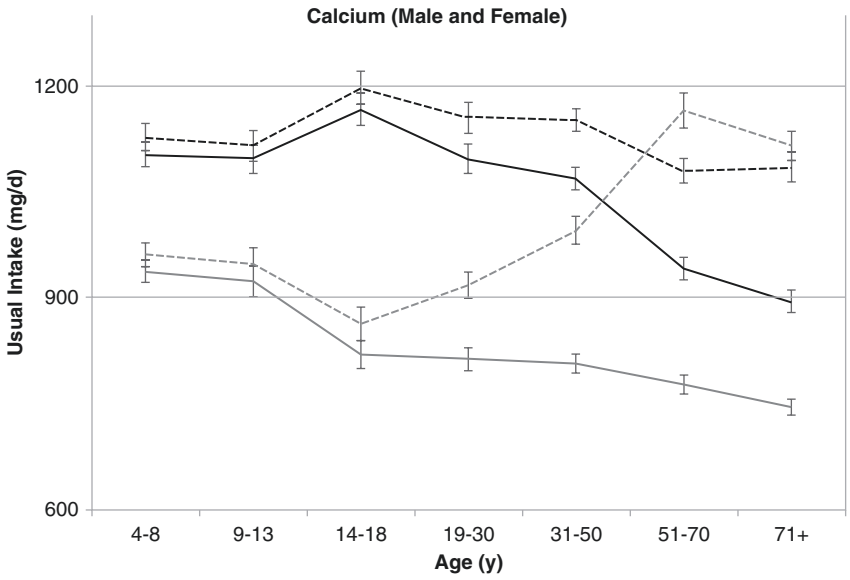


Fig. 4.7 Usual calcium intake estimated from NHANES 2001–2008 intakes among users and non-users of calcium dietary supplements. *Black lines* male, *Grey lines* female; *solid lines* with error bars indicate calcium intake from food only and *dashed lines* indicate calcium intake from food + dietary supplements. RDA for adults is shown by the vertical line at 900 mg/day and that for growing adolescents and young adults is shown at 1200 mg/day (Reproduced with permission from [68])

data from the ingredients list on the label of food products available in most grocery stores to estimate the number containing one or more of three widely used GRAS phosphate ingredients. The results in Table 4.8 for different categories of foods show a total of 589 containing phosphoric acid, 8447 containing sodium phosphate, and 1478 containing sodium polyphosphates (www.foodessentials.com). The number of

Table 4.8 Total number of retail food products containing one of three specific GRAS phosphate ingredients determined from product label information screened from foods available in retail markets, circa April 2012

Food category	Phosphoric acid	Sodium phosphate	Sodium polyphosphate
Cake, cookies, and cupcakes mixes	0	89	16
Breads	103	97	2
Breakfast sandwiches, biscuits, and meals	3	136	0
Cakes, cupcakes, and snack cakes	52	260	104
Canned and bottled beans	1	28	1
Chilli and stews	1	45	17
Canned meat	2	65	16
Canned seafood	1	5	22
Canned vegetables	5	27	1
Cereals	0	629	81
Cheese	47	681	68
Cream	2	163	5
Dairy	13	158	35
Vegetables/lentils mixes	0	14	2
Deli products	27	758	50
Eggs and egg substitutes	0	9	14
Fish and seafood	4	40	272
Frozen dinners	28	994	143
Frozen pizza	10	377	30
Frozen vegetables	0	33	2
Frozen meals	72	1068	307
Frozen snacks	2	121	26
Sausages/hot dogs/brats	0	330	11
Ice cream	112	154	7
Bacon/sausages/ribs	0	280	3
Milk	0	92	43
Noodles	0	26	59
Other meats	0	79	4
Other pastries: croissants/bakery	16	29	25
Packaged deli meats: pepperoni, salami, cold cuts	2	944	2
Prepackaged fruits/vegetables	25	93	13

(continued)

Table 4.8 (continued)

Food category	Phosphoric acid	Sodium phosphate	Sodium polyphosphate
Pancakes, waffles, French toasts	7	9	2
Pasta	30	330	67
Poultry, chicken, turkey	0	137	14
Rice	0	47	9
Subs, sandwiches	21	88	5
Yogurt	3	12	0
Total number of items	*589*	*8447*	*1478*

Data compiled using LabelINSIGHT® by FoodEssentials [http://www.labelinsight.com/]. Database was last accessed April 2012

foods processed with phosphate ingredients has clearly increased; however, it remains unclear if this represents a distortion in the Ca:P intake ratio sufficient to trigger a safety reassessment of specific phosphate GRAS ingredients.

Summary and Conclusions

We believe that the emerging evidence of an association between current levels of dietary phosphorus intake and poor health outcome in CKD patients and even in healthy subjects compels us to reexamine the safety of the existing cumulative use of phosphate-containing food additives. This problem needs to be approached using modern sensitive toxicology endpoints that would capture evidence of the endocrine disruption of mineral homeostasis [39], and needs to be approached from different perspectives [9, 64]. An important barrier to improving awareness of CKD patients of the health risks with excess phosphorus intake is that much of the phosphorus added during processing remains unaccounted for in the databases used to estimate intake. An obvious tool to correct this problem would be to enforce the listing of phosphorus content on the product's Nutrition Facts Panel.

Making such labeling changes mandatory on all processed foods takes time for public dialogue and cost/benefit analyses, thus other approaches to correct this problem should be considered in the interim. Another simple, but effective idea would be to create a Kidney-Friendly Shelf, grouping nutritionally appropriate foods for kidney health and patients with kidney disease in one place in local supermarkets [51]. Some supermarkets have separate shelves for different conditions such as sugar-free products for diabetics and gluten-free products for people with celiac disease. Another interesting suggestion is the incorporation of an icon such as a small kidney on product packaging to indicate CKD-friendliness of the particular food, similar to the small heart created by the American Heart Association [4]. Of interest, the FDA has recently enacted legislation to define and label the gluten content in food, a very important addition to food labels for the estimated 4 million celiac disease sufferers in this country [21]. With the CKD population estimated at

over 20 million, one would expect that the FDA will eventually enact similar regulation to precisely define phosphorus content in food, essential to be able to limit dietary intake of this mineral.

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Nutritional Aspects of Phosphorus Compounds in Foods

5

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Abbreviations

ATP	Adenosine triphosphate
AI	Adequate Intake
Ca	Calcium
DRI	Dietary Reference Intakes
DXA	Dual energy x-ray absorptiometry
EAR	Estimated Average Requirement
ECF	Extracellular fluid
ELBW	Extremely low birth weight
FGF23	Fibroblast growth factor 23
GFR	Glomerular filtration rate
Pi	Inorganic phosphorus
IOM	Institute of Medicine
ICU	Intensive care unit
MBD	Metabolic bone disease
NHANES	National Health and Nutrition Education Survey
NICE	National Institute of Health and Clinical Excellence
PTH	Parathyroid hormone
PN	Parenteral nutrition
P	Phosphorus
RDA	Recommended Dietary Allowance
RFS	Refeeding syndrome
UL	Tolerable Upper Intake
VLBW	Very low birth weight

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Key Points

- Phosphorus is an almost ubiquitous nutrient and plays an essential role in energy metabolism and bone structure.
- The recommended phosphorus intake varies with the age, developmental state, nutritional status, and the presence of kidney disease as well as other illnesses (e.g., intestinal malabsorption syndromes).
- Both excess phosphorus intake and dietary phosphorus deficiency are associated with poor outcomes.

Introduction

Healthy lifestyle and dietary choices are essential to long-term bone and general health. There are a few, specific nutrients implicated in bone health. These include calcium; phosphorus; vitamins A, D, K; magnesium; and sodium. However, it is critical to monitor the overall dietary pattern since, with the exception of phosphorus and sodium, the other nutrients may not be easily obtained in sufficient quantities from meals, particularly over the short term [1, 2]. Phosphorus, along with calcium, is a structural component of bone and teeth. A primary function of dietary phosphorus is to maintain a normal level of phosphorus in the extracellular fluid (ECF) to support skeletal growth and remodeling and to replace excretory losses [3]. The primary sources of dietary phosphorus are both readily available and abundant in the food supply. Thus, whole organism phosphorus deficiency is rare [4, 5]. In fact, phosphorus consumption is increasing in many countries due to the use of phosphate-based additives in processed foods [6]. However, in specific patient populations at high risk for malnutrition (e.g., chronic alcoholism), phosphorus deficiency is a serious problem as is the risk for the refeeding syndrome (RFS), which may occur when patients are treated to correct malnutrition. Knowledge of the optimal phosphorus intakes for normal people and those with various clinical disorders is important since, in addition to the need to manage nutritional deficiencies, long-term excessive phosphorus consumption may have adverse effects on bone and cardiovascular health [1, 2, 6] This knowledge is especially relevant for patients with renal impairment since increased total body phosphorus burden and hyperphosphatemia have been identified as risk factors for accelerated progression of chronic kidney disease, vascular calcification, left ventricular hypertrophy and cardiovascular mortality [7].

Distribution of Body Phosphorus

The human adult body is about 1 % phosphorus by weight (10 g/kg). Phosphorus in the body is bound to both inorganic (Pi) and organic cations but is almost all present in the body covalently bound to oxygen as phosphate (PO_4) [1, 8].

Approximately 80–85 % of total body phosphorus is found in mineralized tissue such as bone and teeth as inorganic phosphate and 15 % is contained within soft tissue, as organic and inorganic phosphates [1, 8]. In addition to providing structural support as bone mineral, organic phosphorus plays an important role in multiple biological processes, including as an integral component of nucleotides (adenosine triphosphate (ATP)), phospholipids, phosphoproteins and phosphoglycans [9]. The extracellular fluid (ECF) contains 0.1 % of total body phosphorus, which is virtually exclusively present as inorganic phosphorus [1]. This inorganic phosphate compartment in ECF is essential to phosphorus homeostasis and is derived from the diet and resorption from bone. Most urinary phosphorus and hydroxyapatite mineral phosphorus in bone are derived from ECF and particularly from plasma phosphorus. The plasma compartment is also the primary source from which the cells derive both structural and high-energy phosphate [3]. Since inorganic phosphate is the starting substrate for the generation of organic phosphorus-containing molecules, it is important to consider how the body regulates its external balance to prevent total body phosphorus excess or deficiency [4]. Similarly, internal balance must be maintained to ensure that phosphate is retained in its normal compartments, for example keeping solid phase phosphate in mineralized tissue and not in soft tissue [4, 10].

Criteria for Dietary Reference Intakes (DRI): Phosphorus [11]

Between 1941 and 1989, the Institute of Medicine's Food and Nutrition Board released the Recommended Dietary Allowances (RDA), which established single values for recommended daily intakes for each nutrient, adjusted for age, gender, and physiological condition. In 1995, the Food and Nutrition Board replaced this single set of values with multiple sets of values, collectively referred to as the Dietary Reference Intakes (DRI). The DRIs are composed of four nutrient-based reference values that are used to assess and plan the dietary nutrient intakes for healthy people.

- Estimated Average Requirement (EAR): The median usual intake value that is estimated to meet the requirement of half the healthy individuals in a life-stage and gender group. The EAR is based on a specific criterion of adequacy that varies based on the nutrient, life stage, and gender. Reduction of disease risk is considered along with other health parameters in the selection of that criterion.
- Recommended Dietary Allowance (RDA): The average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life-stage and gender group. The EAR is used to calculate the RDA.
- Adequate Intake (AI): The recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate. AIs are used when an RDA cannot be determined.

- Tolerable Upper Intake Level (UL): The highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects may increase.

RDAs, AIs, and ULs are dietary guidelines for individuals, whereas EARs provide guidelines for groups and populations. In addition, factors that might modify these guidelines, such as bioavailability of nutrients, nutrient-nutrient and nutrient-drug interactions, and intakes from food fortificants and supplements, are incorporated into the guidelines. This change was justified by new research showing the importance of higher intakes of some nutrients for promoting health (preventing chronic disease) and performance, the fact that precise dietary requirements for some nutrients are not known and may be difficult to ascertain (e.g., trace element needs in healthy infants or in sick individuals), the recognition that for a number of essential nutrients excessive intakes, as well as insufficient intakes, may be unhealthy, and the inability of existing RDAs to distinguish guidelines for groups and populations from those for individuals.

These dietary reference values were subsequently published in a series of reports released between 1997 and 2005, titled the *Dietary Reference Intakes*. DRIs for *Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride were published in 1997.* In the past, recommendations for dietary phosphorus intake were tied to calcium intake. The Institute of Medicine (IOM) deemed this approach unsatisfactory, and instead three indicators were considered for the EAR: phosphorus balance, serum Pi, and the factorial approach [3]. The IOM established dietary phosphorus recommendations in adults based on phosphorus balance studies and serum inorganic phosphate concentrations. The dietary intake needed to maintain serum levels within an optimal range that does not result in dysfunction or disease was considered the most logical indicator of phosphorus requirements [12]. For infants and children, phosphorus recommendations were based on a factorial approach, which takes into account the need for phosphorus during growth [12]. For infants less than 12 months, recommended intakes of phosphorus are based on Adequate Intakes (AIs) that reflect observed mean intakes of healthy, term, infants fed principally with human milk.

Table 5.1 shows the 1997 US Dietary Guidelines and the previous 1989 RDA for phosphorus intakes across life-stage groups. The main changes between 1989 and 1997 are the establishment of both a RDA and EAR for each age group, reductions in intake recommendations for all age groups other than the 9–18 year olds, and the establishment of Tolerable Upper Intakes Levels (UL) [3, 12]. Data from the National Health and Nutrition Education Survey (NHANES 2005–2006) show that for all ages and both genders, except for adolescent girls, phosphorus intake is significantly greater than the EAR. These data most likely underestimate actual intake since the nutrient content databases do not reflect the recent increase in phosphorus additives to foods [13, 14]. Excess phosphorus intake is a real public health concern based on epidemiologic evidence of an association between cardiovascular disease risk and serum phosphorus [15] and between phosphorus intake and the development of osteoporosis [16].

Table 5.1 Dietary Reference Intake values for phosphorus by life-stage group

Life stage group[a]	Indicators of requirement	1997 RDA[c]/ AI[c] (mg/day)	1997 EAR[d] (mg/day)	1989 RDA (mg/day)	UL[e] (g/day)
0–6 months	Human milk	100[c]	ND	300	ND
7–12 months	Human milk + solid food	275[c]	ND	500	ND
1–3 years	Factorial approach[f]	460	380	800	3
4–8 years	Factorial approach	500	405	800	3
9–13 years	Factorial approach	1250	1055	1200	4
14–18 years	Factorial approach	1250	1055	1200	4
19–30 years	Serum Pi[g]	700	580	1200	4
31–50 year	Serum Pi	700	580	800	4
50–70 years	Extrapolation of serum Pi from 19 to 50 years	700	580	800	4
>70 year	Extrapolation of serum Pi from 19 to 50 years	700	580	800	3
Pregnancy					
≤18 years	Factorial approach	1250	1055	1200	3.5
19–30 years	Serum Pi	700	580	1200	3.5
31–50 years	Serum Pi	700	580	1200	3.5
Lactation					
≤18 years	Factorial approach	1250	1055	1200	4
19–30 years	Serum Pi	700	580	1200	4
31–50 years	Serum Pi	700	580	1200	4

[a]All groups except Pregnancy and Lactating are males and females
[b]RDA = Recommended Dietary Allowance. The intake that meets 97–98 % of individual nutrient needs in a group
[c]AI = Adequate Intake. AI is the estimated mean intake
[d]EAR = Estimated Average Requirement. The intake that meets 50 % of individual nutrient needs in a group
[e]UL = A Tolerable Upper Intake Level is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population
[f]Factorial Approach = An approach based on measuring the amount of a specific nutrient that must be replaced when the intake of that nutrient is zero or minimal (see text)
[g]Pi = Serum inorganic phosphate concentration

Table 5.1 shows the criteria used to establish Dietary Reference Intakes (DRI) for *phosphorus* across life-stage groups [3].

Phosphorus Balance

Phosphate balance is the difference between phosphorus intake (normally from the diet) and phosphorus losses from the body (normally from urine, feces, the integument – exfoliated skin, nail and hair growth, and sweat, but also from saliva and dialysate in dialysis patients) and to a very small degree from blood drawing. Phosphorus balance is affected by changes in dietary phosphorus intake, intestinal

uptake of dietary phosphorus, renal phosphorus excretion and reabsorption, and the exchange of phosphorus between bone and ECF pools [17]. Unlike calcium, where changes in balance depends mainly on dietary intake and intestinal absorption, body phosphorus balance is strongly affected by renal excretion as well as phosphorus intake and intestinal absorption [18, 19]. Fibroblast growth factor 23 (FGF23) and Klotho closely work in association with one of the principal modulators of phosphate balance, parathyroid hormone (PTH) to reduce the renal tubular absorption of the phosphorus filtered by the glomeruli. The result is decreased renal tubular phosphorus absorption and increased urinary phosphorus excretion. The other major modulator of phosphorus balance 1,25-dihydroxy vitamin D_3 (1,25-dihydroxycholecalciferol) increases intestinal phosphorus absorption and may also reduce urinary phosphorus excretion by suppressing secretion of PTH and FGF23 [4].

The dietary intake of phosphorus-containing substances varies both in terms of amount and bioavailability. However, only inorganic phosphate is absorbed. Fractional absorption by the intestine is fairly constant due to modest gastrointestinal regulation. With a net zero external balance, urinary excretion of phosphorus essentially equals intestinal phosphorus absorption. As dietary phosphorus increases, PTH increases and so does urinary excretion, as indicated above primarily due to reduced renal tubular reabsorption of phosphorus filtered by the glomerulus. Thus, in healthy states, serum phosphorus concentrations and ECF phosphorus content tend to remain rather constant [20–22]. Intake of glucose and other nutrients may decrease serum phosphorus by stimulating insulin and enhancing the intracellular movement of phosphate [3, 8]. With decreasing glomerular filtration rate (GFR), serum phosphorus may remain normal, even with a deficiency of 1,25-dihydroxyvitamin D. This is due to the rise in serum PTH and FGF23 and the subsequent decrease in renal tubular reabsorption of phosphorus, which thereby maintains normal renal excretion of phosphorus. If the decrease in GFR leads to an advanced state of kidney failure, however, the glomerular filtration of phosphorus will fall to the level where the decrease in renal tubular reabsorption of phosphorus will no longer maintain adequate urinary excretion and serum phosphorus levels will rise.

Low phosphorus intakes, such as in semistarvation or alcoholic patients, may reduce serum, ECF and intracellular phosphorus levels. Hydroxyapatite in bone and teeth represents the largest pool of calcium and phosphate in higher vertebrates. This pool of calcium and phosphate continuously moves into and out of the ECF compartment. The skeleton is in constant flux as part of bone continues to undergo modeling in the growing child and remodeling in the adult [4]. Internal phosphorus homeostasis can be viewed as the maintenance of a normal or healthy burden and distribution of phosphorus within the body. With dysfunction of any of the phosphorus regulatory hormones, phosphate homeostasis may be lost, which in turn may contribute to osteodystrophy, cardiovascular disease, secondary hyperparathyroidism, and progression of chronic kidney disease [4].

There are several limitations to the use of phosphorous balance as an indicator of the phosphorus intake that is adequate both for growth and for maintenance of body

phosphorus mass. Phosphorus balance can be positive, neutral, or negative, depending on a number of factors, including growth, aging, acquired or inherited disorders, and availability of other essential nutrients. An adult can be in net zero phosphorus balance at a dietary intake that is inadequate to maintain serum phosphorous within normal limits [3]. During growth, if phosphorous is sufficiently available, balance can be positive and reflect soft tissue and bone accumulation of phosphorus. However, several factors can reduce the potential for growth and in turn limit the use of phosphorus balance as a marker of nutritional adequacy: (a) availability of other essential nutrients, (b) resistance to or inadequate growth hormone, or (c) a clinical disorder which impairs normal growth (such as advanced chronic kidney failure or liver failure). For example, a growing child who is either deficient in another essential nutrient or has an illness that suppresses bone formation can be in a state of positive phosphorus balance that may be adequate to support their current state of growth but would not support optimal growth. There are also many metabolic disorders associated with negative phosphorus balance, such as catabolic illnesses or hyperparathyroidism, where increasing dietary phosphorus intake may not make phosphorus balance neutral or positive. During the aging process, there is net loss of bone or soft tissue mass and a net phosphorus loss, although the daily loss from the body may approach the limits of detectability by balance measurements. In people who are ill or wasting due to the aging process, it is possible, but not very well documented, that increasing the phosphorus load might attenuate bone and soft tissue loss, whereas in many disease states one does not want a person to be in positive phosphorus balance because it may lead to an excess phosphorus burden and adverse outcomes. In fact, there is growing evidence that serum phosphate is an independent risk factor for cardiovascular events and mortality in people both with and without chronic kidney disease (CKD) [23]. In advanced kidney failure, phosphorus balance can be negative because of bone losses or catabolism in soft tissue even though serum phosphorus levels are normal or even high. Finally, balance data needed to define phosphorus requirements during growth are not available.

Serum Phosphorus

Serum phosphorus levels vary with age. Infants have both the highest dietary requirements (per kg body weight – due to bone growth and soft tissue buildup) and the highest serum concentrations (4.5–8.3 mg/dL (1.50– 2.65 mmol/L)) of phosphorus. Both phosphorus needs (per kg body weight) and concentrations fall toward adulthood (2.5–4.5 mg/dL (0.8–1.5 mmol/L)) [24–27]. The Institute of Medicine considered serum phosphate to be the most logical indicator of the nutritional adequacy of phosphorus intake [3] because dietary phosphorus intake may directly affect serum phosphate levels [28], and both hypo- and hyperphosphatemia result in dysfunction or disease. People with certain renal diseases, renal failure or hyper- or hypoparathyroidism can be exceptions to this contention. The rate of bone remodeling is another determinant of the concentration of serum phosphorus; a rise in bone resorption may increase serum phosphorus concentrations, whereas increased

mineralization of bone may decrease serum phosphorus [24]. An adequate dietary phosphorus intake is one that meets both the cellular and bone formation needs of healthy individuals [3]. According to the DRIs, in a healthy adult, the phosphorus intake that maintains serum phosphorus within the normal range can be defined as adequate. Insufficient evidence exists as to the optimal serum phosphate concentration. The EAR was based on the intake associated with maintenance of serum phosphorus at the lower end of the normal range [3]. Deficient dietary intake may result in altered bone structure and soft tissue dysfunction and can be associated with critical serum phosphate levels as low as or lower than 0.3–0.5 mmol/l (0.9–1.6 mg/dl). The lower limit of the normal range is therefore set safely above that level (0.8–0.9 mmol/l (2.5–2.8 mg/dl)) [3].

The circulating level of phosphate is the only clinically measurable pool, but an abnormal serum phosphorus concentration may not always reflect a phosphate imbalance in which total body phosphorus is either inadequate or excessive for optimal health [29]. Rather it represents a dynamic balance between intestinal absorption, renal excretion, and exchange between bone, soft tissue and ECF pools [29]. Dietary phosphorus intake closely reflects integrated 24-h serum phosphorus levels in healthy individuals, which constitutes the actual exposure that the tissues experience [30–32]. But when fasting serum phosphorus is measured, only a weak correlation with phosphorus intake is observed [31]. A major limitation to the use of serum levels as a marker of nutritional adequacy is that most available data concerning serum phosphorus in healthy adults has been obtained in the fasting (postabsorptive) state. Further evidence that serum phosphate levels alone may not reflect phosphate homeostasis is the finding that vascular calcification has been observed in early stages of kidney disease when fasting serum phosphorus levels are still normal or near normal [33]. It has also been shown in early kidney disease that FGF23 [34, 35] and Klotho [36] can change independently of serum phosphate levels. Finally, the relationship between dietary intake of phosphorus and serum phosphorus levels have only been established in healthy adults [3].

Factorial Method

The use of serum phosphorus as an indicator of nutritional adequacy is limited in infants and children because, despite the known negative impact of low serum phosphorus on growth and epiphyseal cartilage maturation, the relationship between phosphorus intake and optimal serum levels is not clearly characterized [3]. As such, dietary phosphorus requirements in infants, children, and adolescents are based on a factorial approach [3]. The factorial method is based on measuring the amount of a specific nutrient that must be replaced when the intake of that nutrient is zero or minimal. This is the minimum possible replacement value that includes (a) replacement of losses via excretion and utilization at zero or low intake, (b) the amount of nutrient that must be absorbed by the intestinal tract for maintenance of body stores, and (c) an intake that is usually sufficient to prevent clinical deficiency [37]. An inherent limitation to the factorial method is that the phosphorus intake

during the measurement period, even if it is zero, will influence the phosphorus outputs.

Dietary phosphorus is needed during periods of growth to support bone and soft tissue accrual. In infants, children, and adolescents, average net daily additions of bone and soft tissue mass can be estimated [3]. The data on bone growth and body composition changes are derived from one of two methods: (a) the phosphorus content of bone tissue gained over this age range using radiological methods or photon absorptiometry [38] or (b) the increments in whole body bone mineral content using dual energy x-ray absorptiometry (DXA) [39, 40]. The EAR is based on the factorial approach where rates of phosphorus accretion in bone and soft tissue are corrected for efficiency of intestinal absorption and urinary losses [3].

EAR = (accretion + urinary loss) divided by fractional absorption

Infants: Birth to 12 Months

From birth to 12 months of age, there are no functional criteria for phosphorus status that can be used to define nutritional adequacy. So the IOM used Adequate Intakes (AIs) for phosphorus that are based on mean intakes of term-born healthy infants fed human milk as the primary source of nutrition [3]. This approach is justified as there are no known reports of phosphorus deficiency in exclusively human milk-fed, vitamin D-replete, full-term infants [3].

Infants: Preterm

There are significant challenges to providing sufficient nutrition to preterm infants in the very low (VLBW, <1500 g) and extremely low birth weight (ELBW, ≤1000 g) categories [41]. In fact, 10–20% of ELBW infants have radiographically defined rickets [42, 43] associated with decreased intake of intestinal absorption of calcium and phosphorus [43, 44]. With respect to the nourishment of the preterm infant, the calcium and phosphorus content of the usual intake or dose of unfortified human milk, parenteral nutrition, full-term infant formularies, amino acid based formularies, and soy-based formularies are insufficient to meet the needs for bone mineralization [41, 43, 44]. The American Academy of Pediatrics suggests 100–220 mg/kg of calcium and 60–140 mg/kg of phosphorus daily (Ca:P ratio of 1.6:1–2:1) [43]. Thus, to prevent rickets in preterm infants, diets containing high amounts of minerals are recommended (i.e., using formulas designed for preterm infants or human milk supplemented with fortifiers). However, few data suggest better alternatives than human milk [41]. The highest bone mineral contents are found in infants who received the most human milk in their diet, even when compared to formula with supplementation [45]. The advantage of human milk lies in the significantly higher bioavailability for both calcium and phosphorus in human milk than in formula and the presence of other nonnutritive factors (e.g., immunoglobulins) [45]. The

management of infants who already have rickets focuses on maximizing calcium and phosphorus intake from intravenous nutrition while minimizing factors that lead to mineral loss. Currently, the optimal and safest forms and doses of calcium and phosphorus that should be added directly to the diet of preterm infants are unknown [43].

Dietary Phosphorus in Bone Health

The following three pertinent facts regarding phosphorus and bone are relevant to this discussion: (a) phosphorus is a structural component of bone and teeth; (b) adequate intake of phosphorus is essential for bone building during growth [6, 28, 46]; and (c) hypophosphatemia is associated with reduced PTH and FGF23 and increased 1,25 $(OH)_2D_3$; changes that limit mineralization of new bone-forming sites at all ages, impair osteoblast function, and enhance osteoclastic resorption [47]. From a nutritional point of view, adequate intakes of vitamin D and calcium are essential for bone health [9], but the role of dietary phosphorus is less clear.

The epiphyseal growth plate has a crucial role in bone growth. In growing children and even adolescents, growth plate thickness is determined in part, by vascular invasion of the growth plate followed by conversion into primary bone spongiosa [48]. This vascular invasion requires mineralization of the growth plate cartilage, and deficiencies in either calcium or phosphorus will interfere with this process [48]. In addition to the growth plate, phosphorus deficiency manifested physiologically as hypophosphatemia, can affect other parts of the skeleton, resulting in osteomalacia in adults or both rickets and osteomalacia in children [10, 49]. Both of these disorders are characterized by a failure to mineralize forming growth plate cartilage or bone matrix, combined with impairment of chondroblast and osteoblast function [50].

If phosphorus intake is significantly greater than calcium consumption and absorption, then secondary hyperparathyroidism leading to a decline in bone mineral content may occur [51–53]. Secondary hyperparathyroidism occurs in part, because high serum phosphorus concentrations may depress serum calcium, thus stimulating the secretion of PTH. The IOM set the upper limit for phosphorus intake based on the adverse effects of hyperphosphatemia, namely, increased serum PTH, changes in hormonal control of calcium [54], calcification of the kidney, increased porosity of the skeleton, and reduced calcium absorption [3]. However, the Institute concluded that there was little evidence to support setting a ratio for the dietary relationship between phosphorus and calcium, and further, that high phosphorus intakes do not result in negative calcium balance or increased bone resorption if calcium intake is adequate [3]. In kidney failure, severe secondary hyperparathyroidism with enhanced bone resorption may prevent low serum calcium levels from occurring when phosphorus intake is high and serum phosphorus levels are increased. Under these conditions, calcium phosphate may be deposited in many soft tissues including arteries.

The overall trend in food consumption is to drink less milk and consume more phosphorus-additive containing processed, convenience foods [16], all of which results in a lower dietary Ca:P ratio. There is extensive data demonstrating the

adverse effects of a low Ca:P ratio in animal diets [16]. In humans, several studies have demonstrated the potential adverse effects of high phosphorus intake on bone health, including hormonal changes equivalent to mild hyperparathyroidism, decreased 1,25-vitamin D_3 concentrations, and disruption of calcium homeostasis [16, 55, 56]. PTH, bone resorption [16, 53] and FGF23 release from bone [57] are increased with low calcium and high phosphorus intakes. These increases in PTH and FGF23 can occur without significant changes in serum phosphorus concentrations [58]. Even if the dietary Ca:P ratio is increased with a high phosphorus diet, the adverse effects on calcium metabolism cannot be completely reversed [51]. While there are no prospective, controlled studies on the effects of different phosphorus doses on bone mass in healthy humans, an epidemiological cross-sectional study suggests that high phosphorus intakes are negatively and independently associated with lower bone mass in young women [59].

The bioavailability of phosphorus and its association with other nutrients in the gastrointestinal tract might represent another way in which dietary phosphorus can affect bone mass. In this regard, several studies have shown that the physical structure of phosphorus-containing foodstuffs has important effects on intestinal absorption of phosphorus [58, 59]. Furthermore, there are data that indicate that lower availability of phosphorus can lead to increased calcium absorption due to a reduction in the binding of calcium by phosphorus in the gut. With the use of phosphate binders in CKD, phosphate complex formation in the gut is accompanied by the disassociation of calcium phosphate complexes that are already present [60]. While the phosphate is captured by the phosphate binder, free calcium becomes available for intestinal absorption and calcium absorption has been shown to increase with the use of some binders [61]. Lotz et al. performed calcium and phosphate balance experiments using radiolabeled calcium, and demonstrated a significant increase in gastrointestinal absorption under phosphate binder treatment [50]. The opposite may also hold whereby a high phosphorus intake may lead to reduced calcium absorption leading to negative bone mass. It has been reported that increased cola consumption (cola beverages contain phosphoric acid) is associated with lower bone mineral density in women [62, 63]. The epidemiological studies that revealed this significant association might be due to this latter factor or alternatively, it might reflect the fact that people who drink cola are drinking fewer calcium-containing beverages (i.e., milk). There is currently no scientific consensus as to whether a high phosphorus intake causes bone loss. Data that would allow for a definitive conclusion to be made about the effect of the dietary Ca:P ratio on bone health in human are limited [12, 52].

Refeeding Syndrome

That the body has nutritional needs for phosphorus is never more evident than in the refeeding syndrome (RFS). RFS describes the clinical and metabolic changes that occur as a consequence of nutrition repletion of underweight, severely malnourished or starved individuals [64, 65]. Its clinical presentation is dependent on the severity of both the malnutrition and the ensuing electrolyte deficiencies and often

includes hypophosphatemia, salt and water retention with edema and heart failure, hypokalemia, depletion of thiamin, and hypomagenesemia [66]. The incidence of RFS is 48 % in severely malnourished patients who are being refed [67], 34 % of all ICU patients [68], and 25 % of cancer inpatients [69]. With starvation, adaptations occur to ensure survival in which metabolic pathways shift from deriving energy from glucose via glycogen and amino acids, to ketone production as a result of free fatty acid oxidation [64, 65]. With starvation, body fat and protein are lost along with depletion of intracellular stores of potassium, phosphate, magnesium, as well as other nutrients [70]. When nutrition is reintroduced, there is a rapid shift back to glucose as the primary fuel source mediated by an increase in insulin [71, 72]. This shift creates a high demand for the production of phosphorylated intermediates of glycolysis which, if phosphorus intake is inadequate, results in hypophosphatemia [64]. Hypophosphatemia is also caused by preexisting low total body stores of phosphorus during starvation and enhanced cellular uptake of phosphorus during repletion of solid tissue mass [64]. RFS is further characterized by fluid and electrolyte dysregulation including, hypokalemia, hypomagnesemia, abnormalities in glucose metabolism, vitamin (thiamin), and trace element deficiencies [73]. Multiple organ systems including cardiac, respiratory, neurologic, and hematologic can be affected leading to multisystem organ failure and death [73].

While there are no internationally validated guidelines for the treatment of RFS, there are numerous regimens based on published literature and expert opinion [65, 66]. The principles of management are to correct fluid imbalances and biochemical abnormalities, especially certain minerals, including phosphorus due to the potential to cause respiratory compromise or failure [64]. The optimum timing to correct abnormalities in RFS is controversial. It was previously thought that correction of electrolyte abnormalities should occur before refeeding [74] but the recent National Institute of Health and Clinical Excellence (NICE) guidelines indicate that feeding and correction of biochemical abnormalities can occur simultaneously. Regardless of which regimen is followed, refeeding of most, if not all, should be instituted carefully and gradually increased over 4–10 days [66].

Treatment of hypophosphatemia depends on the degree of hypophosphatemia, whether or not the patient is symptomatic, and the routes of administration that are available (i.e., enteral or parenteral) [64]. Asymptomatic patients with mild hypophosphatemia and a functioning gastrointestinal tract can be treated with oral or enteral phosphates, otherwise intravenous phosphate supplementation is used. The required dose of phosphate is essentially empiric, because serum levels may not reflect total body phosphorus stores. Dosing may need to be adjusted in patients with impaired renal function who are not treated with dialysis [64].

Phosphorus and Specialized Nutrition Support

Specialized nutrition support, including oral nutritional supplements, enteral nutrition and parenteral nutrition is indicated for patients who are malnourished or at risk of developing malnutrition when it would benefit patient clinical outcomes

or quality of life [75]. It can be used in the short term as a bridge until patients are able to return to normal food or may be continued as long-term home enteral or parenteral nutrition [75]. The use of specialized nutrition support is not without risk and both hypophosphatemia and hyperphosphatemia are possible complications. As discussed earlier, RFS-associated hypophosphatemia, is a complication that can occur with aggressive administration of incomplete or insufficient formulations of enteral or parenteral nutrition in malnourished patients [75]. Hypophosphatemia tends to occur less often in enterally fed patients than in those who receive total parenteral nutrition (TPN), as TPN constituents are specified and do not contain a standard amount of phosphorus; however, serious hypophosphatemia may occur during aggressive enteral feeding of starving patients. But RFS remains a serious problem during aggressive enteral feeding of malnourished or starved patients [75]. With enteral or parenteral nutrition, serum phosphorus levels should be closely monitored to determine the phosphorus requirement of the patient.

Another complication of specialized nutrition support is metabolic bone disease (MBD), which has been associated with long-term total parenteral nutrition (TPN) [76]. Although the precise prevalence is unknown, it is estimated that 40–100 % of adult patients receiving chronic PN may have some degree of bone demineralization [77]. Inadequate intake of calcium and phosphorus may be the primary cause of bone loss in patients who are dependent on PN. But adequate delivery of calcium and phosphorus in PN solutions may not be simple. Optimal supplementation is restricted by the pH of the solution, which in turn is determined primarily by its amino acid concentrations [78]. Furthermore, calcium and phosphorus requirements may be greater than the solubility of these two minerals and lead to precipitation which limits the amounts of these electrolytes that can be provided in TPN [76]. This is especially problematic for neonates because their calcium and phosphorus needs are high, yet fluid requirements are limited [79]. In infants, a Ca:P ratio of 1.7:1 (weight:weight) decreases calciuria and promotes positive calcium balance and is considered optimal for adequate bone mineralization [80]. In adults, a daily supply of at least 15 mEq(300 mg) calcium and 15 mmol(465 mg) phosphorus can promote retention of these elements [76]. Other causes of bone demineralization in long-term TPN patients include gross physical inactivity, inadequate vitamin D intake, and underlying diseases, such as chronic kidney failure.

Hypophosphatemia and Phosphate Depletion

Hypophosphatemia and phosphate depletion are reviewed extensively in Chapter XX: *Phosphorus deficiency in health and disease: alcoholism and other forms of malnutrition* and will be only briefly mentioned here with regard to nutritional aspects of this disorder. Many investigators including Bartter et al, in 1968, have described the effects of phosphorus depletion. This group reported the effects of prolonged treatment with antacids in normal and hypoparathyroid subjects.

Hypophosphatemia, hypophosphaturia, hypercalciuria, increased resorption of skeletal calcium and phosphorus, weakness, and bone pain were described as part of the phosphorus depletion syndrome [50]. Chronic hypophosphatemia can cause proximal myopathy, intention tremors in addition to hypophosphatemic rickets, and osteomalacia [81]. Additional studies report respiratory failure, decreased myocardial contractility, arrhythmias, hemolysis, leukocyte dysfunction, and neuromuscular disorders including seizures and rhabdomyolysis as symptoms of severe hypophosphatemia [82]. Critically ill and postoperative patients, in addition to those with RFS, are at especially high risk for these complications given their low nutritional intake, increased nutrient needs, frequent metabolic derangements (i.e., metabolic alkalosis), and frequent requirement for artificial nutritional support. These factors may engender an intracellular shift of phosphate or increased losses (e.g., from dialysis therapy, draining fistula, and burns) [83]. Hypophosphatemia is associated with increased morbidity and mortality in critically ill and postoperative patients [84–86]. Aggressive care to prevent and replete hypophosphatemia is recommended to avoid the aforementioned complications.

As indicated previously, refeeding causes phosphate to shift into cells during synthesis of glycogen, protein and such other nitrogenous compounds such as creatine and adenine. If phosphorus intake is inadequate, plasma phosphate levels may drop precipitously. Despite the phosphate content of enteral formulas, patients with protein-energy wasting can develop severe hypophosphatemia during enteral feeding; additive risk factors include chronic alcoholism and intestinal malabsorptive conditions [1]. In tube-fed or intravenously nourished patients who have renal failure, either hypophosphatemia or hyperphosphatemia can be observed. It is therefore important to monitor serum phosphate levels as frequently as daily or even more commonly, in addition to glucose and potassium, for at least 1 week after commencement of feedings in malnourished or severely ill patients.

Treatment of hypophosphatemia depends on the acuity of illness in the patient and on the patient's ability to take oral medications. Felsenfeld et al recommend therapy as aggressive as 0.6 mmol/kg of intravenous phosphate for repletion over 6 h for patients with severe acute hypophosphatemia (serum phosphorus <1 mg/dl), 0.4 mmol/kg for moderate hypophosphatemia (serum phosphorus 1–1.7 mg/dl), and 0.2 mmol/kg for mild hypophosphatemia (serum phosphorus 1.8–2.2 mg/dl) in an intensive care setting [87]. For nonintensive care patients with acute mild to moderate hypophosphatemia (serum phosphorus 1.0–2.2 mg/dl), 0.32–0.16 mmol/kg of phosphate for repletion over 24–72 h is suggested [83]. Given that the volume of distribution of the administered phosphorus may vary from patient to patient, frequent monitoring and dosage adjustments will be required to achieve normal serum phosphorus levels [88]. For chronic hypophosphatemia, a combination of oral phosphate supplements and oral vitamin D is the recommended treatment [87].

Hungry Bone Syndrome

The hungry bone syndrome is another clinical condition associated with hypophosphatemia. After parathyroidectomy for hyperparathyroidism, the osteoclast activity in high turnover bone is curbed, leading to calcium, magnesium, and phosphorus absorption by bone. This, in turn, can lead to rapid and prolonged hypocalcemia, often associated with hypophosphatemia and hypomagnesemia. Aggressive repletion with these nutrients may be necessary to maintain normal serum levels and avoid the adverse consequences of hypocalcemia in addition to the risks of hypophosphatemia described above.

Dietary Phosphorus Needs in Patients with Chronic Kidney Disease (CKD)

There is no established difference in the minimum phosphorus intake required for normal metabolism in CKD patients as compared to the normal population. The goals for dietary phosphorus prescription in CKD patients are generally to normalize bone metabolism and avoid abnormal serum phosphate levels with its risk for other adverse outcomes. In chronic kidney disease, the decreased glomerular filtration of phosphate is initially compensated by a reduction in renal tubular reabsorption which is stimulated by elevation of serum PTH and FGF23 [89]. This compensation maintains normal daily urinary phosphorus excretion and normal serum phosphorus levels until kidney failure becomes advanced [89]. Current recommendations are that phosphorus intake should be restricted in CKD patients when serum parathyroid hormone or serum phosphate levels become elevated. Phosphate binders can also be prescribed. In CKD stages 3, 4 and 5, it is recommended that phosphorus intake should be restricted to between 800 and 1000 mg/day when serum levels of phosphorus and parathyroid hormone are above the recommended range [90]. In physically small CKD patients in whom the prescribed protein intake is low, phosphorus intake may be reduced further without seriously impeding the palatability of the diet. Regulation of protein intake is a mainstay of the nutritional management of CKD stages 3–5. Because of the linear relationship of the protein and phosphorus contents of food, the low protein diets recommended in advanced CKD patients (0.6–0.8 g protein/kg/day) will have a lower phosphorus content than the high protein (1.2–1.4 g protein/kg/day) intake recommended for CKD stage 5 patients who are receiving chronic dialysis therapy [91]. Serum phosphorus levels should be monitored regularly in patients ingesting phosphorus-restricted diets, those receiving phosphorus binders, and patients undergoing regular dialysis treatments. Hypophosphatemia should be carefully evaluated, as there is a J-shaped association between serum phosphate levels and mortality in maintenance dialysis patients, with higher levels of mortality occurring with both very low and very high serum phosphorus levels [92]. After kidney

transplantation, renal phosphate wasting may occur [93], which can increase the dietary needs to normalize serum phosphorus levels. Posttransplant increased phosphaturia and hypophosphatemia can be caused by high serum PTH and FGF23 levels; this usually resolves within 1 year after transplantation but can persist for years in some patients. The management of phosphorus intake, prescribed intestinal phosphate binders, and serum phosphorus levels in CKD patients requires continual assessment of the disease state, the phosphorus burden, and the nutritional needs of the patient.

Conclusions

Phosphorus is an almost ubiquitous nutrient and is found in virtually all organic food matter. In healthy individuals, dietary intake of phosphorus is more likely to be too high than too low. Phosphorus plays an essential role in energy metabolism and bone structure. The recommended phosphorus intake varies with the age, developmental state, nutritional status, and the presence of kidney disease as well as other illnesses (e.g., intestinal malabsorption syndromes). The lack of a close correlation between serum phosphorus levels, total body phosphorus burden, phosphorus balance and nutritional phosphorus requirements makes it difficult to precisely identify specific minimal nutritional requirements. The close association of phosphorus metabolism with calcium, vitamin D, and FGF-23 metabolism also limits the usefulness of evaluating phosphorus needs in isolation. Despite these limitations, assessments of extracellular phosphate levels, bone physiology, and phosphorus absorption have been used to develop current dietary guidelines. Serum phosphorus can be used to monitor adequacy of phosphorus intake or the adequacy of phosphorus restriction and the appropriate prescription of phosphate binders in patients with chronic renal insufficiency. Serum phosphorus can also be used to assess the adequacy of phosphorus intake in individuals with high phosphorus requirements, including the RFS. Other markers of phosphorus nutrition and metabolism, such as Klotho and FGF-23, in the future may help to help refine the recommended phosphorus intakes and methods for monitoring adequacy of phosphorus nutrition.

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Phosphorus Additives in Food Processing

6

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Key Points

- Phosphates are used in food processing for a variety of chemical and biochemical functions to produce a consistent product that meets consumers' desires and expectations.
- The use of phosphates in the United States is regulated by the USDA in meat, poultry, and catfish, and by FDA in all other foods.
- The real attributes of food additives containing phosphates are often either overlooked or misunderstood by many outside of the food manufacturing sector.

Introduction

The inorganic food-grade phosphates such as phosphoric acid and the sodium (Na), potassium (K), and calcium (Ca) salts and their chemical properties are described in Table 6.1. These phosphates are generally recognized as safe (GRAS), and are eligible for Kosher and Halal designations. The food-grade phosphates impart numerous physicochemical properties in foods, such as pH adjustment of meats for moisture retention, buffering of coffee whitener, protein dispersion in the preparation of frankfurters and bolognas, moisture adsorption to maintain the free-flow

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Table 6.1 Common name, acronym, chemical formula, solubility in water, and pH of selected food-grade anhydrous phosphates

Phosphate	Acronym	Formula	Solubility (g/100 ml)	pH (1 % solution)
Monosodium phosphate	MSP	NaH_2PO_4	80	4.4–4.8
Disodium phosphate	DSP	Na_2HPO_4	10	8.6–9.4
Trisodium phosphate	TSP	Na_3PO_4	12	11.9–12.5
Monopotassium phosphate	MKP	KH_2PO_4	33	4.4–4.8
Dipotassium phosphate	DKP	K_2HPO_4	167	8.6–9.4
Tripotassium phosphate	TKP	K_3PO_4	90	11.9–12.5
Monocalcium phosphate	MCP	$Ca\ (H_2PO_4)_2$	min.	2.7–3.0
Dicalcium phosphate	DCP	Ca_2HPO_4	insol.	7.2–8.2
Tricalcium phosphate	TCP	$Ca_5\ (OH)\ (PO_4)_3$	insol.	7.0–8.0
Sodium acid pyrophosphate	SAPP	$Na_2H_2P_2O_7$	12	4.0–4.4
Tetrasodium pyrophosphate	TSPP	$Na_4P_2O_7$	6.5	9.9–10.7
Tetrapotassium pyrophosphate	TKPP	$K_4P_2O_7$	184	10.0–10.5
Calcium acid pyrophosphate	CAPP	$CaH_2P_2O_7$	Insol.	3.0–4.0
Magnesium pyrophosphate	MGDP	$MgHPO_4$	Insol.	7.0–8.0[a]
Sodium tripolyphosphate	STPP	$Na_5P_3O_{10}$	15	9.5–10.2
Potassium tripolyphosphate	KTPP	$K_5P_3O_{10}$	180	9.5–10.2
Sodium hexametaphosphate	SHMP	$(NaPO_3)n$ $n=7–10$	∞	ca. 7.3
Sodium hexametaphosphate	SHMP	$(NaPO_3)n$ $n=12–15$	∞	ca. 6.9
Sodium hexametaphosphate	SHMP	$(NaPO_3)n$ $n=18–21$	∞	ca. 6.3
Sodium aluminum phosphate	SALP-A	$Na_2Al_2H_{15}(PO_4)_8$	min.	3.3–3.5
Sodium aluminum phosphate	SALP-4	$NaAl_3H_{14}$ $(PO_4)_8$-$4H_2O$	insol	2.7
Sodium aluminum phosphate, basic[b]	SALP, basic	Na_8Al_2 $(OH)_2(PO_4)_4$	insol	9.3[c]
Monoammonium phosphate	MAP	$NH_4H_2PO_4$	38	4.5–4.7
Diammonium phosphate	DAP	$(NH_4)_2\ HPO_4$	58	7.9–8.1

Modification of Ref. [6]
[a]10 % suspension
[b]Manufactured as an autogenous blend with ca. 30 % DSP
[c]25 % suspension

properties of spice blends, ion exchange in the manufacture of even melting cheese slices, sequestration of minerals to maintain the natural potato color of strips, flavor of colas, improved whipping of egg whites, foam stability of whipped toppings, cryoprotection of the protein in surimi, and texture development of baked goods. The uses and applications of the food-grade inorganic phosphates have been discussed in great detail elsewhere [1–6].

Bakery and Cereal Products

Bakery products can be leavened by one or a combination of yeast, steam, air, and acid–base reactions (chemical leavening). Traditional breads and some rolls are yeast leavened (a microbial fermentation of sugar to produce CO_2 and ethanol); cream puff shells and choux pastry are steam leavened; frozen "rising" crust pizzas may be leavened using yeast and chemical leavening; and most cookies, cakes, crackers, pancakes, waffles, biscuits, muffins, quick breads, and batters and breadings are chemically leavened. Chemical leavening may also be used in extruded products such as breakfast cereals, some croutons, and grain-based fibers textured for meat analog use.

The most commonly used chemical leavening is an acid–base reaction to produce a neutral salt, carbon dioxide, and water.

HX +	$NaHCO_3$ →	NaX +	H_2O +	CO_2
Acid or acid salt	Sodium bicarbonate	Neutral salt	Water	Carbon dioxide

The acid or acid salt is either an organic or inorganic acid or its salt, such as monocalcium phosphate (MCP), sodium acid pyrophosphate (SAPP), sodium aluminum phosphate (SALP), calcium acid pyrophosphate (CAPP), a blend of magnesium phosphates (MGP), sodium aluminum sulfate (SAS), citric acid, and tartaric acid. There is also a chemical leavening reaction that is restricted to very low moisture products such as gingerbread, because the residual ammonia will have an off flavor in high moisture (cakes and soft cookies) baked goods [7].

NH_4HCO_3 +	*ca. 60°C*→	NH_3 +	H_2O +	CO_2
Ammonium bicarbonate	Heat	Ammonia	Water	Carbon dioxide

The increase in volume during baking is attributed to heat expanding the air that was incorporated during mixing, expansion from the CO_2 evolved from the chemical leavening, water that turns to steam when heated, and CO_2 contributed by the flour. The crumb structure is set by baking time and temperature.

Acids or their salts used in chemical leavening are selected for the speed of CO_2 generation once mixed with $NaHCO_3$ and hydrated; this is the rate of reaction (ROR) or in a standard biscuit dough, the dough rate of reaction (DRR). Different chemical forms of some leavening acids will react differently when mixed with a base and hydrated. For example, MCP-1 (MCP-A) reacts very quickly while its anhydrous, coated counterpart (cMCP-0) will have a delayed rate of reaction. The SAPPs (DRRs of 22, 26, 28, 37, 40, and 43) are manufactured with dopants which are usually minerals to slow the DRR when mixed with a base and hydrated. A slow DRR would be selected for a refrigerated biscuit dough or cookie (SAPP 22 or SALP-A) where approximately 22 %, 11 %, and 67 % of the CO_2 is generated during mixing, at bench time, and baking, respectively. A moderate rate (SAPP 40) is used in donuts and results in CO_2 generation of 40 %, 8 %, and 52 % during mixing,

bench action, and baking, respectively. The most rapid DRR (MCP-1) reacts with $NaHCO_3$ to generate 60 % CO_2 during mixing and 40 % CO_2 during baking. As a result, MCP-1 is blended with a slower acting leavening acid, such as SAPP 28 or SAS, for use in baking powders, cakes, cookies, and pancake mixes. MCP-0 may be coated to slow its reactivity with the base and may be used in many prepared mixes.

The first chemical leavening agent, cream of tartar or potassium acid tartrate, has been used since 1835 [8]. Its use is limited because 70 % of the gas is released in 2 min, which is undesirable for many applications. The same is true for citric acid, which also adds an objectionable bitter flavor. SAS has a very low DRR and is commonly blended with MCP-1 in double acting baking powders used in the home to accommodate the desired CO_2 generation. SAS used alone provides a blistered appearance of soft tacos and the tunneling of English muffins, while the presence of SALP provides a firmer structure and a finer crumb in cakes. DCP-2 is used as an adjunct in dense batters with high sugar content and frozen doughs when the temperature of the batter exceeds 60 °C (140 °C) because the DCP is decomposed to tricalcium phosphate (TCP) and phosphoric acid to provide the "crown" of the cake [7]. CAPP and MGP are the newer of the leavening acids and are promoted for reduced sodium-baked goods [9, 10]. MGP may offset SALP-0 and CAPP may be modified to replace different grades of the SAPPs.

The amount of leavening acid used in a formulation is calculated based upon the neutralizing value (NV). NV is calculated by the weight of NaHCO3 (a) neutralized by the weight of the acid and (b) times 100 or $NV = (a/b) \times 100$. As a rule of thumb, chemical leavening is usually less than 2 % of a formulation. Exceptions may be refrigerated doughs which may contain up to 2.5 % chemical leavening [11].

Phosphates in Meat, Poultry, and Seafood Processing

The process of converting muscle to meat occurs upon slaughter or harvest. In vivo, the pH of live muscle is neutral. Postmortem biochemical events profoundly alter the properties of the meat. One impact is on the water holding capacity (WHC) or the ability of the meat to hold its natural moisture or any added water via brines, injection pickles, and marinades. It is estimated that two-thirds of the reduction in WHC is attributed to the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and downstream metabolites and one-third is related to the depletion of glycogen with a concomitant increase in lactic acid [12]. As the lactic acid accumulates, the pH falls from neutrality to ca. 5.6–5.8, which approaches the isoelectric point (I.P., 5.0) of the myofibrillar or functional protein fraction of meat. At the IP, there is no net charge on the protein and water holding capacity is minimal. During this process, the muscle is undergoing rigor mortis, which is the irreversible formation of actomyosin and a contraction or stiffening of the muscle. During subsequent aging, there is a degree of resolution of rigor; however, this does not return the meat to the prerigor state which had a very high WHC.

Historically, sodium chloride (NaCl) was added to induce the swelling of protein associated with the absorption of moisture for improved WHC. The Cl^- ions interact with positively charged groups of actin and myosin and induce swelling of the meat by electrostatic repulsion [13]. Offer and Trinick [14] and Offer [15]

have also shown that NaCl is associated with extraction of the Z and M lines of the sarcomere. Maximum WHC is reported to occur in the presence of 8 % NaCl [13]. In practice, 3–3.5 % NaCl is used because higher levels cause palatability issues. Shults et al. [16] demonstrated the synergistic effect of condensed phosphate and NaCl; the effective amount of NaCl could be reduced to 1.6–1.8 % if condensed phosphates (0.5 %) were included in meat formulations. Another benefit to the use of phosphates was that they would compensate for the oxidative effect of NaCl [17]. The most often used phosphate is STP, which the alkaline phosphatase enzymes in fresh meat hydrolyze to diphosphate that has been shown to mimic the effect of ADP on the swelling of the myofibril and moisture retention. Alternatively, TSPP or TKPP (to reduce Na level) are alkaline diphosphates with a pH near ten that may be directly added. Often blends of phosphates are used so that benefits of each, such as sequestration of minerals (STPP, SHMP), cure acceleration (SAPP), solubilization of myofibrillar protein (TSPP), and a targeted pH will be achieved.

Hall [18] filed a patent in 1945 to use condensed phosphates in meat processing to reduce cooking losses and was followed by Brissey [19] with a patent to use food-grade orthophosphates to reduce cooking losses in hams. The Brissey patent was assigned to Swift and Company (now JBS USA). Bendall [20] reported sodium phosphate salt effects on the water holding capacity (WHC) of minced rabbit muscle; sodium pyrophosphate had a greater impact than either orthophosphate or sodium hexametaphosphate (Calgon), sodium pyrophosphate and NaCl had a synergistic effect on WHC, and actomyosin was split into its component parts (actin and myosin) with at least partial conversion of these components from gel to sol. Swift and Ellis [21] applied these principles to stabilizing beef sausage emulsions shortly thereafter. Tims and Watts [22] compared precooked pork with and without condensed phosphates and demonstrated that a warmed-over flavor (WOF) developed in the absence of phosphates. Younathan and Watts [23] later reproduced this work and identified iron induced lipid oxidation as the mechanism in the development of WOF. The technical impacts of phosphate use include a reduction in cook-cool loss and thus a higher moisture content, which results in a more succulent product. Oil in water emulsions (frankfurters and bologna) are developed via the separation of actin from myosin where the hydrophobic myosin tail wraps around the fat droplet while the hydrophilic end binds water. Marinades containing NaCl and condensed phosphates provide a reduction in thaw-drip loss and subsequent cook-cool loss in uncooked meat and poultry. The use of phosphates and NaCl can extract myosin to form a sol with a tackiness that aids in binding chunks of meat together when forming boneless hams and delicatessen poultry loaves. Of great importance is the fact that Trout and Schmidt [24] showed that the presence of condensed phosphate raised the thermal transition temperature from 70 to 87 °C which imparts greater thermal stability of the myosin with reduced moisture loss during cooking. For more detailed information related to meat and poultry, see Lampila [6].

Phosphates were permitted for use in ham and bacon to reduced cooking losses; however, phosphates were not allowed in other value-added meat and poultry products until 1982 [25] as a result of the need to reduce total sodium in processed meats, as a means to reduce nitrite (to prevent nitrosamine formation) and for enhanced microbial safety [4].

The addition of phosphates to ground meat, hamburger, and fresh sausage is expressly prohibited by the USDA (9CFR319.15 and 319.140) [26]. Phosphates permitted for use in meat and poultry products are listed in 9CFR 424.21 [26]. The

addition of any phosphate into a meat or poultry product must be listed on the ingredient statement. Surveys on the amounts of phosphorus in foods containing added phosphates [27, 28] had limitations to their usefulness, including the evaluation of only one package of a given meat, poultry, or seafood entrée; failing to indicate if the glassware had been washed with 20 % nitric acid prior to use; not indicating the purity of the reagents; and describing if distilled, deionized water had been used for solubilization and dissolution of the products. Sherman and Mehta [28] reported an amount of 2 gm of potassium in a 200 gm sirloin steak. If the potassium phosphate used was TKPP, this would translate to 10 gm TKPP in the 200 gm steak. This amount of potassium would not only render the steak unpalatable, but would likely cause laxative effects. Sherman and Mehta [28] suggested that food-grade sodium and potassium phosphates are used in meat and poultry products but are not labeled. In meat and poultry products, where phosphates are permitted, their presence must be identified in the ingredient statement and are highly regulated as to amounts that are allowed to be used. Excessive sodium phosphates result in a soapy off-flavor and are, thus, self-limiting. Potassium phosphates give a bitter off-flavor when used in excess of 0.3 % in meat and poultry products and are also self-limiting. There has been an increase in sodium and potassium containing ingredients (primarily acetates, diacetates, and lactates) in ready-to-eat meat and poultry products to inhibit the pathogen *Listeria monocytogenes*, for which there is a zero tolerance allowed for its presence in RTE foods.

The food-grade phosphates have very specific functional attributes in seafood processing. Unfortunately, overuse in some sectors has resulted in scrutiny by the US Food and Drug Administration because abuse leads to excessive addition of moisture and may lead to charges of economic fraud. It is important to note, however, that responsible use of the phosphates can prevent charges of economic fraud. The net weight is the amount of food in the container; failure to provide the stated weight of product is a form of economic fraud. Rockfish (*Sebastes* spp.) myofibrillar proteins rapidly denature postmortem and lose up to 80 % of their water holding capacity after 5 days of storage on ice [29], while similar changes in beef muscle take 45 days at >20 °C [30]. A reduction in water holding capacity means that the fluids in the rockfish fillets are continually lost and the usable fish weight is reduced due to desiccation or moisture loss. Crawford [31, 32] dipped rockfish fillets in either chilled water (control) or a chilled 12 % solution consisting of STPP, SHMP, citric acid, and potassium sorbate for 60 s. Each treatment adsorbed an increase of 4 % in weight of either the control or the phosphate-base solution, however; the control treatment lost weight or failed to meet the net stated weight in 4 days while the phosphate treated fillets met the net stated weight (green weight plus adsorbed solution) after 14 days storage. The proper application of phosphates to seafood is useful to promote retention of natural juices and thus prevent charges of economic fraud by meeting the stated net weight.

In the Pacific Northwest, shrimp (*Pandalas jordani*) is given a brief (ca. 5 min) dip in a 3–5 % solution of chilled (<5 °C) STPP [33]. The shrimp are then cooked with atmospheric steam for approximately 90 s followed by movement over a series of nips and rollers with water spraying from jets onto the shrimp to dehead and peel the shrimp. The connective tissue of the shrimp is immature or not highly cross-linked. The combination of the alkaline STPP treatment and steam causes more

efficient rupture of the connective tissue to allow for a more complete separation of the flesh from the shell. This process resulted in an increase (8–12 %) in the amount of edible tail flesh yield without an increase in moisture. Crawford [34] estimated that the increased (8–12 %) yield over the first 8 years of using the STPP dip prior to the steam cook resulted in an ex-plant (value-added processed and packaged) value of an additional $64 million dollars. A secondary benefit was a reduced biochemical oxygen demand of the effluent due to more efficient extraction of the edible flesh from the shell. Additionally, it was determined that the process results in no net increase in phosphorus in the shrimp and, in this case, the STPP treatment qualified as a processing aid as defined in 21CFR101.100 and would not require listing as an ingredient on the product label.

In canned tuna with a pH 6.2 or greater containing magnesium ammonium phosphate, there is a tendency for the formation of these phosphate compounds into crystals, commonly called struvite. Struvite is problematic since the crystals resemble shards of glass. SAPP (ca. 0.25 %) is added to adjust the pH slightly more acidic which prevents struvite formation. In pasteurized and canned blue crab (*Callinectes sapidus*), SAPP is added to prevent a blue discoloration to the flesh. The blue color is caused by a complexing of copper with the amino acid tryptophan and SAPP sequesters the copper to prevent development of this discoloration.

Dairy Foods

Cheeses are the main dairy food in which phosphates are used. Natural cheese is a weak emulsion that undergoes phase separation or breaks when heated. The heated protein coagulates and the fat "oils off" or weeps away from the protein, giving an appearance typically considered undesirable by the consumer. Elmer Eldridge of the Phenix Cheese Company was issued the first patent for use of phosphates in the manufacture of process cheese [35]. The Phenix Cheese Company subsequently merged with J.L. Kraft. Tartrates were used in early process cheeses, but have a tendency to crystallize which results in a sandy textured cheese. Either orthophosphates or citrates, or a combination of the two (termed "melting salts"), may be added to create a cheese that exhibits an even melt when heated. Young, natural cheese with intact protein may be blended with aged cheeses for flavor before grinding. The ground cheese is then heated in a ribbon mixer, cooker-cutter, or similar vessel followed by the addition of a modest amount of water and up to 3 % "melting salts" (21CFR133.169) [26]. The native cheese is a protein gel that is transformed to a sol by the orthophosphate induced dispersion of the protein in the presence of heat and agitation (shear). Since the protein is a calcium caseinate, it is not water soluble. During heating and mixing, the sodium of the disodium phosphate is exchanged for the calcium of the caseinate thus forming a water soluble sodium caseinate in a matrix with dicalcium phosphate bridges. Ideally, melting salts have neutral flavor, have protein dispersing properties, are good buffers, and are blended so that the ideal process cheese has a pH near 5.8. The most often melting salts used include the orthophosphates, DSP, TSP and SALP basic and the citrate, disodium

citrate. Process cheese made with orthophosphates has a stable pH that can be measured immediately; the pH of cheeses prepared with citrates will stabilize after 2–3 days. More acidic cheeses become brittle and have a grainy appearance and more basic cheeses have a soapy surface and a tendency for precipitation of the orthophosphate crystals to the surface [35]. Disodium phosphate is the protein dispersant and primary ion exchange substrate. Trisodium phosphate is usually added to adjust the pH toward neutrality. Sodium aluminum phosphate (SALP basic) is typically added to improve sliceability. More water is added to formulations to prepare a homogenous, spreadable cheese and the standard of identity is 21CFR133.180 [26]. Approximately 10 years ago, processed cheeses with double and triple amounts of calcium were introduced with the added mineral content typically from tricalcium phosphate (TCP). Some mozzarella cheese is processed with SHMP in order to raise the temperature stability of the protein for use in stuffed crust pizza.

Cottage cheese may be produced by the addition of lactic acid bacteria with or without rennet or by direct acidification with or without rennet. Acids, if used, must be food grade and may be chosen from a group consisting of phosphoric acid, lactic acid, citric acid, or hydrochloric acid to reach a pH of 4.5–4.7, with or without rennet and/or any other suitable clotting enzyme in order to form the desired curd (21CFR133.128; 21CFR133.129) [26]. When food-grade acid is used to prepare cottage cheese, the label shall bear the statement "Directly set" or "Curd set by direct acidification" [26]. Each of the ingredients used in the food shall be declared on the label except that milk-clotting enzymes may be declared by the word "enzymes" [26].

Fermented dairy products may tend to separate into two phases over time. Consequently, "safe and suitable" ingredients may be added to improve texture and prevent syneresis of sour cream and acidified sour cream (21CFR131.160 and 131.162) [26]. Multiple use GRAS substances, such as STPP and SHMP, may be used to fulfill this role. Full fat, low fat, and nonfat yogurt may also contain stabilizers (21CFR200.131.203 and 131.206, respectively) [26]. Typically either DSP is used alone or STPP or SHMP may be incorporated if hydrocolloids are included in the formulation. Buttermilk may be stabilized with either sodium citrate or TSPP.

Dairy Desserts

Instant pudding and cheesecake mixes typically contain tetrasodium pyrophosphate to set the milk-based gel and disodium phosphate or monocalcium phosphate is often added to hasten the set of the gel. The presence of either modified or pregelatinized starches also facilitate gelation [36]. The pudding is a soft gel after two minutes of stirring and sets within another five minutes. The pudding is prepared with cold milk so there is little temperature rise. Traditional, scratch puddings involve the heating of the milk, sugar, and native cornstarch with constant stirring, the careful stirring of the hot mixture into the egg yolks without coagulating the egg protein and cooling; the preparation typically takes approximately 30 min followed by several hours for complete chilling. When these puddings are used in cream pies, formulations containing native starch tend to undergo retrogradation while those containing starch phosphate diesters (a form of modified starch) have been shown to undergo ten freeze-thaw cycles without separation of water from the matrix [3].

Ice cream that is constantly whipped, such as soft serve ice cream may undergo a phenomenon termed "churning" which is an agglomeration of the fat particles to the extent that the ice cream develops a sandy or gritty texture. DSP, TSPP, or SHMP at a level of 0.1–0.2 % may be incorporated to prevent this texture defect.

Whipped toppings may be stabilized with DSP, TSPP, or SHMP. These phosphates stabilize the foam during transportation, distribution, and retail. Chocolate and or malted milk may have TSPP added to keep the cocoa in suspension. Polyphosphate may be added to strawberry milk to bind iron and prevent a purple discoloration. DKP is the phosphate type most often added to nondairy creamers to prevent "feathering" of the protein as it is introduced into the acidic coffee medium. This is one application in which the potassium salt is preferred over the sodium salt for flavor neutrality. DSP is added to evaporated milk to maintain protein dispersion through the retorting process and during its shelf-life.

Beverage Applications

Phosphoric acid is the acidulant in cola-type beverages, sarsaparilla, and sometimes root beer (citric may be used), and it contributes to the distinctive flavor profile. The use level is typically around 0.05 % in the colas and 0.01 % in root beer and sarsaparilla [1]. In some powdered beverage mixes, MCP may be used as an acidulant to partially off-set citric acid in the formulation [1]. In other powdered beverage mixes, TCP may be added as either a free-flow (anticaking) agent or a clouding agent or both. SHMP or STPP may be used in noncarbonated beverages as a sequestrant of minerals that may precipitate or cause changes in color. SHMP, short chain, is effective in the prevention of age gelation of UHT milk. The effective level of use is ca. 0.1 %. Calcium-fortified orange juice has been on the market for approximately 20 years. Inorganic TCP is the calcium source in some juices while organic calcium is selected for other brands. TCP interacts with the pectic acid in the juice to result in a slight increase in viscosity which results in a richer mouth-feel. As a rule, isotonic beverages will use MSP, MKP, or both. Enteral feedings and liquid diets may include the aforementioned phosphates and TCP as a source of nutrients.

Potato Processing

Potatoes are the most common vegetable in which phosphates are used. SAPP is used to inhibit the blackening of the potato surface after cooking and cooling. The black discoloration is caused by oxidation of o-diphenolic compounds in the presence of iron [37]. Commercially, potato strips are sent through a flume of SAPP (ca. 1 %) at 60–71 °C with a residence time of <60 s in order to complex the ferrous ions into a colorless form. The concentration of SAPP and treatment time will vary because iron content will fluctuate by region and from year to year while the diphenol content increases with the size of the potato [38, 39]. It has been shown that this process results in no significant increase in phosphorus. Battered French fries will contain more phosphorus because of the presence of chemical leavening in the coating.

Other Uses of Food-Grade Phosphates

The real attributes of food additives containing phosphates are often either overlooked or misunderstood by many outside of the food manufacturing sector. Further, there are some circular references that continually exaggerate the actual consumption levels of the food-grade phosphates and phosphoric acid. There are inferences that these valuable food ingredients lead to a variety of disease states ranging from obesity, osteoporosis, heart disease, and renal disease when, in fact, renal disease (most commonly linked to the secondary effects of diabetes mellitus and hypertension) causes bioconcentration of phosphorus from the diet. Assumptions based on production data of food-grade phosphates lead to erroneous conclusions. There is a plethora of uses of food-grade phosphates which include nonhuman food use, indirect food contact, and as processing aids (Table 6.2). Additionally, there are inferences that all food starch is modified by phosphates when, in fact, only 3 of the 36 methods described in 21CFR172.892 involve inorganic phosphates and 3 other methods use phosphorous oxychloride [40]. There

Table 6.2 Selected nonhuman food applications, indirect food contact, and processing aid uses of food grade phosphates[a] in food processing

Miscellaneous	Indirect food contact	Processing aids
Cosmetics (Na)	Adhesives and coatings (Na, PWA)	Carcass washes (Na)
Dentifrice (Na, Ca, K)	Sanitizers (Na, PWA)	Denuding hogs (Na)
Mouthwash (Na)	Food contact polymers (Na)	Poultry scald water (Na, K)
Anticoagulant (Na)	Packaging materials (Na, Ca)	Rendering of lard (Na, Ca)
Bowel evacuant (Na)	Scale removal in dairy and beer facilities (PWA)	Oil degumming (PWA)
Petfood (Na, PWA)	Plastic wrap extrusion (Na)	Nonbattered French fry processing (Na)
Petfood supplement (Ca)	Antiwear agent in lubricants (Ca)	Mechanical peeling of Pacific Northwest shrimp [(*Pandalas jordani*), (Na)]
Animal feed (Na, PWA, Ca)	Lubricating/can manufacturing (PWA)	Egg cleaning (Na)
Yeast and bacterial nutrient (PWA, K, A)	Styrofoam polymerization (Ca)	Hydration limiting agent for chewing gum (Ca)
Metal finishing (Na, PWA)		Cane sugar refining (PWA)
Photocopier toner (Ca)		
Tobacco products to control burn (A)		

Modification of Ref. [6]

[a]*Abbreviations*: *Na* sodium phosphates, *PWA* purified wet acid, *Ca* calcium phosphates, *K* potassium phosphates, *A* ammonium phosphates

are also physical methods employed to modify starch that do not require the use of chemicals.

Conclusions

Phosphates are used in food processing for a variety of chemical and biochemical functions in order to produce a consistent product that meets consumers' desires and expectations. Phosphate acidulants used in chemical leavening are responsible for the rise and texture of cakes, cookies, muffins crackers, pancakes, and waffles. The condensed phosphates in addition to NaCl are essential in the extraction of myofibrillar protein to bind pieces of meat in the preparation of delicatessen meat and poultry products; the emulsion development of frankfurters and bolognas; to compensate for the oxidative impact of NaCl; and the inhibition of warmed over flavor in cooked meats. DSP and TSP are essential in the manufacture of process cheese with an even melt while the SALP enhances the sliceability of process cheese. Beverages benefit from phosphates due to protein dispersion, suspension of flavor, nutrient content, and flavor. Potato strips retain a desirable light color due to the brief residence time in a flume containing SAPP that sequesters iron. Many foods have highly desirable characteristics attributed to the presence of phosphates and not all could be covered in this brief chapter. Foods would be less expensive without the use of phosphates, however, in many cases, consumers might be dissatisfied with their quality. Phosphate intake cannot be based upon production data because there are many uses of food-grade phosphates that are not consumed by humans, are indirect food contact substances, or are processing aids.

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Effects of Natural and Added Phosphorus Compounds in Foods in Health and Disease

7

Eiji Takeda, Hironori Yamamoto, and Yutaka Taketani

Key Points

- Inorganic phosphorus in processed food contributes significantly to the phosphorus burden of individuals eating highly processed diets.
- A higher occurrence of vascular calcification and secondary hyperparathyroidism are two of the most commonly hypothesized consequences of phosphorus toxicity.
- Available nutrient databases may not adequately reflect the extra phosphorus content as a result of the dietary additives.

Introduction

In recent dietary survey data, phosphorus intake has been increasing, while calcium intake has been decreasing [1]. High protein foods, like meat, milk, eggs, and cereals are generally high in phosphorus and contribute the largest amounts to the total dietary phosphorus intake. Furthermore, the amount of phosphorus from phosphorus-containing additives in convenience and fast foods has considerably increased [1]. These patterns of food intake are suggested to interfere with the normal calcium, phosphorus, and vitamin D homeostasis and to affect bone, kidney, cardiovascular functions, and quality of live (QOL) [1–3].

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Phosphorus in Food

Phosphorus in Natural Foods

Natural sources of phosphorus are associated with the protein content of the foods and are often referred to as organic phosphorus (Table 7.1). Approximately 20–30 % of the dietary phosphorus for most adults come from milk and milk products and another 20–30 % from meat, poultry, fish, the grain products, and legumes [1]. The relationship between high protein foods and natural phosphorus content has been largely consistent with a mean total intake of 15–17 mg of phosphorus for every gram of protein [4, 5].

Phosphorus in meat is well absorbed since it is found mostly as intracellular organic compounds, which are easily hydrolyzed in the gastrointestinal tract, releasing inorganic phosphorus for absorption. In milk, about one-third of the total phosphorus is inorganic phosphorus. All the phosphorus in casein (20 % of total) is of lower availability. Phosphorus in plants, especially in beans, peas, cereals, and nuts, is mostly in the form of phytic acid or phytate [6, 7]. Phytate phosphorus is not available in humans because the small intestine of animals and humans does not secrete the enzyme phytase for releasing phosphorus from phytate. Many fruits and vegetables contain only small amounts of phosphorus.

Added Phosphorus in Processed Foods

Phosphates are currently being added to a large number of processed foods including meats, cheeses, dressings, beverages, bakery products, restructured meats (chicken nuggets etc), processed and spreadable cheeses, instant products (puddings and sauces), refrigerated bakery products, and beverages [8]. The phosphorus and protein content of 44 foods, including 30 refrigerated or frozen precooked meat, poultry, and fish items were measured and it was found that the ratio of phosphorus to protein ranged from 6.1 to 21.5 mg/g [9]. The mean ratio of phosphorus to protein was 14.6 mg/g in 19 food products that were labeled as having phosphorus as an additive as compared with 9.0 mg/g in the 11 items that did not list phosphorus additives. The quantity of phosphorus in a 50-g portion of cheese varies from 100 mg in Brie cheese to almost half a gram in processed soft cheese, which contains a significant amount of phosphorus salt [10, 11]. The presence of inorganic phosphate is often obscured by the use of complex names for the ingredients [12].

Functions of Added Phosphorus in Processed Food

Phosphorus additives play an especially important role in the meat industry, where they are used as preservatives. Since phosphate salt retains moisture and tenderness of beef, pork, lamb, and chicken during cooking, there has been a significant increase in the use and consumption of phosphorus-containing food additives,

Table 7.1 Energy, protein, and phosphorus contents of a variety of foods

Food item (100 g)	Energy (kcal)	Protein (g)	Phosphorus (mg)
Cereal			
White rice	356	6.1	94
Brown rice	350	6.8	290
Barley	340	6.2	110
White bread	264	9.3	83
Croissant	448	7.9	67
Wheat noodles, dehydrated	348	8.5	70
Macaroni/spaghetti, dehydrated	378	13.0	130
Chinese noodle	281	8.6	60
Buckwheat noodle	274	9.8	170
Potato and starch			
Potato	76	1.6	40
Sweet potato	132	1.2	46
Legumes			
Soybean, domestic, dehydrated	417	35.3	580
Firm tofu	72	6.6	110
Itohiki natto	200	16.5	190
Nuts and seeds			
Almond	598	18.6	500
Peanut, parched	585	26.5	390
Vegetables			
Green pea	93	6.9	120
Japanese pumpkin	49	1.6	42
Cauliflower	27	3.0	68
Cabbage	23	1.3	27
Red leaf lettuce	16	1.2	31
Broad beans	108	10.9	220
Onion	37	1.0	33
Corn	92	3.6	100
Tomato	19	0.7	26
Red paprika	30	1.0	22
Spinach	20	2.2	47
Lettuce	12	0.6	22
Fruits			
Strawberry	34	0.9	31
Valencia orange	39	1.0	24
Grape fruit	38	0.9	17
Pineapple	51	0.6	9
Banana	86	1.1	27
Grape	59	0.4	15

(continued)

Table 7.1 (continued)

Food item (100 g)	Energy (kcal)	Protein (g)	Phosphorus (mg)
Mango	64	0.6	12
Apple	54	0.2	10
Seafoods			
Bonito	114	25.8	280
Chum salmon	133	22.3	240
Bluefin tuna	125	26.4	270
Roasted kamaboko	103	16.2	60
Roasted chikuwa	121	12.2	110
Fish sausage	161	11.5	200
Squid	88	18.1	250
Dried cuttlefish	334	69.2	1100
Oyster shell, cultured	60	6.6	100
Meat			
Domestic beef, lean tissue of shoulder	143	19.9	190
Beef liver	132	19.6	330
Chicken tenderloin	105	23.0	220
Pork, lean tissue of shoulder, raw	125	20.9	200
Vienna sausage	321	13.2	190
Pressed harm	118	15.4	260
Loin harm	196	16.5	340
Eggs			
Chicken egg	151	12.3	180
Milk products			
Milk	67	3.3	93
Yogurt	67	4.3	100
Processed cheese	339	22.7	730
Camembert cheese	310	19.1	330
Cottage cheese	105	13.3	130
Parmesan cheese	475	44.0	850
Ice cream, regular fat	180	3.9	120
Confectionery			
Shortcake	344	7.4	120
Cake doughnut	375	7.0	100
Custard pudding	126	5.5	110
Potato chips	554	4.7	100
Milk chocolate	557	7.4	240
Beverage			
Sake (rice wine)	104	0.3	7
Beer, solid color	40	0.3	15
Red wine	73	0.2	13

Table 7.1 (continued)

Food item (100 g)	Energy (kcal)	Protein (g)	Phosphorus (mg)
Coffee, liquid	4	0.2	7
Carbonated drink, cola	46	0.1	11
Condiment			
Tomato ketchup	119	1.7	36
Thousand island dressing	416	1.0	30

especially in high protein sources such as chicken and beef [1, 12–15]. They are also used as a component of melting salts in the production of soft cheese. Phosphates loosen the structure of protein, enabling it to bind more water. Phosphorus additives serve many desirable functions enabling food products to achieve better texture, taste, emulsification, acidification, leavening, acid–base reaction to produce gas, anticaking, moisture binding, antimicrobial action in washes, color stability, iron binding, reduction of cooking time, buffering, maintenance of firmness in freezing and canning, and nutrient fortification [16].

The Amount of Daily Phosphorus Intake and Its Metabolism

The Amount of Daily Phosphorus Intake

The recommended dietary allowance for phosphorus is 700 mg/day in healthy adults and an allowance of up to 1,250 mg/day has been suggested in older children and pregnant women in the United States [17]. The mean daily intake of phosphorus for the typical American diet varies with age and gender and more than half of the young and middle-aged men consume 1,600 mg phosphorus⁄day or more and comparably aged women consume almost 1,200 mg⁄day. More than 50 % of young and middle-aged men consume more than 1,600 mg/day, while comparably aged women consume about 1,000 mg/day [1, 5].

These estimates of dietary phosphorus intake, however, largely reflect the "natural" phosphorus content of foods. When the phosphorus amounts were compared, in only those daily menus with six or more processed foods, the underestimation of phosphorus content deviated by more than 350 mg/day. Similar findings have been reported from Spain [18] and Brazil [19]. These results underscore the difficulty in accurately estimating or predicting phosphorus intake because the phosphorus contribution from food additives cannot be reliably quantified for individuals from food records or dietary recall. Depending on individual food choices, phosphorus intake could be increased by as much as 1,000 mg/day [1, 13, 15, 20, 21]. The major public health implication from these considerations is that the phosphorus burden from inorganic phosphorus–containing food additives is disproportionately high relative to organic phosphorus.

Different Metabolism of Phosphorus in Food

As an essential biologic element, phosphorus is required by all cells for normal function and is a critical component of all living organisms [22]. Dietary phosphorus is efficiently absorbed (60–70 %) throughout the small intestine by both active and passive mechanisms and is linearly related to phosphorus intake over a wide intake range, 4–30 mg/kg/day [5, 23]. Metabolic consequences of phosphorus intake may differ according to the phosphorus source [24, 25]. This may be because of the variation in bioavailability or absorbability of phosphorus. Organic phosphate esters are found mainly in protein-rich foods such as dairy products, fish, meat, sausages, and eggs. They are slowly hydrolyzed in the gastrointestinal tract and then slowly absorbed from the intestine. The bioavailability of vegetable phosphate esters is usually less than 50 % [26, 27] and thus much lower than that of the phosphate esters in protein-rich foods. Inorganic phosphorus additives are almost 100 % absorbed [20] and seem to affect parathyroid hormone (PTH) secretion more strongly than natural organic phosphorus [24, 25].

On phosphorus loading, there is a prompt response of PTH, causing very early phosphaturia. However, within a time frame of 8–16 h on continued high phosphate intake, fibroblast growth factor 23 (FGF23) increases and takes over the phosphaturic effects of PTH. Higher FGF23 levels are associated with phosphaturia and a decline in $1,25(OH)_2D$ levels. Low Klotho activity is associated with a high serum phosphate concentration in CKD [28, 29]. Phosphate causes lasting damage to the cardiovascular system, either by a direct mechanism or by way of these hormonal factors, and accelerates aging processes in animal models [3].

Effects of Phosphorus on Health and Disease

Phosphorus in CKD

It was first recognized in patients with renal disease that a high serum phosphate concentration is a major risk factor for elevated cardiovascular and overall mortality [30, 31]. In a cohort study of 40,538 hemodialysis patients, 12 % of the 10,015 deaths occurring over the period of observation were associated with hyperphosphatemia [30]. Moreover, evidence exists that calcium–phosphate product and serum phosphorus are related to mortality rate in dialysis patients [32]. The study that used a food frequency questionnaire showed that higher dietary phosphorus intake or phosphorus-to-protein ratio was associated with increased 5-year death risk in 224 prevalent hemodialysis patients [33]. A number of epidemiologic studies have shown an association between high serum phosphorus levels and increased death risk in both dialysis-dependent patients with ESRD [34, 35] and individuals with less advanced stages of CKD [36]. Hyperphosphatemia in these latter patients also seems to be associated with a faster rate of CKD progression [37]. In addition, cardiovascular diseases are highly prevalent and severe in CKD. They represent the main cause of morbidity and mortality, which is an average 30-fold higher than in

the general population, particularly in young adult patients [38]. However, the hemodialysis patients whose protein intake rose while their serum phosphorus declined over time showed the greatest survival [39].

Phosphorus in General Population

A controlled trial of young women found no adverse effects of a phosphorus-rich diet of up to 3,000 mg/day on bone-related hormones and biochemical markers of bone resorption as long as dietary calcium intakes were maintained at almost 2,000 mg/day [40]. In contrast, high intake of phosphorus has been shown to increase serum PTH secretion and bone resorption and to decrease bone formation in healthy subjects [41]. Nationally representative and smaller longitudinal epidemiological studies suggest that even small increases in serum phosphorus within the normal range are significantly linked to cardiovascular disease risk even in healthy subjects without evidence of kidney disease [41–46]. Thus, growing evidence now suggests that phosphorus intakes in excess of the nutrient needs of healthy men and women may significantly disrupt the hormonal regulation of phosphorus, calcium, and vitamin D, contributing to impaired kidney function, disordered mineral metabolism, vascular calcification, and bone loss [3, 4, 42, 47]. On the other hand, longitudinal population studies indicate that dairy phosphorus differs in physiologic effects from inorganic phosphates when blood pressure was the main endpoint measured [48, 49].

Protective Management Against Excess Phosphorus Intake for Prevention and Treatment of Diseases

Restriction of Phosphorus Intake in CKD

Serum 1.25 (OH)2D levels can be increased by dietary phosphorus restriction in the early stages of renal failure. When the residual renal function is very poor, serum 1.25 (OH)2D levels cannot be modulated by dietary management [50, 51]. A significant reduction of serum PTH levels can be achieved even in severe chronic renal failure by dietary phosphorus restriction and calcium supplementation [50, 52]. A study compared meat and vegetarian diets in a group of eight CKD patients using a crossover experimental design with meat and vegetarian diets that contained the same concentrations of protein and phosphorus [53]. The results showed that consuming the vegetarian diet over a week resulted in significantly lower serum phosphorus levels compared with the week of consuming the meat diet. Boiling vegetables reduces the amount of minerals including phosphorus—a mean of 21–27 % of phosphorus can be removed from vegetable as well as from animal foods.

In patients with mildly to moderately impaired renal function, the multivariate adjusted risk of hyperphosphatemia (defined as a serum phosphate concentration above 1.45 mmol/L) was higher in persons of the lowest income class than in persons of the highest income class [54]. Inexpensive foods containing additives

(processed food) are consumed in greater amounts by the poor population. Phosphorus-containing additives may also have an impact on patients at earlier stages of CKD. Hyperphosphatemia may also contribute to cardiovascular and bone disease among patients with moderate CKD [55, 56]. In addition to the source of phosphate in the diet, the amount of phosphate consumed by patients with advanced renal failure should not exceed 1,000 mg per day, according to the guidelines.

Awareness of Phosphorus in Foods for Human Health

In healthy individuals, high dietary phosphorus intake has been linked to the risk of poorer bone status [2] and cardiovascular [26, 36, 44] health. Recent European studies suggest the phosphorus intake exceeds dietary recommendations by two to three fold [57–60]. In addition, the studies on meat and poultry indicate that products with phosphorus additives have a substantially higher phosphorus content than products prepared without them [9, 15].

Conclusions

It is possible that interventions aimed at dietary phosphorus restriction may improve cardiovascular profile and survival even in individuals with high-normal or borderline elevated serum phosphorus levels. Comprehensive public education with a scientifically well-grounded explanation of the adverse effects of high phosphate intake along with easily understandable labeling of the phosphate content of food is important to minimize the cardiovascular risk. Relevant information is absolutely required to raise public awareness of the health risks of phosphorus.

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Phosphorus Utilization in Animal Agriculture

8

Ermias Kebreab and April B. Leytem

Key Points

- Phosphorus is an essential mineral for livestock production and is considered to be a macromineral for crop production.
- Monogastric animals including humans have much lower efficiency of P utilization, requiring supplementation of feed with inorganic P sources, resulting in high P excretion to the environment.
- Phosphorus comes from finite nonrenewable sources and so measures will need to be taken to limit phosphorus waste in livestock and crop uses.

Introduction

Phosphorus (P) is an essential macromineral for all animals, including humans. Phosphorus is necessary for several body functions, fundamental to maintenance and repair of all body tissues, and is indispensable, along with calcium (Ca), for proper growth and mineralization of osteoid tissue and muscle development [1]. Phosphorus is a nonrenewable resource and 90 % of the demand for P is for food production [2]. Livestock diets are routinely supplemented with inorganic P sources and agriculture is estimated to use 148 million t of rock phosphate every year [2]. At this rate, it is estimated that the global commercial phosphate reserve will be

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depleted in 50–100 years [3]. This has a geopolitical implication as most of the rock phosphates left are mined in China, the United States, and Morocco [4]. Therefore, it is essential that P use in livestock and crop production be optimized.

One of the reasons for supplementation of P in livestock feed is because availability of P from plant based feed is low and variable. The principal form of P storage in plant seeds is phytic acid or phytate [5]. Phytate is not digestible by humans or monogastric animals such as swine and poultry; therefore, supplementation is often required. This decreases the overall P utilization of monogastric feeds and leads to high concentrations of P being excreted in manure. Although ruminant animals can digest phytate P in feed, the average P use efficiency in ruminants is only approximately 40 % [6], thereby leading to large amounts of P being excreted from these animals as well. This inefficient use of P by livestock makes their manure valuable as a fertilizer source for raising crops; however, globally there are large imbalances in P supply and demand.

In some regions, such as in Europe and North America, there is an oversupply of manure P and high P fertilizer use which has led to a buildup of P in soils which can cause environmental pollution such as eutrophication of water bodies [7]. Alternatively, a lack of manure and fertilizer P in regions such as Sub-Saharan Africa has led to a drawdown of soil P reserves and left these areas in a P-deficient state hindering their ability to produce much needed food [8]. Therefore, both efficiencies in P utilization in food production along with better redistribution of P on a global scale are needed to meet the growing demand for food as populations increase.

Phosphorus Utilization in Livestock

The efficiency of utilization of P in livestock depends on several factors. Bioavailability of P, efficiency of feed conversion, and the amount of P consumed in excess of the animal's requirement influence efficiency of utilization [9].

Bioavailability of Phosphorus Used in Livestock Production

Vegetable feedstuffs, inorganic supplements, and bone, meat, poultry, and fishmeals are the major sources of P for animals. Plants used as animal feed have variable P contents, for example, over 65 % of the P in cereal grains is in the form of phytate (*myo*-inositol hexakisphosphate), which is poorly available to humans and monogastric animals [10]. Ruminants, however, can readily digest phytate because they contain microorganisms in their gut flora that produce an enzyme called phytase. Phytase belongs to a class of phosphatases that enables dephosphorylation of phytate in the digestive tract of the animal or in the feed before ingestion [11]. Phytase naturally originates from plants, gut microbes, and intestinal mucosa, but the activity of the latter two is minimal in monogastric animals. Use of phytase in improving P utilization efficiency is discussed later.

Inorganic P sources such as dicalcium phosphate have high P availability (normally above 90 %) and can easily be absorbed in the small intestine of humans and livestock, which is the main reason for their use as supplements. Animal based feedstuffs (meat and bone meal) also have availabilities ranging from 70 to 94 % [10].

Efficiency of Feed Conversion

Livestock utilize P inefficiently, excreting 60–80 % of that consumed. Therefore, the majority of P brought on to the farm in feed stays on the farm, rather than being exported in meat or milk. Efficiency of utilization of P highly depends on the form in which P is consumed by livestock. As mentioned in the previous section, organically bound phytate P is not available to monogastric animals; therefore, the efficiency of utilization is low. However in ruminants up to 85 % of phytate P is dephosphorelated by microbial phytase; therefore, it is available for absorption and utilization by the animal. Some studies show that available or digestible P can be absorbed from the small intestine at rates greater than 90 % [12]. Even in humans with particularly low P intake, as in some developing countries, higher amounts of phytate P can contribute to mineral deficiency due to the binding of minerals with phytate making them unavailable for absorption. Therefore, one of the greatest limitations to achieving higher efficiency of P utilization in some livestock and humans is the release of phytate P from the feed matrix. For example, in laying hens, utilization of P is closely linked to Ca through a complex mechanism to maintain homeostasis in various nutritional conditions aimed at a proper distribution of available Ca over several metabolic processes. An inadequate supply of Ca or P may lead to excessive withdrawal of Ca phosphate from the medullary bones and could result in excessive excretion of either Ca or P in manure. Kebreab et al. [13] developed a dynamic and mechanistic model of P metabolism in layers and evaluated the effect of Ca supply on P utilization. The authors reported that P retention in bone and egg increased from 8.4 to 25.4 % of absorbable P intake at the lowest and highest concentration of Ca inclusion, respectively.

Improving P Utilization by Livestock

Kebreab et al. [6] reviewed various options currently available to improve the efficiency of animals to utilize P in its various forms. In all species of livestock, P fed in excess of animal requirements is excreted, making reduced overfeeding a powerful tool to improve total P utilization efficiency and reduce the P content of manure. Kebreab et al. [14] found that in Ontario, Canada, farmers were using 30 % more P than nutritionally required and if they reduced the P content to recommended levels, farmers would save $20/cow per year. Moreover, excretion of P would be reduced by 1.3 kt/year. In grazing systems, reduction in grazing intensity has frequently been recommended to meet biodiversity and production goals for a more sustainable system by reducing N and P excretions [15]. The requirement for P in livestock

Table 8.1 Mitigation options to improve P utilization in livestock diet

Mitigation	Increase in available *P*, %	References
Phytase	2.0–205.0	[38]
Low-phytate plant	38.4–41.3	[19]
High-phytase plant	18.2–163.2	[39]
Transgenic animal	81.2–90.4	[20]
Liquid feeding	18.4–34.0	[40]

changes according to their physiological status; therefore, phase feeding or adjusting the P content for different stages of growth has been a successful way of improving P utilization and reducing P excretion.

Some of the mitigation options available to increase availability of P in monogastric animals are summarized in Table 8.1. Microbial phytase can be added to diets to increase availability of phytate bound P. The most common microbial phytase used in swine and poultry diets are from fungi (*Aspergillus niger* and *Peniophoralycii*) and bacteria (*Eschericha coli*) [16]. The activity of phytase depends mainly on pH, temperature, dose, and diet composition [17]. There is a wide variation in the effect of phytase added even at the same phytase dose and type of diet [18], contributing to its unreliability in diet formulation (Table 8.1). This unreliability leads producers and feed manufacturers to add "safety margin" and supplement feed with high amount of inorganic P sources. It is expected that the continued development of phytase through improved understanding of its ability to breakdown organic P will produce more effective classes of phytases leading to a more reliable effect and reduction of supplementation with inorganic P. Liquid feeding, particularly in young animals, has shown to improve P availability by up to 24 % (Table 8.1).

Biotechnology has been used to develop transgenic plants [19] and animals [20] to increase utilization and minimize excretion of P. Transgenic plants improve P utilization either through lower phytate P (such as low phytate corn or soybeans), which has higher available P or higher phytase content plants that release more phytate P when consumed. A transgenic pig producing salivary phytase (*E. coli* phytase) was developed in Canada but its use has not yet been approved by Health Canada or US Food and Drug Administration. In the near future, nutritional genomics is expected to play a role in establishing better nutrient requirement estimates, leading to higher efficiency and lower P excretion to the environment [6].

Phosphorus Utilization in Crop Production

The two main inputs of P in crop production are manure (including green manures) and fertilizer P, which is primarily derived from rock phosphate. However, only 15–30 % of applied P fertilizer is actually taken up by harvested crops [21]. Phosphorus is a highly reactive compound in soils and is easily bound by soil particles and readily precipitates with aluminum, iron, and Ca making it unavailable for plant uptake. Over time, P applied as fertilizer and manure will naturally decrease in bioavailability making it necessary to replenish soil P reserves regularly to meet crop uptake demands [22]. Globally, P deficiency is considered to be one of the

major limitations for crop production particularly in low-input agricultural systems [23]. However, in many developed countries, overapplication of manure and fertilizer P has become a significant source of water pollution. The effectiveness of P fertilizers (both inorganic and organic) will depend on the quantity applied and their placement relative to plant root development [24].

Fertilizer P Utilization

Most phosphate fertilizers have been manufactured by treating rock phosphate (the phosphate-bearing mineral that is mined) with acid to make it more soluble. Much of this P is blended to make fertilizers containing nitrogen and potassium the other plant macronutrients; however in some instances rock phosphate is utilized as a fertilizer. Initially this fertilizer P is quite soluble and available for plant uptake. Gradually reactions occur making the easily dissolved compounds of phosphate more insoluble and the phosphate becomes fixed and unavailable. Residual fertilizer P continues to be available for plant uptake for many years, but freshly applied P is generally most soluble and available for plant uptake. The common practice of building soil P concentrations to appropriate agronomic ranges provides a long-term source of this nutrient to crops.

Fertilizer P sources such as ammonium phosphates, superphosphates, and nitric phosphate are equivalent P sources for optimizing crop yields. In an effort to reduce the amount of soluble P in soils and thereby reducing the potential for offsite losses of P, there has been development of slow release P fertilizers. Leytem and Westerman [25] demonstrated that a polymer coated slow release P source had a very low apparent solubility, but was able to support high levels of plant P accumulation. The coating reduced the dissolution and reaction of the fertilizer P with the soil enabling enhanced solubility and a reduction in potential for losses of P in runoff. These types of fertilizer may enhance P utilization by releasing slow amounts of soluble P over time that can then be better utilized by the growing crop.

Manure P Utilization

Manures have been utilized as a P source for centuries. There is a large variability in the solubility and availability of P from various materials added to soil. These large differences are due to the unique properties of the materials, rather than any unique character associated with a specific soil. The chemical composition of the P in manures determines its solubility and plant availability. As plants only absorb P in the form of phosphate, the availability of P in some manure is limited as the majority of P may be in organic forms that need to be mineralized prior to plant utilization. Additionally, land application of manure applies not only nutrients but also large amounts of organic material that can affect mineralization, mineral solubility, and plant availability. Leytem and Westermann [25] showed that liquid manures tended to be as soluble and plant available as fertilizer P, while solid manures and composts tended to have lower solubility and slightly lower plant uptake efficiencies.

The chemical composition of P in manures is heavily dependent on species and diet. Manure P is predominately inorganic phosphate, followed in descending order by phosphate monoesters (including phytate), phosphate diesters (nucleic acids and phospholipid), pyrophosphates, and in some cases phosphonates. Concentrations of phytate can range from nondetectable to 80 % of the total P in manures from a variety of ruminant (cattle and sheep) and monogastric animals (poultry and swine; [26]). The solubility of manure P is closely related to phytate concentration with increasing levels of phytate P resulting in less water soluble P [26]. It has been shown that the soil sorption capacity differs with various organic P compounds with phytate being very tightly bound by soils while other organic P compounds such as nucleotides, DNA, and glucose phosphates are more mobile in soils [27, 28]. Therefore, variability of the phosphorus composition of manures, either due to differences in species, manures handling techniques, or through dietary manipulation, could affect the solubility of P in soils and thereby affect both plant uptake and potential for offsite losses of P. However, Leytem et al. [29] demonstrated that the effects of phytate in manure on soil P solubility decreased with time on a calcareous soil and after 9 weeks manure characteristics such as the carbon to P ratio of the manure were more important in determining P solubility than the P characteristics of the manures. Microbial activity has a strong influence on soil P solubility. Stimulation of microbial biomass by added organic C is important in determining soil P solubility following manure application, with increasing concentration of carbon added decreasing soil P solubility [30].

In low input and organic cropping systems, the use of "green" manures is common. In this system, either crop residues, crops which are not harvested, or other perennial biomass is incorporated into the soil for use as a fertilizer. These are most commonly used to supply nitrogen to growing crops, but in the short term they may also have the added benefit of enhancing P availability. The main mechanisms for enhancing P availability include the cycling of P from soils into plant materials which can be more soluble/plant available as this material decomposes and the generation of acidic conditions or release of organic acids during decomposition which can dissolve soil mineral P or block P adsorption sites [31–34]. Cavigelli and Thien [35] demonstrated that soil test P increased from 3 to 5 mg kg^{-1} following incorporation of a variety of green manure crops. The use of green manures from perennials grown with crops can supply some available P; however the perennial would need to acquire a large portion of its P from less available soil P pools, rapidly produce biomass without competing with the crop for nutrients, contain high P content in the biomass, and export little P in products exported from the field [36]. These demands are not easily met. In the long term, other P fertilizers will need to be used to sustain crop production.

Enhancing P Utilization in Cropping Systems

As previously discussed, P solubility and plant availability in soils is inherently low and therefore enhancing P utilization in cropping systems is challenging. In systems where there is an excess of P, enhanced utilization can be achieved through

reductions in P application and better matching applications with plant demand. By reducing the overfertilization of crops via manure and fertilizer applications you not only enhance P utilization by default but also reduce the risk of off-site P losses which can negatively impact water quality. Alternatively in P limited systems, the use of specialized fertilizers that release P slowly over the growing season may in some cases allow lower P fertilizer application rates without sacrificing crop production. Genetically modified plants developed through a combination of genetic selection, breeding for nutrient efficient plants, and biotechnological approaches will have the potential to use applied P efficiently and perform better under nutrient-limiting conditions [23, 37].

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Technical Aspects About Measuring Phosphorus in Food

9

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Key Points

- The ability to measure phosphorus in foods is key to understanding the benefits and harm from dietary phosphorus in the food supply.
- Several methods had been proposed for the measurement of phosphorus levels in food, and choosing the best method depends on the analytical target of interest.

Introduction

Phosphorus is an essential nutrient present in food from either vegetable or animal sources, which could be part of the inorganic salts or bound to organic compounds [1]. Organic phosphates are present in foods as phosphoproteins and phospholipids, phytate or starch phosphate monoester in vegetables, as well as several enzymes involved in many different metabolic pathways, namely phosphocreatine, nucleotides, nucleoproteins, and nucleic acids. In addition to the above mentioned compounds, which are naturally present in foods, phosphorus can be added as preservative [2]. These additives can be either organic (like lecithin) or inorganic (like polyphosphate) compounds.

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Phosphorus Determination in Food

Several methods had been proposed for the measurement of phosphorus levels in food. Some of them are suitable for the quantification of total phosphorus, while some others should be used for the determination of the concentration of just a single subtype of phosphorus. As a consequence, the analytical methods in the literature could be divided according to their analytical target.

Total Phosphorus

UV–Vis spectrophotometry probably is the most popular technique for the quantification of total phosphorus in food. Phosphorus in a sample is converted to inorganic phosphate, as orthophosphates, by dry ashing or acidic digestion [3, 4]. Then, phosphate anions are detected using the reaction between orthophosphoric acid and molybdate ion in a strongly acidic medium, in the absence or in the presence of vanadate or antimonite. Heteropoly acids or molybdophosphoric acids, such as phosphomolybdenum yellow or molybdenum yellow and its reduction product (the so-called phosphomolybdenum blue or molybdenum blue), can be formed. In the presence of some reducing agents, molybdenum yellow is reduced to form molybdenum blue, which shows stronger light-absorption than the molybdenum yellow and the maximum absorption wavelengths are at longer wavelengths, around 650–850 nm [5, 6]. However, this method is suitable only for an assay of orthophosphate ions in aqueous solutions [3].

More advanced techniques like atomic absorption spectroscopy (AAS), inductively coupled plasma-optical emission spectroscopy (ICP-OES), or inductively coupled plasma-mass spectrometry (ICP-MS) can be used for the determination of total phosphorus in food [7]. Both ICP-OES and ICP-MS are able to provide the simultaneous quantification of phosphorus and other elements in the matrix with high accuracy and sensitivity, although the latter is much more sensitive and could be coupled to selective chromatographic separation techniques, such as high performance liquid chromatography (HPLC), providing information on different chemical form of phosphorus in the same run. Ion chromatography (IC) has been commonly used for the separation and determination of phosphate species. Nowadays, electromigration methods, such as capillary electrophoresis CE and capillary isotachophoresis CITP, have been tested as complementary methods to IC in the analysis of phosphates [3]. Recently, 31P-NMR analysis has been applied for phosphorus determination in soil, water, and other environmental samples, including food products [8, 9].

Phosphorus from Phospholipids

The classical method for measuring total phospholipid content is by direct determination of phosphorus in the polar lipid extract. First, the organic phosphorus is

converted to inorganic phosphate by dry ashing or acid digestion, afterwards it is further converted to colored complex. By means of spectrophotometer, phosphate standard solutions, and proper phosphorus/phospholipid conversion factor, the phospholipid concentrations can be determined [10, 11]. More advanced techniques like atomic absorption spectroscopy (AAS), inductively coupled plasma-optical emission spectroscopy (ICP-OES), inductively coupled plasma-mass spectrometry (ICP–MS), and Fourier-transformed infrared spectroscopy can be used for the determination of total phospholipid's phosphorus in oil or food extracts. The methods for the total phospholipid determination do not give any information about individual phospholipids. Nowadays, for the analysis of polar lipids, numerous high-performance liquid chromatography methods have been published for the separation of lecithin samples into a single polar lipid class [12, 13]. Recently, 31P-NMR is gaining popularity for absolute quantification of phospholipids in food matrices [8].

Phosphorus from Phosphoprotein

Phosphorus derived from phosphoproteins is measured in precipitate from filtrate of homogenized sample in hot water. After dry ashing, the classic spectrophotometric or capillary isotachophoresis CITP were tested, and phosphorus derived from phosphoproteins was estimated in raw meat from different species [14].

Inorganic Phosphorus: Orthophosphate and Polyphosphate

Phosphate ions, both natural and added inorganic phosphorus like phosphate salts or polyphosphates, are quantified by direct determination in water extract of sample. Ion chromatography (IC), electromigration methods, such as capillary electrophoresis CE and capillary isotachophoresis CITP, have been commonly used for the separation and determination of phosphate species [15]. More recently, 31P-NMR analysis has been used for the determination of added inorganic phosphate in pork meat [16]. However, polyphosphate hydrolysis occurs during shelf life of meat products, which converts polyphosphates to orthophosphate, and the protein-rich food, such as meat, contains natural phosphorus compounds (nucleotides, phospholipids, etc) along with naturally occurring orthophosphate. This is the reason why the qualification of amount of added phosphate is difficult [17]. In fact, all the amount of inorganic phosphate, including that from additives, is determined by the difference between the amount of total phosphate and phosphate bound to protein and phospholipids.

Phytate

Phytates are found mainly in cereals and legumes, where they are concentrated in the seeds and fibrous parts. Actually, phytic acid is the primary storage compound

of phosphorus in seeds accounting for up to 80 % of the total seed phosphorus and contributing as much as 1.5 % to the seed dry weight. In humans, the enzyme phytase is not expressed; therefore phytate degradation occurs only partially by the intestinal bacterial flora or to some extent by nonenzymatic hydrolysis reaction [18]. This results in lower bioavailability of dietary phosphorus of plant origin, which remains below 50 % (usually 30–40 %).

Different analytical methods such as ion-exchange chromatography, complexometric titration, and 31P-NMR technique have been developed to determine the amount of phytates in food [19]. It has to be pointed out that NMR technique allows both qualitative and quantitative analysis and, unlike other techniques such as HPLC, offers the possibility to detect and quantify all the phosphate compounds in the same experiment, without the need for any standards. On the other hand, several factors involved in the sample preparation process could be sources of bias influencing the recoveries of phosphors. In addition, due to the relatively poor sensitivity of NMR, extensive sample enrichment could be necessary prior to the analysis.

Phosphorus in Meat Products: Analytical Method and Results

From a practical point of view, total and different subtypes of phosphorus (inorganic P, P from phospholipids, and P from phosphoproteins) can be quantified by the same detection method, i.e., molybdenum blue detection on wet ashing samples, preceded by a proper sample preparation procedure, specific for each subtype of phosphorus. This approach provides a complete overview of the different type of phosphorus content, making use of conventional instrumentations present in a routine analytical laboratory and of a limited number of reagents and solvents. We report here a synthetic description of a recently published analytical method possessing these features [20]. The sample preparation procedures for the different kind of phosphorus can be summarized as follows:

- *Sample preparation for total phosphorus* (*TP*): the sample (approximately 0.4 g fresh mass, fm) is wet mineralized with 96 % sulfuric acid and 30 % hydrogen peroxide, under heating conditions (up to 700 K). The cool digest is then diluted with bi-distilled water and submitted to the P quantification procedure.
- *Sample preparation for inorganic phosphorus* (*IP*): the sample (5 g fm) is extracted with 1 mM NaOH, using an orbital shaker, and the extract is centrifuged at 4800 rcf. The supernatant is filtered under vacuum and the filtrate is diluted with bi-distilled water. Afterward, 96 % sulfuric acid and 30 % H_2O_2 are added to an aliquot of the sample solution and the obtained mixture is submitted to digestion under heating until it is clear. The cool digest is diluted once again and used for the final measurement of phosphorus by using the phosphorus quantification procedure.
- *Sample preparation for phosphorus from phospholipids* (*PL*): Phosphorus derived from phospholipid should be quantified in the polar lipid extract of samples, which is prepared according to the Folch method [21]. In practice, the

sample (2 g fm) is added with a chloroform/methanol mixture (2:1, v/v) and immediately homogenized by a stainless steel rotating knife homogenizer. The so obtained homogenate is centrifuged at 2000 rcf and then the supernatant is collected and filtered. After water addition, a liquid to liquid extraction is carried out. The organic layer containing the analytes of interest is treated with anhydrous sodium sulfate, submitted to filtration in order to remove the solid matter, evaporated under vacuum to dryness, and dried in a laboratory oven. Cool lipid extract content is then weighed, and half of it (from 40 to 80 mg) is then added with 96 % sulfuric acid and 30 % H_2O_2, and treated as described for IP.

- *Sample preparation for phosphorus from phosphoprotein* (*PP*): the sample (1.5 g fm) is added with bi-distilled water and homogenized by a stainless steel rotating knife homogenizer. The obtained mixture is boiled on a hot plate, cooled, and treated with 100 g/L trichloroacetic acid solution. After filtration of the precipitate, the filter paper containing the solid matter is dried in an oven (373 K), the solid stuff weighted, and about 50 % of it (equivalent to 0.75 g fm) is digested by the same procedure described for IP.
- *Phosphorus quantification procedure*: orthophosphate ions in the wet mineralized sample solution from TP, IP, PP, and PL are detected by the molybdenum blue method. In practice, the mineralized sample solutions is added with water, a 4 M sulfuric acid solution, a 18 g/L ammonium molybdate solution, and a 25 g/L ascorbic acid solution. The flask containing the mixture is gently swirled and then placed in boiling water for the formation of characteristic molybdenum blue species. After having cooled the solution at room temperature and having diluted it properly with bi-distilled water, the absorbance is measured by spectrophotometry at 650 nm against a blank and the quantification is carried out by a suitable calibration curve.

The above described analytical method was used to measure TP, as well as IP, PP, and PL in cooked ham samples. Samples from 24 different brands provided the following results, expressed as average phosphorus content with standard deviation:

TP: 251 ± 59 mg P/100 g sample
IP: 154 ± 50 mg P/100 g sample
PP: 39 ± 14 mg P/100 g sample
PL: 37 ± 16 mg P/100 g sample

These results basically are in agreement with data from the literature. In order to investigate the influence of additives and preservatives on phosphorus concentration levels, the 24 sample were divided in three different groups that were characterized by a different use of phosphorus-containing ingredients and additives during the production processes. They were classified according to the ingredient declared on the package labeling, as follows: “regular”, when phosphorus-containing ingredients and additives were not declared, “enhanced with phosphorus-containing additives” (EWP), when the words “containing polyphosphates” or the coding “E338–E341, E450–452” were present in the ingredient list, “enhanced with

Table 9.1 Results of phosphorus analysis into three different groups of cooked ham (mean ± standard deviation)

Group	TP	IP	PP	PL
(mg P/100 g sample)				
Regular	191 ± 10	103 ± 12	35 ± 8	36 ± 13
EWP	279 ± 60	199 ± 50	27 ± 4	25 ± 14
EWPIA	282 ± 40	159 ± 18	55 ± 7	48 ± 11

Table 9.2 Results of phosphorus determination in certified reference materials

Parameter	TP	PP	PL
Reference sample av. (mg P/100 g sample)	870	131	433
uncertainty (mg P/100 g sample)	50	2	4
(w ± μ)[a] (mg P/100 g sample)	876 ± 14	128 ± 2	404 ± 6
Standard error of mean	5.1	0.7	2.1
Coefficient of variation (%)	1.3	1.2	1.2
Recovery (%)	100.7 ± 1.3	97.5 ± 0.1	93.1 ± 1.1
LOD (mg P/100 g sample)	13	2	6
LOQ (mg P/100 g sample)	37	20	16

Notes: [a]Measured average phosphorus content with the confidence limit for $p = 95\,\%$

phosphorus-containing ingredients and additives" (EWPIA), when the words "containing polyphosphates" or the coding "E338–E341, E450–452" together with the key words "milk proteins", "plant protein extracts", "soy protein isolates", and "wine" were mentioned. Results shown in Table 9.1 confirm that TP content was higher both in EWP and EWPIA with respect to regular ham. Moreover, no significant difference for the same parameter was observed between EWP and EWPIA. On the contrary, differences in IP concentration were found among all groups. In particular, as expected, regular hams exhibited the lowest concentration values, while EWP samples showed the highest ones. As a consequence of TP and IP values, it could be hypothesized that the addition of phosphorus-containing ingredients, such as milk proteins and plant protein extracts or isolates, decreased the amount of functional additives, such as polyphosphates and phosphate salts, used in the production processes of EWPIA with respect to EWP. In addition, higher PP concentration levels for EWPIA with respect to regular samples and EWP, which were quite similar, could be related to the addition of ingredients, such as milk proteins and plant protein extracts or isolates, containing significant amounts of phosphorus from phosphoprotein. Also PL content was higher for EWPIA than EWP and regular hams. A dilution effect due to the presence of water in ingredients (reported on the package label) of each EWPIA and EWP with respect to regular (in which water was not listed on the product label) may explain even lower PL and PP values measured in EWP with respect to those of regular items.

This method was developed for boiled ham, but it could be used for the quantification of different subtypes of phosphorus in several kinds of meat product. As far

Table 9.3 Results of inorganic phosphorus (IP) determination in a spiked sample

Parameter	Level 1	Level 2
Spiked sample av. (mg P/100 g sample)	100	150
uncertainty (mg P/100 g sample)	1	2
$(w \pm \mu)^a$ (mg P/100 g sample)	90 ± 1	135 ± 4
Standard error of mean	0.2	1.6
Coefficient of variation (%)	0.2	2.6
Recovery (%)	90 ± 1	90 ± 2
LOD (mg P/100 g sample)		
LOQ (mg P/100 g sample)		

Notes: [a]Measured average phosphorus content with the confidence limit for $p = 95\,\%$

as the method performances are concerned, they were obtained by detecting TP, PL, and PP in lyophilized pork muscle powder as a certified reference material. The main features are reported in Table 9.2. The performances for IP detection, obtained by analyzing a spiked sample at two different concentration levels, are reported in Table 9.3.

Analysis of Phosphorus in Foodstuffs by 31P NMR: Qualitative and Quantitative Investigation

To this point, discussion was mainly focused on quantitative phosphorus determination. Anyway, a quantitative analysis could require a preliminary qualitative study for the elucidation of the structures of unknown phosphorus containing compounds. In this frame, high resolution 31P NMR could be considered a valuable tool, able to carry out either qualitative or quantitative investigations with high accuracy. Due to the sophisticated and expensive equipment required, NMR spectroscopy is mainly used for research purpose, although it is gaining popularity also for applicative investigations. This technique is currently considered as an important tool in food science, also for authentication and quality control of foodstuff. [8] Reviews and papers about NMR applications in specific field of food analysis, such as dairy products, meat, fruits and vegetables, cereals, and lipids have been published [9, 22]. The increasing use of NMR in this field is largely due to the availability of modern NMR spectrometers, equipped with high field magnets, able to detect compounds of interest at a lower concentration level with respect to instruments belonging to the previous generation.

Sample preparation prior to analysis, which depends on the matrix and on the kind of analyte under investigation, strongly affects final results. As an example, phospholipids extracted from food have the tendency to self-aggregate both in polar and in apolar solvents. As a consequence, NMR spectra became unsuitable, mainly for quantitative determinations. In 1979, it was demonstrated that aqueous solutions of EDTA combined with surfactants overcome this problem, making the spectrum well resolved. Nevertheless, spectrum shape is strongly dependent on surfactant

concentration, as well as pH. It was also reported that several solvent mixtures could be suitable with different results. One of them is chloroform/methanol/water-EDTA. 31P NMR could be used to study both the factors effecting muscle metabolism in meat animals, such as age and genetic type, and the technological processes used in the meat production, such as animal feeding, slaughter conditions, and storage temperature. In particular, spectra from intact muscles provided information on level of phosphorylated metabolites and their change post-mortem [8]. Actually, NMR spectroscopy, together with complementary techniques, such as mass spectrometry, could be used for metabolomics, as well as other -omic sciences.

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Part III

Phosphorous: Nutrient Interactions

Interaction Between Calcium and Phosphorus and the Relationship to Bone Health

10

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Abbreviations

$1,25(OH)_2D$ 1,25	Dihydroxyvitamin D calcitriol
BALP	Bone-specific alkaline phosphatase
BMD	Bone mineral density
Ca	Calcium
CTx	Carboxy-terminal telopeptide of type I collagen
DPyr	Deoxypyridinoline
FGF23	Fibroblast growth factor 23
iCa	Ionized calcium
K	Potassium
Npt	Sodium-dependent phosphate transporter
NTx	Amino-terminal telopeptide of type I collagen
OC	Osteocalcin
P	Phosphorus

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Key Points

- Studies have shown that an increased phosphorus intake may have negative effects on the skeleton, whereas higher calcium intake may protect against the adverse skeletal effects of excess phosphorus.
- Animal and human data indicate that maintaining an appropriate dietary calcium to phosphorus ratio is key to preserving skeletal health.
- There is some evidence that high calcium intake does not completely counteract the adverse effect of a high phosphorus intake, particularly from food additives.

  Many studies have shown that an increased phosphorus (P) intake may have negative effects on the skeleton, whereas calcium (Ca) intake may have a protective effect on it.

Introduction

Calcium (Ca) interacts with phosphorus (P) in many ways in the body. High Ca intake inhibits P uptake from the gut, and a high P intake may decrease the absorption of Ca, both due to the formation of Ca-P salts. P influences Ca metabolism on many levels. From a dietary perspective one of the best sources of Ca, milk products, is also one of the best sources of P, which makes it difficult to differentiate between the nutrients in epidemiological studies. The bioactive vitamin D metabolite, 1,25-dihydroxyvitamin D (1,25 $(OH)_2D$) together with parathyroid hormone (PTH) plays key roles in both Ca and P metabolism.

Calcium

In the adult human body, Ca content comprises approximately 1000 g in women and 1200 g in men. Most of it (>99 %) is in the skeleton and the teeth as hydroxyapatite. The remaining Ca is found in blood, extracellular fluid, muscle and other tissues and cells. Besides being an elemental part of hydroxyapatite in bones, Ca is an important regulator of several body functions: intracellular signalling, muscle contraction, functioning of the nervous system, hormone and enzyme secretion and blood clotting. Therefore, the concentration of both intra- and extracellular Ca is tightly regulated. In serum, around 50 % of Ca is in ionised form (S-iCa),which is the biologically active form, and the other 50 % is bound to serum protein, mainly in albumin and globulins [17]. As a result of normal daily bone turnover, around 500 mg of Ca is released from the bone and the same amount is accreted. Excess absorbed Ca is excreted in urine, feces and sweat, while non-absorbed Ca is excreted in feces. The relation between dietary Ca and Ca loss depends on intestinal Ca absorption efficiency, skeletal turnover and balance and urinary Ca excretion in the kidneys, as

endogenous and dermal Ca loss remain low. In the skeleton, 99.5 % of Ca is in the form of insoluble hydroxyapatite, and only 0.5 % is released by resorption or deposited during bone formation.

Calcium Homeostasis and Status

Ca homeostasis is tightly regulated by PTH and 1,25 $(OH)_2D$ in the intestine, bone and kidney. Low S-iCa increases the secretion of PTH and 1,25 $(OH)_2D$ whereas high S-iCa has a inhibitory effect. Ca-sensing receptors exist in parathyroid and kidney cells [5, 6]. In response to low S-iCa concentration, PTH secretion increases rapidly [72], and 1,25 $(OH)_2D$ is produced more in the kidneys. PTH enhances 1-α -hydroxylase activity, thus inducing conversion of 25 (OH)D to 1,25 $(OH)_2D$ [19]. These actions lead to increased Ca absorption and decreased urinary Ca excretion, the end result of which is a rise in S-iCa concentration to normal levels. In addition, PTH and 1,25 $(OH)_2D$ act together to mobilize Ca from the skeleton. PTH secretion decreases due to a feedback mechanism induced by increased 1,25 $(OH)_2D$ and S-iCa. Recent findings in mice suggest that Na-phosphate-cotransporter (NPT) type 2c may also maintain normal Ca metabolism, probably by modulating the vitamin D/fibroblast growth factor 23 (FGF23) axis [73].

With normal dietary Ca intakes (about 1000 mg/d), around 10 g of Ca is filtered daily through the kidneys and more than 98 % of this is reabsorbed [17]. PTH is the most important regulator of urinary Ca excretion, and an increase in serum PTH concentration decreases Ca excretion into urine by increasing tubular reabsorption of Ca [47]. Understandably, high dietary Ca intake increases urinary Ca excretion (e.g., [32, 54]), whereas high dietary P (e.g., [29, 44]) and potassium [51, 67] intakes decrease absorption.

Nutritional Calcium Status

As the concentrations of intra- and extracellular Ca are tightly regulated, and Ca, when needed, is available from the skeleton, assessment of Ca status is difficult. In healthy individuals, S-Ca is rarely ever low due to Ca deficiency. As S-Ca is tightly controlled and kept within a narrow range, S-Ca poorly reflects total body Ca. S-iCa functions as an intracellular Ca regulator, and S-iCa concentrations are strictly regulated and follow a circadian rhythm [8, 53]. In follow-ups of 24 h or less, oral Ca intake (dose 500–1500 mg) increased S-iCa and decreased serum PTH concentrations as well as increased urinary Ca excretion in both men and women [30, 33, 45].

As S-iCa concentration is maintained at normal concentrations, by inducing increases in PTH secretion, serum PTH concentrations give useful information about Ca homeostasis when measured together with S-iCa, S-Ca and urinary Ca. In fact, PTH response to an oral Ca load has been used as an indicator of Ca bioavailability from Ca supplements [20] and foods [46]. In research, urinary Ca excretion

has been used as a marker of Ca absorption, although urinary Ca excretion does not equal the amount of absorbed Ca [57]. Calvo et al. [8] demonstrated diurnal variation in urinary Ca excretion, with a decrease at nighttime.

Effects of Dietary Calcium on Calcium Metabolism

Oral Ca intake has in several studies been found to increase S-iCa concentration acutely in healthy men and women of varied ages (e.g., [31, 45, 70]). Oral Ca intake (dose 172 mg) has been demonstrated to acutely (within hours) suppress PTH secretion (e.g., [23, 24, 30, 45, 46]). In fact, acute dose-dependent effects on S-iCa and PTH concentrations after 250- and 1000-mg Ca doses [45] as well as after 500- and 1500-mg Ca doses [22] have been reported. However, with administration of a single oral 1000-mg and 2000-mg Ca dose, S-iCa increased in a similar manner, with the maximal increase occurring after 2 h of Ca administration, indicating saturation of the active Ca absorption mechanism [30]. When Ca intake was decreased from 900 to 170 mg/d for four days, serum PTH increased from 24 to 41 ng/L in premenopausal women [66]. An increase in urinary Ca excretion is found in response to higher dietary Ca intake in experimental situations [22, 25, 45, 54]. In a controlled situation, urinary Ca excretion strongly correlates with acute Ca intake (for review, see [12]). In addition, Ca intake has indirect effects on 1,25 $(OH)_2D$, as PTH, the central regulator of Ca metabolism, mediates the impact of Ca intake on 1,25 $(OH)_2D$; elevated PTH increases the production of 1,25 $(OH)_2D$ in the kidneys. Serum PTH and serum 25 (OH)D concentrations correlate negatively (e.g., [48]).

Effects of Dietary Phosphorus on Calcium Metabolism

Most of the ingested calcium remains in the gut lumen, where it can bind other nutrients such as phosphorus. Heaney and Nordin [27] presented that each 500 mg of ingested Ca binds 166 mg of dietary P. The capacity of Ca to bind P in the gastrointestinal tract has been used in the treatment of kidney patients, as Ca supplements are widely used as P binders (for review, see [59]).

An elevation of serum P stimulates PTH secretion both directly and indirectly. The indirect mechanism of P to PTH secretion through lowering serum Ca concentration has been known for decades [68]. The decrease in serum Ca by dietary P is attributed to the formation of calcium phosphate complexes in the blood [71]. The direct effect of P on parathyroid glands both in vitro [1] and in vivo [16] was discovered more recently.

Calcitriol has both direct and indirect effects on P metabolism. Calcitriol affects serum P directly by increasing its intestinal absorption, but also indirectly by increasing its tubular reabsorption through suppressing PTH. The opposing effects of PTH and calcitriol on the kidney and the intestine, respectively, balance serum P concentrations while preserving Ca ion homeostasis [61].

Earlier studies imply that dietary P might interfere with Ca metabolism in several ways: by directly affecting S-iCa concentration [31] and urinary Ca excretion [49] or through PTH secretion [38] and 1,25 $(OH)_2D$ production [83]. Conflicting results exist concerning the effects of P on Ca absorption [26, 28, 77, 84], and only a few studies have been conducted on this topic, usually with a small number of subjects. An increase in dietary P intake (2000 mg/d) increased fecal Ca excretion in some but not all study subjects, when daily Ca intake was 2000 mg, but not when Ca intake was < 1500 mg [77]. No association was present between Ca absorption efficiency and P intake in women of different age groups [26, 28]. Different phosphate additives may vary in their effects on Ca absorption, as polyphosphates have decreased Ca absorption compared with orthophosphates [84]. While it is uncertain whether P directly affects Ca absorption, P might influence absorption through 1,25 $(OH)_2D$ synthesis, as P directly and independently determines the 1,25 $(OH)_2D$ production rate by affecting the function of 1-α-hydroxylase in vivo [80]. These effects have been demonstrated also in healthy humans; P supplementation (3000 mg/d) for 10 days decreased serum 1,25 $(OH)_2D$ concentration, whereas P restriction (500 mg/d) increased the concentration [65]. Based on the findings of Portale and co-workers [62–65], P regulates the production rate of 1,25 $(OH)_2D$, thus affecting the serum 1,25 $(OH)_2D$ concentration. In postmenopausal women, the association between serum PTH and serum 1,25 $(OH)_2D$ was significant only with a moderate dietary P intake, but the association diminished with high or low P intakes [15].

Serum Ca decreases after P loading in humans, and it has also been demonstrated that P per se increases PTH secretion in vitro [76] and in vivo in rats [40], probably through the sodium-dependent phosphate co-transporters in parathyroid glands [56, 78]. Strong evidence has emerged in animals that high-P diets increase PTH secretion (for review, see [11]). In some studies with humans, high dietary P intake increased serum PTH concentration in longer term situations (e.g., [65, 74]), but no studies have properly investigated the dose–response effects of dietary P intakes. In addition, P sources may differ in their effects on serum PTH; acutely, P originating from phosphate additives alone increased serum PTH concentration more than P from cheese, meat and whole-grain products [35].

Effects of Dietary Calcium-to-Phosphorus Ratio on Mineral Metabolism

In studies with mice, rats and dogs, a low dietary Ca:P ratio increased PTH secretion in a chronic manner [14, 34, 41, 43, 73]. In humans, only two studies conducted over 50 years ago with a low number of subjects specifically investigated the effects of dietary Ca:P ratios on Ca metabolism [50, 60]. Later intervention studies have evaluated only high-P, low-Ca diets [9, 10, 44] or high-P, adequate-Ca/high-Ca diets [21, 81]. Patton et al. [60] described urinary Ca excretion to increase with an increasing Ca:P ratio and a constant Ca intake. At varying levels of Ca intake, when P intake was increased, no significant effect on Ca retention was observed. However, when P intake was kept constant, an increase in Ca intake resulted in an increase in

Ca balance. In intervention studies, low-Ca, high-P diets decreased S-iCa and increased serum PTH concentration in healthy young men and women [9, 10, 44]. In these studies, increased serum PTH concentrations similar to those observed in animal studies (for review, see [11]) were demonstrated, suggesting the adverse effects of low dietary Ca:P ratios on Ca metabolism. Although dietary Ca intake was adequate (800 mg), by increasing the daily P intake from 800 to 1800 mg serum PTH increased [81]. However, when Ca intake was high (1995 mg/d), high P (~3000 mg/d) intake had no effect on serum PTH [21]. A diet high in P and low in Ca may cause alterations also in other Ca-regulating hormones, as Calvo and co-workers [10] found that after a 4-week low-Ca, high-P diet serum PTH levels increased, but no changes occurred in serum 1,25 $(OH)_2D$ concentrations, which usually increase in response to low Ca intake.

Dietary Calcium-to-Phosphorus Ratio and the Skeleton

The adverse effects of low Ca:P ratios in animal diets on bone metabolism are quite convincing, as animals fed with high-P and low-Ca, i.e., low Ca:P ratio, diets manifested secondary hyperparathyroidism (seen as increased serum PTH and decreased serum Ca concentrations), loss of bone and osteopenia (for review, see [11]). Bell and co-workers [3] found that by increasing Ca intake of mature mice the adverse effects of high P intake on bone could be partially diminished. Mice receiving a low-P diet with varying Ca intakes had higher bone weight and mineral content than mice with a high-P diet with varying Ca intakes. Koshihara et al. [42] reported that a high Ca:P ratio due to low P intake was favorable for bone mineralization in adult rats since it increased intestinal Ca absorption. A reduction in dietary Ca:P ratio, in turn, decreased bone mass and strength in estrogen-deficient rats [41]. In an 8 week controlled study in male rats, Huttunen et al. [34] showed that decreasing the Ca:P ratio (1:1; 1:2; 1:3) affected the skeleton adversely as shown by pQCT, μCT, and histomorphometry.

Some cross-sectional studies in humans have also described an association between dietary Ca:P ratios and BMC or BMD. A positive correlation was noted between dietary Ca:P ratio and BMD in perimenopausal women [52] and between dietary Ca:P ratio and BMC in older men, but not in older women [82]. In a more recent cross-sectional study, Basabe et al. [2] concluded that high Ca intake (1000 mg/d) and a Ca:P weight ratio exceeding 0.74 were associated with better BMD in young females. These findings are in accord with epidemiological studies in young females conducted in the 1990s by Metz et al. [55] and Teegarden et al. [79]. Interestingly, the Ca:P ratio of a single foodstuff might affect bone metabolism, as the consumption of cheese, which has a high Ca:P ratio, decreased serum PTH and bone resorption (urinary NTx), unlike the other P sources examined (phosphate salts, meat, whole-grain products) [35].

The combined effects of dietary calcium and phosphorus on calcium and bone metabolism have not been studied in a large extent. In a controlled 24-h study [37] where the P intake was high (1850 mg/d, 1500 mg as P-Salt), increasing the total Ca

intake from 480 to 1080 mg and further to 1680 mg at four different sessions, several beneficial effects on Ca and bone metabolism were noted as compared to the effect of high P intake only. However, not even the high Ca intake (1680 mg/d) could affect the decrease in bone formation causes by the high P intake.

In a cross-sectional study [39], low habitual dietary Ca:P ratios (Ca:P molar ratio 0.50) had unfavorable associations with markers of Ca metabolism in healthy women with an adequate Ca intake. In fact, the lowest quartile, with a Ca:P molar ratio 0.50, differed significantly from the other quartiles by being associated with both increased serum PTH concentration and increased urinary Ca excretion. None of the women in this study achieved the suggested dietary Ca:P molar ratio of 1, although their habitual dietary Ca intakes were in general adequate or good. This is discussed in greater detail below.

While dietary P intake increases serum PTH concentrations by decreasing S-iCa concentration [31] and by directly affecting PTH secretion [76], Ca administration has been demonstrated to decrease serum PTH in young adults [45, 70] via an increase in S-iCa [31]. In a controlled intervention study (all study subjects took part in four different sessions) focusing on the interaction of calcium when P intake is high, by Kemi et al. [37], serum PTH concentration decreased in a dose-dependent manner with increasing Ca intake (1060 and 1680 mg), indicating that Ca supplementation can reduce the rise in serum PTH induced by higher dietary P intake (1850 mg/d). Serum PTH concentration was above the upper reference limit (>65 ng/L) in 50 % of subjects on the control day (Ca 480 mg, P 1850 mg), suggesting adverse effects of the low dietary Ca:P ratio and the low Ca intake on serum PTH. In the cross-sectional study by Kemi et al. [39], higher serum PTH concentrations were found in participants with low dietary Ca:P ratios (Ca:P molar ratio 0.50). While the mean intake of P in all quartiles of this cross-sectional study was over two fold higher (>1200 mg/d) than the dietary guidelines [58], the mean intake of Ca was slightly below recommended levels [58] in the lowest quartile only (742 mg/d). Excluding the lowest quartile revealed similar associations with serum PTH in the other quartiles. The finding of higher mean serum PTH only in the lowest quartile supports the importance of higher dietary Ca:P ratios and the vital role of adequate Ca intake simultaneously with high dietary P intake in habitual diets.

The effects of high P intake on bone formation markers in humans have been contradictory, with bone formation markers showing a decrease (serum bone-specific alkaline phosphatase [s-BALP], serum procollagen type I carboxyterminal peptide, osteocalcin) [21, 44], an increase (osteocalcin) [4], or no change (osteocalcin) [10]. However, in these studies dietary Ca intake varied, being low [10, 44], high [21], or unknown [4]. The acute effect of Ca on bone formation has also yielded inconsistent findings (e.g., [45]). High dietary P intake increases serum PTH, and PTH is well known to increase bone resorption. In a study in a young women with similar Ca intake, no change was found in the bone resorption markers, serum type I collagen terminal telopeptide (serum CTx) or in the free form of urinary deoxypyridinoline/urinary Cr (UDPD/U-Cr) excretion after a 1500-mg P dose [44]. The discrepancy in this earlier study could have been due to neither serum CTx [24] nor U-DPD/U-Cr [71] being very sensitive markers of bone resorption. In different settings, other

markers of bone resorption, such as urinary hydroxyproline (U-Hyp), either increased [9] or showed no change [74] after P supplementation. Kemi et al. [36, 37] showed in two different short term intervention studies that the effect of P on the bone formation marker, S-BALP activity, resulted in decreased activity with increasing P doses (from 500 to 1500 mg on three doses over the day), and when P intake was maintained at the same level (1850 mg/d) and Ca intake was varied, the effect of this level of P intake on the bone formation marker was decreased despite the varying dietary Ca intake. One possible explanation for the finding of an unchanged bone formation rate is that high Ca intake cannot counteract the effects of higher-than-recommended P intakes. In the study focusing on dose–response of P on calcium and bone metabolism, bone resorption (U-NTx/U-Cr) increased with a high-P (1995 mg/d) and low-Ca (250 mg/d) treatment [36]. In the second study [37], in which the P intake was high and Ca intake varied, although there was no change in bone formation, Ca supplementation decreased bone resorption, as indicated by the decreased excretion of U-NTx/U-Cr. Therefore, the decrease in bone resorption indicates that Ca supplementation can diminish the effects on the bone resorption marker, when P intake is three-fold higher than the recommended 600 mg/d [58]. In other Ca administration studies, increased Ca intake was found to decrease bone resorption in adolescent girls [80], young adults [45, 69], and male athletes (Guillemant et al. 2004), while the effects of high P intake on bone metabolism have been demonstrated to be the opposite in a controlled P study [44] and an 8-day intervention study [9].

Determinants of Habitual Dietary Calcium-to-Phosphorus Ratios

Speculation has arisen whether the dietary Ca:P ratio is clinically significant in adult humans [18, 71] since in adult diets the Ca:P ratio varies, being the highest in dairy products. Although some foods rich in P are also good sources of Ca, e.g., dairy products, many others contain very little Ca. Furthermore, P is added to foods as a phosphate additive, further increasing already high P intake. If the habitual diet lacks dairy products, the dietary Ca:P ratio will easily be low. Very low Ca:P weight ratios (0.25) have been reported in diets of young girls and boys in Poland [13] as well as in teenagers and young adults in USA [7]. However, whether it is necessary to reach a Ca:P molar ratio of 1 (e.g., SCF 1993 [58]) in diets is unknown. In the habitual diets in the cross-sectional study by Kemi et al. [38], even when the dietary Ca intake among participants was mostly adequate or high (mean Ca intake 1056 mg/d), none of the participants achieved the suggested Ca:P molar ratio of 1. This was mainly because of the high P content in their habitual diets, rather than a low dietary Ca intake, as mean dietary P intake exceeded 2.4-fold and mean dietary Ca intake 1.3-fold the Nordic nutritional recommendations for P (600 mg/d) and Ca (800 mg/d) [58]. Results of that study also imply that a cut-off Ca:P ratio may exist, below which the effects on mineral metabolism and bone health are more severe. In this study, such a cut-off Ca:P molar ratio was 0.51. However, higher Ca:P ratios might be needed if dietary Ca intake drops markedly below nutritional recommendations.

Conclusions About the Combined Effects of Dietary Calcium and Phosphorus

Calcium and phosphorus metabolism are interconnected with effects on PTH, 1,25 $(OH)_2D$, serum, and urinary concentrations of both calcium and phosphorus including intestinal absorption urinary excretion and skeletal actions. Many studies have shown that an increased P intake may have negative effects on the skeleton, whereas calcium intake may have a protective effect on it. As there may be an optimal balance between the nutrients in relation to bone health, interest has been focused on the dietary Ca:P ratio. Both animal and human research data indicate that a low Ca:P ratio has a negative impact on the skeleton, but there is also suggestive evidence that if P intake is high, a high Ca intake does not completely counteract the adverse effect of a high P intake, especially if it derives from food additives.

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Phosphate Deficiency and the Phosphate-Depletion Syndrome: Pathophysiology, Diagnosis, and Treatment

11

Suzanne M. Boyle and Stanley Goldfarb

Key Points

- Phosphorus depletion describes a state of substantial phosphorus deficiency within all body compartments.
- While hypophosphatemia is rare in the general population, there are several high-risk subgroups, including alcoholics and the severely malnourished.
- Important consequences of phosphorus depletion include respiratory and cardiac muscle dysfunction, rhabdomyolysis, osteomalacia, and hemolysis.

Introduction

This chapter will explore the relationship between phosphate deficiency and the phosphorus-depletion syndrome, including causes, consequences, and treatment. It will emphasize the risk in vulnerable populations such as alcoholics and the severely malnourished.

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What Is Phosphorus Depletion?

Phosphorus is distributed broadly throughout the body. Approximately 80 % is in the skeleton in the form of crystalline hydroxyapatite; 10.9 % is in viscera; 9 % is in skeletal muscle; and a mere 0.1 % is in the extracellular compartment, comprising the interstitial and intravascular space [5]. Phosphorus itself refers to the elemental form, while phosphate refers to the ionic form complexed with oxygen (PO_4^{3-}) [6]. It is present in the body as phosphate in both organic and inorganic states. Phosphorus depletion is a state of severe negative phosphorus balance leading to total body deficiency. This state encompasses the sum of phosphorus stores across all compartments of the body, both intra- and extracellular. On the contrary, hypophosphatemia is a term to specifically denote a deficiency of serum phosphate, typically <2.5 mg/dl (<0.81 mmol/L). While serum phosphate levels are usually reflective of total-body stores, it is imperative to recognize that this is not always the case; a patient who has depletion of his total-body phosphorus stores may or may not have hypophosphatemia. The reverse may also be true: a patient with adequate total-body phosphorus stores may present with severely low serum phosphorus. This distinction becomes critically important when evaluating and risk-stratifying patients.

To illustrate this point, let us consider the case of a healthy human subject who voluntarily hyperventilates. This situation was simulated by Mostellar and Tuttle in 1964. In their experiment, Mostellar and Tuttle recruited 11 healthy subjects to hyperventilate for a period of 320 min. During this time, serial levels of arterial pH, serum phosphate, glucose, and calcium were measured. Within approximately 1 h of hyperventilation, phosphate levels declined from a mean baseline value of 3.2 mg/dl to a nadir 0.8 mg/dl. This occurred with a concomitant rise in arterial pH to nearly 7.80. At 1 h after cessation of hyperventilation, serum phosphate levels returned to baseline, while arterial pH normalized. These rapid changes in serum phosphate were not due to net loss or gain in total-body phosphate. Rather, they were due to redistribution of phosphate between the intracellular and extracellular compartments, driven by acute changes in pH. In this experiment, levels of severe hypophosphatemia were precipitated in the absence of any obvious untoward physiologic effects [7]. Thus, this example underscores the fact that hypophosphatemia can occur without total-body phosphate depletion, purely due to phosphate redistribution.

One may also encounter the converse circumstance: an elevated serum phosphate levels in the face of true phosphorus depletion. To illustrate this, consider a patient with a history of alcoholism and several alcohol-related disorders including alcohol withdrawal seizures and pancreatitis, who is admitted to the hospital with an altered mental status [1]. On exam, the patient is thin with temporal wasting and manifests extracellular volume depletion. Laboratory studies may show elevated serum ketones, normal serum glucose levels, low pre-albumin, and high-anion gap metabolic acidosis with mild acute kidney injury. Initial serum phosphate level is in the high normal range at 5.5 mg/dl. A diagnosis of alcoholic ketoacidosis is made. The patient is treated with thiamin as well as dextrose and saline solutions in order to correct ketoacidosis and volume deficits, respectively. On the following day of

hospitalization, the patient may begin to exhibit signs of alcohol withdrawal with a significantly increased respiratory rate. Laboratory values now reveal alkalemia, a normalized serum creatinine, and serum phosphate level of 1.0 mg/dl. In this example, the patient initially presents with hyperphosphatemia. This is likely due to a tendency for extracellular redistribution of intracellular phosphate stores due to a generalized catabolic state. Additionally, the mild acute kidney injury and volume depletion may decrease renal phosphate clearance. Once treatment for the ketoacidosis and volume depletion is begun, the tendency for extracellular phosphate redistribution will reverse, anabolism will commence, and phosphate may enter the intracellular space as glycogen begins to accumulate [4, 8]. The respiratory alkalosis which often accompanies alcohol withdrawal also favors phosphate movement intracellularly [9]. This along with the enhanced renal clearance from volume repletion causes serum phosphate levels to significantly decrease to the severe range. The lower level of serum phosphate is likely more closely reflective of total-body phosphorus stores given that alcoholism is often associated with poor nutritional status. While the initial elevated level of serum phosphate was accurate due to the reasons described above, it was deceptive with respect to indicating total-body phosphate stores.

These two examples demonstrate that clinicians must interpret serum phosphate levels with caution as they are not necessarily representative of total-body phosphorus content. It is incumbent upon the clinician to recognize common risk factors for phosphate depletion as well as situations when serum phosphate levels are not likely to reflect true phosphate stores.

Pathways to Phosphate Depletion

Phosphate balance is regulated by the interaction of the gastrointestinal tract and the kidney. Save a few exceptions (e.g., dialysis), these are the sole routes for phosphorus to enter and exit the body.

Gastrointestinal Tract

Phosphorus depletion can result from poor dietary intake of phosphate-rich foods if ongoing phosphate losses occur. However, it is quite difficult to become phosphorus deficient by lack of dietary intake alone given that phosphate is nearly ubiquitous in the food supply [4]. It is highly present in grains, dairy products, meat, fish, eggs, colas, nuts, and chocolate. There are, though, certain populations who are at risk for chronic dietary deficiency as well as other concomitant depletion mechanisms. In the industrialized world, these often include alcoholics and the severely malnourished (e.g., those with eating disorders such as anorexia nervosa).

Once phosphate-rich foods are ingested, they are absorbed by the small intestine, mostly in the jejunum. This is done via an active transport mechanism facilitated by 1, 25-hydroxy-vitamin D as well as a passive transport mechanism. Thus, in order

to absorb phosphorus one must have a functional intestinal wall. For example, in the patient who suffers from chronic alcoholic pancreatitis because a substantial amount of his daily calories come from alcohol, the patient likely has a minimal daily phosphorus intake [5, 9]. In addition, because of chronic pancreatitis and the resultant insufficiency in certain digestive enzymes, there may be further vitamin D deficiency due to fat malabsorption. The potential for vitamin D deficiency likely further impedes phosphate absorption. However, the extent to which it influences absorption in this situation is unclear, especially when one can observe normal to high serum phosphate levels in patients with chronic kidney disease who are known to have decreased production of active vitamin D. Even beyond the dietary and malabsorptive challenges that face chronic alcoholics, exposure to ethanol in and of itself likely confers intracellular phosphate deficiency and predisposition to adverse effects. In studies in dogs that were fed daily intoxicating doses of ethanol for a 2-month duration with an otherwise well-balanced, phosphate-rich diet, chronic alcohol exposure led to cellular phosphate deficiency. The hypothesized mechanism for this finding is that ethanol disrupts normal sodium transports in cells. Because phosphate transport is tightly coupled to that of sodium, intracellular phosphate deficiency ensues [4]. In the absence of chronic alcoholism, patients with predisposition to phosphate malabsorption in the gastrointestinal tract include those with inflammatory bowel disorders such as Crohn's disease or surgical short-gut syndrome. These patients might lack the functional absorptive capacity for both vitamin D and phosphate.

Drugs may reduce intestinal phosphorus absorption. The classic example is nonabsorbable antacids such as, calcium carbonate and magnesium-aluminum hydroxide bind phosphate in the intestinal lumen, and thus, inhibit absorption. This effect was originally noted by Bartter et al. in 1968. In their experiment, normal human subjects and subjects with hypoparathyroidism were given high doses of antacids daily for several weeks with close measure of dietary intake and fecal/urinary output. In one normal subject, serum phosphate levels gradually declined over about 6 weeks time to severe levels ($\leq$1.0 mg/dl). At these levels, symptoms of weakness, bone pain, and anorexia appeared. These symptoms were alleviated as the serum phosphate levels rose again after discontinuation of the antacid therapy. This experiment was the first to provide evidence of symptoms related to phosphate depletion in humans [3].

Renal Phosphate Handling

Phosphate is freely filtered at the glomerulus. Its reabsorption occurs almost exclusively in the proximal tubule coupled to sodium. The rate of phosphate transport is primarily regulated by parathyroid hormone (PTH) action to induce phosphaturia. In addition, there are several other phosphaturic factors that have emerged, collectively known as phosphotonins [5]. Most prominent among these is fibroblast growth factor (FGF)-23, which is thought to act by decreasing sodium phosphate cotransporters in the proximal tubule. Abnormally high levels of FGF-23 have

Table 11.1 Phosphaturic medications and their proposed mechanisms of action

Therapy	Proposed mechanism of action
Ifosfamide	Acquired Fanconi syndrome
Tenofovir	Acquired Fanconi syndrome
Azacitidine	Acquired Fanconi syndrome
Valproic acid	Acquired Fanconi syndrome
Acetazolamide	Impairment of sodium reabsorption in proximal tubule
Thiazide diuretics	Impairment of sodium reabsorption in distal tubule
Normal saline infusion in euvolemic or volume-expanded state	Impairment of sodium reabsorption in proximal tubule
Bisphosphonates	Stimulation of PTH due to decrease in serum calcium with resultant hypophophaturia
Imatinib	Tubulopathy
Glucocorticoids	Enhancement activation of the Na+−H+ exchanger in the proximal tubule with resultant dissipation of the sodium gradient that drives phosphate reabsorption

recently been implicated in hypophosphatemic bone diseases such as X-linked and autosomal dominant hypophosphatemic rickets as well as tumor-induced osteomalacia [10]. Several phosphotonins are metabolized by the liver. Thus, it has been reported that in cases of partial hepatectomy, hypophosphatemia is seen [11]. Certain acquired or genetic disorders of the sodium phosphate cotransporters also result in phosphaturia. These include Fanconi syndrome, which can be seen most commonly in multiple myeloma and is also accompanied by urinary wasting of amino acids, bicarbonate, and uric acid [5, 10]. Agents that enhance phosphate transport in the proximal tubule include decreased dietary phosphorus intake and vitamin D.

Several medications are also known to increase urinary phosphate excretion. Many of these cause decreased phosphate reabsorption through a variety of mechanisms, including an acquired Fanconi syndrome. Table 11.1 lists some of these medications and the proposed mechanism of phosphaturia [12].

Intracellular Redistribution of Phosphate

There are several stimuli for redistribution of phosphate from the extracellular to the intracellular compartment in the acute setting. As noted before, redistribution will alter serum phosphate levels but not necessarily total-body phosphorus stores. The two most important agents that induce a redistribution of phosphate from extracellular to intracellular fluid are glucose infusions and acute respiratory alkalosis [4].

The awareness of a linkage between glucose load and hypophosphatemia was well known approximately 100 years ago as a fall in serum phosphate following a glucose load was used as a crude measure of the presence of insulin release. However, the potential for severe consequences of acute glucose loading in patients who were chronically malnourished and phosphate deficient was first noted by

Schnitker et al., who described a group of Japanese prisoners of war who developed signs of heart failure after being acutely fed after months of severe malnutrition. They developed severe hypophosphatemia as part of what came to be known as the refeeding syndrome [13]. In malnourished states, the body predominantly utilizes lipid and protein stores as sources of energy. When carbohydrates are reintroduced into the diet, insulin is secreted thereby shifting glucose and phosphate into cells. The process of glycolysis is then stimulated as a means to generate energy for the cell. This energy is primarily in the form of ATP. During glycolysis itself, without regard to subsequent oxygen-dependent energy-generating processes such as the citric acid cycle, one ATP molecule is consumed per molecule of glucose to yield a net gain of one ATP molecule. These metabolic pathways require a supply of inorganic phosphate in order to be sustained. Thus, in response to this intracellular demand for phosphorus, phosphate stores are quickly mobilized from hydroxyapatite in bone and moved into cells, mostly those of muscle and liver. If adequate intracellular are present, a net gain in ATP ensues. Studies by Brautbar et al. in well-nourished dogs receiving hyperalimentation and glucose infusion demonstrated that phosphorus stores increased in muscle and liver but were depleted in bone. To the contrary, studies by Knochel et al. demonstrated that by feeding dogs a phosphate-deficient diet prior to hyperalimentation, muscle, liver, and bone phosphorus stores were globally depleted. Thus, in response to a glucose/insulin load, serum phosphate levels will decrease as phosphate is mobilized from bone and the extracellular compartment in order to comply with metabolic demand [4, 14]. Without adequate total-body phosphorus stores, this demand cannot be met as existent stores are quickly mobilized and exhausted. Because of this, adverse clinical consequences can potentially ensue.

Patients who are at risk for refeeding syndrome have low total-body phosphorus and a carbohydrate load can lead to cellular ATP deficits as noted above. In addition to hypophosphatemia, patients may also exhibit hypokalemia, hypomagnesemia, and glucose intolerance as part of the consequences of their malnutrition. In particular, patients with anorexia nervosa (especially patients <70 % of ideal body weight), alcoholics, and patients with sudden and dramatic weight loss, including those who have received bariatric surgery or with malignancy, are a highest risk. In order to mitigate risk, such patients should initiate a daily caloric intake of 600–1000 kcal with weekly increases of 500 kcal/day. Weight gain should be targeted in the range of 0.5–0.9 kg (1–2 pounds) per week [15]. In addition, nutrition should include adequate amounts of phosphorus. For patient receiving hyperalimentation, phosphate must accompany the nutritional preparation in order to prevent depletion of inorganic phosphorus stores.

Increased pH of body fluids is the other driving force for intracellular redistribution of phosphate. This was illustrated in the example of the healthy volunteers with hyperventilation [7]. In cases of alkalemia, especially respiratory alkalosis, the rise in intracellular pH stimulates glycolytic pathways that require inorganic phosphate. This in turn shuttles phosphate intracellularly [4]. As mentioned previously, it is quite common for alcoholics to develop respiratory alkalosis, a part of the alcoholic

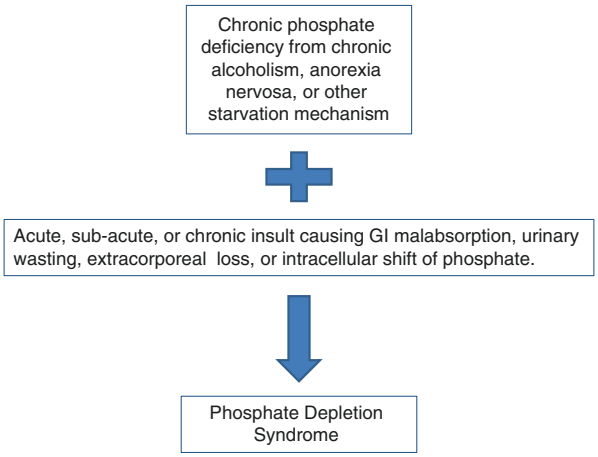

Fig. 11.1 This demonstrates the requisite conditions for development of the clinical syndrome of phosphate depletion. For deleterious clinical consequences to occur, a chronic state of phosphate deficiency is typically present at baseline. This baseline state is then exacerbated by variety of other potential stimuli, which then culminate in clinical manifestation of phosphate deficiency

withdrawal syndrome. This, coupled with poor nutrition and infection, creates a very high risk for the development of hypophosphatemia, especially when "refed," even if initial serum phosphate levels are not reduced [1, 9].

There are several other stimuli which induce intracellular redistribution of phosphate. One example is a rapidly proliferating malignancy. Specifically, there are multiple case reports in the literature of profound hypophosphatemia in patients with acute myeloid leukemia as well as after stem cell transplant for myeloid leukemia. In the proliferative state, the leukemic cells have high rates of aerobic metabolism, requiring a phosphate shift intracellularly [12, 16]. A similar phenomenon mediates the sharp fall in serum phosphate that has been reported with hematologic reconstitution following stem cell transplant [17]. Beta-catecholamines also induce acute redistribution of phosphate to for the intracellular fluid; this effect contributes to the hyposphosphatemia observed in sepsis. Finally, the hungry bone syndrome, a clinical state which is observed after parathyroidectomy, is characterized by rapid uptake of both calcium and phosphate into bone [12] (Fig. 11.1).

Extracorporeal Removal of Phosphate

While gastrointestinal and renal routes are the primary mechanisms by which phosphorus balance is altered, it is important to be aware that renal replacement therapies including intermittent hemodialysis (iHD), peritoneal dialysis (PD), and continuous renal replacement therapies (i.e., continuous veno-venous hemofiltration [CVVH]. and continuous veno-venous hemodialysis [CVVHD]) have the potential to induce

the phosphorus-depletion syndrome in certain acutely ill patients. Phosphate removal with both iHD and PD is limited. However, with CVVH and CVVHD, it is highly efficient. The incidence of hyposphosphatemia with CVVH and CVVHD is estimated to be as high as approximately 60 % [18, 19]. As patients with acute critical illness are more likely to receive CVVH and CVVHD, this extracorporeal mechanism for phosphate removal will tend to exacerbate the risk for phosphorus-depletion syndrome in patients with increased baseline risk, including alcoholics, postoperative patients with prolonged courses of bowel rest in the absence of parenteral therapy, the chronically malnourished, and patients with sepsis.

Clinical Consequences of Phosphorus Depletion

Phosphorus is an essential constituent of many energy-rich organic molecules, including ATP. It is also a component of 2–3 diphosphoglycerate (2,3-DPG), a molecule that is produced as a by-product of anaerobic glycolysis in red blood cells. It serves to bind to hemoglobin and decrease its affinity to oxygen, thereby promoting oxygen delivery to tissue. It is also an integral part of the phospholipid bilayer of cells and serves as a buffer for pH homeostasis [5]. Given that phosphorus is so crucial in a variety of metabolic pathways, it is hardly surprising that that phosphate deficiency could be associated with organ dysfunction.

Clear insights into the pathophysiology of phosphorus depletion derive from various animal studies completed in the 1970s and 1980s. Several investigators demonstrated that in order for hypophosphatemia to cause cellular injury, a certain vulnerable “precondition” must be present. This “precondition” is a threshold level of phosphorus depletion and/or possibly preexistent cellular injury from chronic insults such as ethanol exposure. Knochel et al. performed a pair of experiments in dogs which examined this “precondition” and its effect of the presence or absence of rhabdomyolysis in skeletal muscle. In the first experiment, two groups of dogs were fed phosphorus-deficient diets for several weeks. Once they had lost 30 % of their initial body weight, they received intravenous hyperalimentation. One group received a nutritional preparation with 1.87 g of phosphorus per day and the other group received a preparation devoid of phosphorus. In the latter group, severe hyposphosphatemia ensued (<1.0 mg/dl). Dogs started to manifest weakness, difficulty swallowing, muscle fasciculations, and in some, seizures. Creatine phosphokinase (CPK) levels were increased in the latter group and skeletal muscle biopsies demonstrated evidence of frank rhabdomyolysis. This was not the case in the former group who received phosphorus supplementation. The same research team then performed a complementary crossover experiment in which dogs were fed a phosphorus-rich diet for 2 weeks. Following this 2-week period, the dogs’ caloric intake was reduced by half until they reached 30 % of their initial weight. At that point, they were given phosphorus-deplete hyperalimentation. Serum phosphorus level fell to 1.0 mg/dl or less. However, unlike the animals that received phosphorus-deplete hyperalimentation following a phosphorus-deficient diet, the group of dogs that had received phosphate supplements lacked severe symptoms and did not show biopsy

or laboratory evidence of rhabdomyolysis. While severe hypophosphatemia occurred in both experiments in dogs who did not receive phosphorus supplement during hyperalimentation, severe cellular damage was only apparent in those dogs who had a history of a chronically phosphorus-deficient diet [4]. Thus, the required "precondition" for hypophosphatemia-associated cellular injury with symptoms was chronic phosphate depletion.

Knochel et al. uses the concept of phosphorylation potential to capture the threshold by which a cell will succumb to the damage of phosphate depletion. Phosphorylation potential is the ratio of ATP to the product of adenosine monophosphate (ADP) and inorganic cytosolic phosphate (P_i): [ATP]/[ADP][P_i] [4, 20]. If the phosphorylation potential remains stable during the stress of hyperalimentation or "refeeding," the animals are resistant to cell injury. If, however, the phosphosphorylation potential increases, this indicates a vulnerability to cellular injury following refeeding. Thus, if cytosolic inorganic phosphate stores are replenished at an ample rate to fuel ATP production (i.e., replenished via intracellular redistribution and mobilization from other storage depots such as bone), animals are protected. If the ratio is high, then cytosolic inorganic phosphate stores have been depleted.

In both humans and dogs, it seems that the serum threshold at which symptoms of the phosphate-depletion syndrome appear is <1.0 mg/dl. This was the case in the aforementioned animal studies as well as in the work of Bartter et al., in which normal and hypoparathyroid human subject received phosphate-deficient diets combined with antacid therapy [3]. However, as addressed previously, the serum phosphate level alone does not mark the threshold at which the syndrome will occur; the precondition of chronic phosphorus depletion must be met. Since one cannot directly and routinely measure intracellular stores of ATP, ADP, and inorganic phosphate in order to determine phosphorylation potential, the clinical setting must be considered to identify at-risk patients.

In chronic hypophosphatemia, it is well appreciated that clinical consequences include proximal muscle weakness and osteomalacia [4]. For example, in children with hereditary hypophosphatemic rickets, osteomalacia, and short stature occur in the absence of treatment with phosphate and calcitriol [21]. There is also limited observational data that supports a negative correlation between serum phosphorus and glucose intolerance and obesity [2, 22].

As muscle is a highly metabolically active tissue, one could extrapolate the effect of phosphate depletion on skeletal muscle to other types of muscle, namely cardiac and respiratory muscle. Numerous observational studies suggest an association between hypophosphatemia and respiratory and cardiac dysfunction, but these studies are plagued by small sample sizes, different definitions of hypophosphatemia, and poor adjustment for potential confounders, which thus limits their ability to demonstrate causality [2]. In 1977, O'Connor et al. were among the first groups to suggest an association between myocardial performance and serum phosphate levels. His group described a heterogeneous cohort of seven patients with normal baseline left ventricular (LV) function and baseline serum phosphate levels of <2.0 mg/dl. Using measurements of cardiac volume and stroke work obtained via thermodilution, they found a trend toward improved cardiac function

after repletion [23]. Similarly, Darsee et al. described a cohort of four patients with clinical congestive heart failure, all of whom had significant alcohol histories and serum phosphate levels <1.0 mg/dl. After restoration of serum phosphate levels to ≥3.0, LV function reported improved as did clinical symptoms. Darsee and his group attributed this improvement and the etiology of the heart failure to hypophosphatemia [24]. Subsequently, these results were called into question when allegations of fraudulent data reporting surfaced [2]. These studies were followed by a controlled trial by Davis et al. in 1988, in which hospitalized patients with moderate and severe hypophosphatemia had assessments of LV function before and after phosphate repletion. Compared to normophosphatemic controls, the hypophosphatemic patients demonstrated an improvement in function proportionate to their degree of hypophosphatemia. Of note, this trial was also relatively small ($n = 33$), all male, and comprised of nearly half the alcoholics [25]. With respect to the association between respiratory muscle dysfunction and hypophosphatemia, studies are also limited by small sample size, heterogeneous cohorts and definitions of hypophosphatemia, and varied metrics of diaphragmatic muscle function, including maximal inspiratory pressure and transdiaphragmatic pressure [26–28]. A recent single-center prospective cohort study by Demirjian et al. ($n = 329$) explored the association between hypophosphatemia (<2.0 mg/dl) with the endpoint of time to prolonged respiratory failure (defined by tracheostomy) and all-cause 28-day mortality in patient with acute kidney injury on CRRT. It was found that there was a significant association between hypophosphatemia and prolonged respiratory failure. However, there was significantly higher mortality in the non-hypophosphatemic group than the hypophosphatemic group. These results may have been influenced by the competing risk model, which captured the first-occurring endpoint, tracheostomy, or death [29].

Aside from muscle dysfunction, there is also evidence in the way of rare human case reports and animals studies that there exists a possible association between hypophosphatemia and hematologic dysfunction. There are a few case reports of hemolytic anemia occurring in patients with severe hypophosphatemia (<0.5 mg/dl) with the hypothesis being that depleted ATP stores disturb the integrity of the red cell membrane. However, patients within these rare reports also had competing comorbid conditions such as alcoholic ketoacidosis which may have contributed [30]. Experiments in dogs by Craddock et al. suggested that severe hypophospatemia led to chemotactic and phagocytic impairment in leukocytes, again owing to depletion in cellular ATP levels [31] Signs and symptoms of phosphate depletion are in (Table 11.2).

Despite the numerous studies that relate hypophosphatemia and certain surrogate outcomes like muscular dysfunction, the broader question is whether or not hypophosphatemia affects mortality. To this purpose, there are several observational studies which examined mortality as a clinical endpoint and all have found significant associations [32, 33]. Like studies which examined surrogate outcomes, these studies were fraught with similar challenges that limit the ability to infer causality. In particular, examined cohorts are comprised of critically ill, septic patients in whom unmeasured confounding is a ubiquitous issue. However, given our

Table 11.2 Potential signs and symptoms associated with the clinical syndrome of severe phosphate depletion

Bone and muscle pain
Respiratory failure in the critically ill with impaired ventilator weaning
Exacerbated cardiac failure
Hemolytic anemia
Impaired leukocyte function
Global weakness
Poor appetite
Rhabdomyolysis
Growth impairment in children

knowledge of physiology and the epidemiologic analyses available, it is appropriate to conclude that phosphorus depletion is an important clinical problem with an association with vital end-organ damage and mortality. Clinicians must be aware of vulnerable populations and common clinical presentations so that treatment can be instituted. While treatment comes with a few important risks, it is relatively easy and should be employed when indicated.

Treatment of Hypophosphatemia

In the approach to treatment, the clinician must first identify at-risk patients. In patients with conditions such as hereditary hyposphosphatemic rickets, it is standard of care to provide repletion with both activated vitamin D as well as oral phosphate supplements [1, 21]. Thus, in this situation, the decision to provide phosphate repletion is relatively objective. However, many times, identifying patients who might warrant treatment is less clear. In patients with chronic alcoholism, anorexia nervosa, or intestinal abnormalities which severely impair absorption, stressors which increase the demand for ATP production such as hyperalimentation, respiratory alkalosis, sepsis, or intracellular inorganic phosphate stores are particularly vulnerable to severe drops and the risk for cellular damage in the setting of acute stressors like sepsis and refeeding. Again, since it is not feasible to measure intracellular inorganic phosphate stores or ATP levels in clinical settings, one must anticipate the risk to these patients. In these settings, other labs values might be useful. For example, low serum pre-albumin levels have been associated with more severe hyposphosphatemia during hyperalimentation [34].

Once high-risk individuals are identified, uncertainty remains regarding when and at what interval to check serum phosphate levels. Patients with known or suspected malnutrition, including alcoholics, should have a serum phosphate level drawn on acute presentation to the inpatient setting, and it should be monitored at least daily during treatment. Patient receiving nutritional support in the way of TPN or enteral feeding should have a serum phosphate checked daily. Those who receive nutritional support in the setting of malnourishment are at the highest risk for the

refeeding syndrome, and thus, should have serum phosphate measured every 6–12 h. It may be prudent for surgical patients with a relatively long anticipated period of bowel rest, especially those undergoing hepatic or gastrointestinal tract procedures, to have a perioperative serum phosphate measured [1]. For those patients who receive CRRT and have other risk factors such as sepsis and nutritional support, it is unclear how frequently levels should be checked. Typical CRRT protocols include basic metabolic panels as well as serum phosphate every 6–8 h.

Just as there is no strong evidence by which to guide screening for hypophosphatemia, there is also a lack of evidence regarding the threshold at which to treat. Based on case reports and animal studies, it appears that symptoms in patients with known chronic phosphate depletion universally occur at levels less than 1.0 mg/dl, which is the recognized threshold for severe hypophosphatemia. Clinical symptoms include muscle weakness, anorexia, bone pain, and fatigue [3]. In the experiments previously referenced by Knochel et al., dogs with known chronic phosphate deficiency prior to hyperalimentation with phosphorus-poor formulations showed biopsy-proven rhabdomyolysis at serum phosphate levels of <1 mg/dl. However, other observational studies defined hyposphophatemia with higher serum phosphate levels and given their inability to adequately adjust for a multitude of confounders, it is difficult to ascertain whether or not symptoms are caused by phosphorus depletion at higher serum values. Based on this, there is no dispute that serum phosphate be treated in patients with levels ≤1.0 mg/dl, or in those whose symptoms are suspected to be related to hypophosphatemia. In the acute setting, this should be done via the intravenous (IV) route [2]. For patients requiring intensive care with acute hypophosphatemia and lack of enteral access, there are weight-based IV dosing recommendation for each grade of hypophosphatemia, including mild (2.0–2.5 mg/dl) and moderate (1.0–1.9 mg/dl) [35]. The intravenous form is available either as sodium phosphate or potassium phosphate. These recommendations derive from studies conducted with the knowledge that the volume of distribution of phosphate is highly variable, and thus, it is often difficult to achieve target levels. Recently, a barrier to implementing these recommendations has come with production shortages of sodium phosphate. This has forced clinicians to modify their practice habits and again raise the question of what threshold of serum phosphate warrants urgent repletion with intravenous phosphate.

For chronic hyposphosphatemia, hypophosphatemia in ambulatory patients, and acute hyposphatemia that is mild to moderate in a patient with a functioning gastrointestinal tract, oral repletion is appropriate. Oral repletion can come in the form of sodium or potassium-based preparations. Depending on the severity of deficiency, variable treatment courses might be required. For example, 32.3–64.6 mmol/day of phosphate for 7–10 days is typically sufficient to replete stores in the setting of acute mild to moderate hypophosphatemia. However, the daily dose might be as a high as 96.9 mmol if there is a more severe deficiency. While repletion can come in the form of commercially prepared potassium and sodium-based supplements, it many times can be achieved with phosphorus-rich foods. The recommended source is generally skim milk, which contains 1 mg/ml of phosphorus. In cases of suspected total-body phosphate depletion, especially when a patient is to be "refed," or when parenteral hyperalimentation is to be used,

simultaneously phosphate supplementation must be provided. The recommended amount of supplementation for prophylaxis of severe hypophosphatemia with hyperalimenation is 11–14 mmol of potassium-phosphate per 1,000 cal of parenteral formula [1]. While intravenous phosphate repletion strategies are often advocated in the critically ill with severe hypophosphatemia and/or lack of enteral access, they are associated with important potential complications. There is risk for hypocalcemia from chemical precipitation with phosphate in body fluids and soft tissues. Calcium phosphate precipitation can then cause potentially catastrophic consequences such as acute kidney or cardiac injury [1, 2, 36]. With respect to oral phosphorus, the main concerns are adequate gastrointestinal absorption as well as tolerability given that diarrhea is a common side effect [1]. Because of concern for these side effects, phosphate was historically repleted intravenously in a very ginger manner over 8–12 h. However, several investigations demonstrated that more rapid administration was also safe and efficacious. In addition, because phosphate is often incompatible with other intravenous infusions, a more rapid rate of administration enables efficient administration of other needed medication to a critically ill patient. Charron et al. conducted a trial of intravenous repletion studies in patients with moderate ($\leq$2.0 meq/L and $\geq$1.2) and severe hypophosphatemia (<1.2 meq/L). Those with moderate hypophosphatemia were randomized to either 30 mmol of potassium phosphate in 50 ml of saline over 2 h or 30 mmol of potassium phosphate in 100 ml of saline over 4 h. The severe hypophosphatemia group was randomized to either 45 mmol of potassium phosphate in 50 ml of saline over 4 h or 45 mmol of potassium phosphate in 100 ml of saline over 6 h. There were no major adverse events in any of the groups and no statistical difference in serum phosphate levels. Thus, this demonstrated that a faster infusion protocol was safe and can be utilized [37]. The potassium-based preparation can be considered for administration if serum potassium is <4.0 mmol/L and if the patient has normal renal function in the absence of oliguria [37].

Conclusions

Phosphorus depletion describes a state of substantial phosphorus deficiency within all body compartments. Several groups of individuals are at high risk of developing hypophosphatemia, including alcoholics and the severely malnourished. This can lead to important consequences such as respiratory and cardiac muscle dysfunction, rhabdomyolysis, osteomalacia, and hemolysis. Prompt identification and treatment of phosphorus depletion is necessary to avoid morbidity and mortality in individuals with total-body phosphorus deficiency.

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Dietary Phytate and Interactions with Mineral Nutrients

12

Felix Grases, Rafel M. Prieto, and Antonia Costa-Bauza

Key Points

- Phytate serves as a storage form of phosphorous and minerals and constitutes the main source (80 %) of phosphorous in seeds.
- Important beneficial properties of phytate have been described: antioxidant, anticancer, inhibition of calcium salt crystallisation, prevention of renal stone formation and reduction of starch digestion.
- Phytate shows great potential for complexing positively charged multivalent cations, especially iron, zinc, magnesium and calcium.
- Under non-balanced dietary conditions, phytate may affect the bioavailability, and in consequence the status of such ions.
- The presence of phytate in adequate amounts in the diet is remarkable and must be favourably considered.

Introduction

The first information about phytate dates from 1855 to 1856 when Hartig reported small round particles in various plant seeds similar in size to potato starch grains [1, 2]. Salts of phytate are widely distributed in the plant kingdom. Phytate serves as a storage form of phosphorous and minerals and contains around 75 % of total

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phosphorous of the kernels [3]. Other parts of plants such as roots, tubers and turions, however, are very low in phytate (0.1 %) [4, 5]. In fact, phytate constitutes the main source (80 %) of phosphorous in seeds [5].

For decades, phytate has been regarded as an antinutrient, as, during gastrointestinal passage, it may inhibit the absorption of some essential trace elements and minerals, which under certain dietary circumstances leads to calcium, iron and zinc deficiencies [5–9]. Thus, intensive research has been carried out to remove phytate from food, by appropriate processing, to improve the bioavailability of essential trace elements and minerals and to avoid their deficiencies. In the last 25 years, however, important beneficial properties of phytate have been observed. Thus, antioxidant [10] and anticancer activities [11–13] have been reported. Inhibition of calcium salt crystallisation and prevention of renal stone formation [14], reduction of starch digestion along with slowing down of the glycaemic index of foods [15, 16], as well as positive effects on blood glucose and blood cholesterol [17, 18] have also been reported. These findings have revived discussions about the significance of phytate and other inositol phosphates in human nutrition and for human health.

Dietary Phytate Intake

The mean daily phytate intake in humans from all over the world is strongly variable. Thus, for infants (<1 year) in Sweden, the mean daily phytate intake reaches 26–189 mg, and this represents an idea of the level of phytate intake in infants in Europe. Children in the United States and Canada (1–5 years) show a mean daily phytate intake of 166–501 mg, which is much lower compared to Egypt, Kenya and Mexico, where infants (1.5–2.5 years) show a mean daily phytate intake of 796 mg, 1066 mg and 1666 mg and pupils (7–9 years) show a mean dietary intake of 1270 mg, 2390 mg and 3380 mg, respectively. For children from Malawi (4–6 years), a very high mean daily dietary phytate intake of 1622–1729 mg (females) and 1857–2161 mg (males) is also reported [5, 19].

For adults, studies from Sweden, Finland and Italy report a low mean daily phytate intake of 180–370 mg if Western style diets are consumed, and for vegetarians (Sweden) a much higher intake of 1146 mg is reported. In the United Kingdom, two levels of phytate intake are present: one in the range of 504–848 mg and another one in the range of 1436 mg. Similar data exist for the United States, indicating for women a low range of the mean daily phytate intake of 395 mg (18–24 years) and a high intake of 1293 mg (19–35 years). In 1971, the mean phytate intake of average Americans was estimated at 750 mg. In Nigeria and Guatemala, much higher daily phytate intakes of 2200 mg and 2254 mg, respectively, have been described and the maximum daily phytate intake ever reported in humans has been 5770 mg for lacto-ovo vegetarians [5, 19].

This means that for adults three groups of daily dietary phytate intake can be considered: a low level (200–350 mg) for Western-style diets low in phytate-rich plant foods; a medium level (500–800 mg) for Western-style diets with enhanced portions of cereals, whole grain products and other phytate rich foods and a high

level (>1000 mg) for diets rich in plant and phytate-containing foods such as vegetarian diets [5, 19].

It can be assumed that in developing countries, the high content of cereals and legumes in the traditional diet contribute to obviously quite high levels of phytate intake upto 2000 mg and more, although only few studies are available [5, 19].

Consequently, strong differences of daily phytate intake can be found between developing and industrialised countries, between females and males, between young children and older children and between omnivores and vegetarians.

Influence of Phytate on Intestinal Mineral Absorption

Phytate is present at important amounts in all edible plant seeds such as grains, legumes, oilseeds and nuts, and also at lower content in roots, tubers and vegetables. Because phytate is strongly negatively charged under physiological conditions [5], it shows great potential for complexing positively charged multivalent cations, especially iron, zinc, magnesium and calcium. These complexes are soluble under the acidic conditions in the stomach and insoluble at neutral pH in the intestine, resulting in poor absorption of minerals and trace elements. Other components like inorganic phosphate, polyphenols and non-digestible dietary fibre also reduce the absorption of mineral and trace elements [20, 21]. Thus, numerous studies have described the negative effect of phytate on the bioavailability of minerals and trace elements [5, 22–24].

Considering the chelating capacity of phytate on ions such as zinc, iron and calcium, under non-balanced dietary conditions, phytate may affect their bioavailability and, in consequence, the status of such ions [6–9, 25–27]. It should be considered that in many countries whole grain cereals and legumes are among the most important food sources for minerals and trace elements but they also contain high amounts of phytate and polyphenols. Thus, the interactions between the minerals and trace elements and phytate have to be taken into account to ensure proper bioavailability and adequate supply of minerals and trace elements.

Iron deficiency is one of the most prevalent deficiencies in the world being mainly caused by insufficient iron intake [28]. Moreover, the sources of iron, haem or non-haem iron, and the total composition of the diet are of great importance for iron bioavailability. The content of phytate in food has been closely related to iron absorption and high phytate content results in low iron absorption [29]. Phytate decreases the solubility of iron by forming low-soluble iron phytate [30]. This inhibition can be counteracted by other complexing agents such as proteins, peptides, beta-carotene, organic acids and ascorbic acid [31–35]. Ascorbic acid, moreover, stops the oxidation of ferrous to ferric iron preventing the formation of the very low soluble Fe^{3+}-phytate and, by keeping up the concentration of ferrous iron in the chyme, the intestinal iron absorption can be improved.

Organic acids obtained by food fermentation counteract the inhibitory effects of phytate and enhance zinc absorption in the presence of phytate [36]. The same effect has been reported for dietary protein, although the content and type of protein

and the content of zinc are important for the improvement of zinc absorption [37]. Diets low in animal protein result in low zinc absorption in the presence of phytate [38], and high calcium content increases the inhibitory effect on zinc bioavailability by forming calcium–zinc phytates. Insufficient zinc intake, however, is the main cause for zinc deficiencies in humans [39].

The effects of phytate on mineral bioavailability have been widely studied with different and opposite results. Some studies have found that the element absorption is not modified [40–46], and others have observed a decrease in the element bioavailability [47–55]. In fact, the obtained results on mineral bioavailability must be attributed to three different aspects: (a) the amount of phytate contained in the diet, (b) the chemical form in which the phytate is contained in the diet (sodium phytate, calcium–magnesium phytate, calcium phytate, etc.) and (c) the composition of the diet used (macronutrients and minerals). As can be deduced from the literature data [41, 45, 46, 51], the presence of phytate accounting for 0.1 % of the diet, using balanced diets with an adequate mineral content, does not produce any adverse effect on mineral and trace elements bioavailability. Thus, in humans, it has been reported that the intake of 2 g of phytate per day does not generally affect mineral balances when levels of mineral intake are sufficient [56, 57]. In this aspect, it is interesting to consider that humans consuming the so-called 'Mediterranean diet', famous for its healthy properties, consume 1–2 g of phytate daily. Nevertheless, when high phytate doses together with unbalanced mineral content diets, e.g. some soy-based diets, have been used, a deficit in mineral absorption has been detected in some cases [50, 54, 58]. It has to be taken into account that, apart from meat and eggs, the major sources of zinc, for example, are considered to be nuts, whole grains and legumes and they also are an excellent source of phytate.

On the other hand, it has also been previously reported [59] that the consumption of high phytate amounts does not produce an increase of its absorption. Thus, it is clear that phytate levels in the body are related to its oral intake through the diet. Nevertheless there is an ingested amount for each diet that gives a maximum absorption, and from this amount, in spite of increasing the phytate ingestion, the absorbed amount does not increase. Consequently high phytate doses do not imply an increase of phytate levels in the organism. Precisely, the phytate doses that gave maximum levels in the organism correspond to moderate consumption for which no negative effects on status of minerals can be expected.

Studies have been carried out on element bioavailability and phytate concentrations when sodium phytate is added to the diets [45, 46, 55, 60]. In such studies, it has been considered that this is not the naturally occurring form of phytate in foods. In fact, phytate is mainly found in vegetable seeds as calcium/magnesium salt called "phytin" [61–63]. The results suggest that if the essential elements are present in balanced ratios with respect to phytate, as occurs with phytin, there is no reason for a modification of the calcium magnesium balance [41, 64].

Table 12.1 [45] shows the concentrations of calcium, magnesium, zinc, iron and copper in the brain liver, kidney, bone, plasma and urine of rats fed three controlled diets (AIN-76A, AIN-76A + 1 % of phytate as sodium salt, AIN-76A + 6 % carob

Table 12.1 Mineral content (calcium, magnesium, zinc, iron and copper) in the brain, liver, kidney, bone, plasma and urine of rats fed with AIN-76A, AIN-76A + 1 % sodium phytate and AIN-76A + 6 % carob germ for 12 weeks

Diet	AIN-76A	AIN-76A + 1 % sodium phytate	AIN-76A + 6 % carob germ
n	12	12	10
Calcium content			
Brain (μg/g)	33.3 ± 2.4	32.8 ± 1.7	33.4 ± 2.7
Liver (μg/g)	18.6 ± 0.8	18.4 ± 0.7	18.7 ± 1.0
Kidney (mg/kg)	17.9 ± 2.1	6.3 ± 1.8[a]	6.4 ± 2.3[a]
Bone (g/kg)	331 ± 9	323 ± 10	329 ± 10
Plasma (mg/L)	22.1 ± 1.1	21.4 ± 0.9	22.0 ± 1.1
Urine (mg/L)	154.8 ± 6.8	135.2 ± 9.6[a]	117.0 ± 11.0[a]
Magnesium content			
Brain (μg/g)	1.83 ± 0.02	1.78 ± 0.03	1.80 ± 0.09
Liver (μg/g)	2.35 ± 0.13	2.20 ± 0.09	2.18 ± 0.10
Kidney (mg/kg)	22.8 ± 1.3	18.4 ± 0.8[a]	19.4 ± 0.8[a]
Bone (g/kg)	3.83 ± 0.23	3.69 ± 0.17	3.67 ± 0.18
Plasma (mg/L)	1.30 ± 0.05	1.18 ± 0.05	1.27 ± 0.06
Urine (mg/L)	100 ± 1	101 ± 2	125 ± 4[a, b]
Zinc content			
Brain (μg/g)	17.2 ± 0.6	156.5 ± 0.8	17.7 ± 0.7
Liver (μg /g)	11.4 ± 0.4	11.3 ± 0.5	11.8 ± 0.6
Kidney (μg/g)	12.2 ± 0.7	11.4 ± 0.5	12.7 ± 0.6
Bone (mg/g)	1.39 ± 0.04	1.28 ± 0.05	1.42 ± 0.08
Plasma (mg/L)	6.2 ± 0.4	6.3 ± 0.2	6.3 ± 0.3
Urine (mg/L)	0.74 ± 0.03	0.70 ± 0.02	0.61 ± 0.08
Iron content			
Brain (μg/g)	24.7 ± 0.9	21.8 ± 0.7[a]	23.4 ± 0.8
Liver (μg/g)	25.7 ± 0.7	24.6 ± 0.9	24.7 ± 0.9
Kidney (μg/g)	18.9 ± 0.6	17.9 ± 0.6	18.2 ± 0.7
Bone (mg/g)	0.90 ± 0.08	0.84 ± 0.06	0.85 ± 0.08
Plasma (mg/L)	8.5 ± 0.6	8.2 ± 0.4	8.4 ± 0.5
Urine (mg/L)	1.20 ± 0.07	0.97 ± 0.07[a]	1.07 ± 0.10
Copper content			
Brain (μg/g)	9.7 ± 0.5	9.6 ± 0.4	9.8 ± 0.4
Liver (μg/g)	8.4 ± 0.4	8.2 ± 0.3	8.1 ± 0.3
Kidney (μg/g)	5.4 ± 0.3	5.6 ± 0.2	5.7 ± 0.3
Bone (mg/g)	0.50 ± 0.06	0.46 ± 0.06	0.51 ± 0.07
Plasma (mg/L)	3.4 ± 0.2	3.3 ± 0.3	3.3 ± 0.3
Urine (mg/L)	0.40 ± 0.04	0.42 ± 0.05	0.48 ± 0.06

Data obtained from Grases et al. [45]
Results are mean ± SE
[a] $p < 0.05$ vs. AIN-76A
[b] $p < 0.05$ vs. AIN-76A + 1 % sodium phytate

seed germ). AIN-76A is a purified diet in which phytate is absent. No important or significant differences in the mineral status (Zn, Cu, Fe) of blood, kidneys, liver, brain and bone, are observed, except the presence of iron in the brain. Thus, the amounts of iron found in the brain of rats fed AIN-76A + 1 % sodium phytate are significantly inferior to those found in rats fed AIN-76A diet. The amounts of phytate found in organs of group fed AIN-76A diet become very low or even undetectable, while the ones found in rats fed diets that contained 1 % sodium phytate and 0.12 % phytate (AIN-76A + 6 % carob seed germ) are considerably higher and similar. Moreover, the majority of rats fed AIN-76A diet exhibit calcifications at the corticomedullary junctions, whereas no calcifications have been detected in rats fed the other two diets. From these results, it can be deduced that there are no important adverse effects on mineral status as a consequence of the presence of phytate in the studied diets. Besides, considering that a 0.12 % phytate contained in the AIN-76A purified diet, through the addition of a 6 % of carob seed germ to this diet, produces the same beneficial effects as the direct addition of a 1 % of sodium phytate and no negative effects on the mineral status has been observed, it can be concluded that the value of the presence of phytate at adequate amounts in the diet is remarkable and must be favourably considered.

Conclusion

The inhibition of the intestinal metal absorption of phytate can be counteracted by many food compounds such as organic acids and complexing agents, ascorbic acid, food fermentation products, etc., competing with phytate in the binding of minerals and trace elements. Thus, it is assumed that in well-balanced diets the inhibitory effects of phytate on mineral absorption are very low, and little evidence exists from nutritional surveys that in well-nourished population groups, dietary phytate may really affect the status of iron, zinc and calcium. Under malnutrition and non-balanced diets, low in minerals and essential trace elements but high in phytate, however, the situation is completely different. Vulnerable groups in developing and developed countries, with inadequate intake or deficiencies of minerals and trace elements, need to increase total intake of these elements. This can be accomplished via the daily diet or to improve their bioavailability through modification of factors inhibiting or enhancing the bioavailability of the minerals and trace elements in the diet. Adequate strategies to prevent deficiencies of these essential elements, adjusted to the specific situation, are required and different approaches are possible by supplementation of the respective elements, by increasing the content of competing and complexing agents or by removing phytate from food, although this latter option can lead to other health problems.

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Part IV

Phosphorus and Disease

Phosphorus and Kidney Disease: Mechanisms for Perturbed Phosphorus Homeostasis in Chronic Kidney Disease

13

Anna Jovanovich and Michel Chonchol

Key Points

- Abnormalities in phosphorus metabolism occur early in chronic kidney disease patients.
- Compensatory changes in renal phosphate handling help maintain serum phosphorus within the normal laboratory range in early kidney disease, but in a more advanced kidney disease, these mechanisms no longer suffice and hyperphosphatemia ensues.
- Fibroblast growth factor 23 and parathyroid hormone increase in response to abnormal phosphate homeostasis, and together elevations in these hormones help restore serum phosphorus levels to normal until very late stages of disease.

Introduction

Phosphorus is an essential mineral that regulates multiple metabolic processes, including signal transduction, energy production, and mineral metabolism [1]. Serum phosphorus concentrations are tightly regulated in healthy individuals through several mechanisms including dietary absorption, bone flux, and renal excretion. It is known that overt hyperphosphatemia results in adverse effects on the bone and parathyroid glands [2, 3]. Over the past several years, higher serum

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phosphorus concentrations have been found to associate with death, cardiovascular events, and vascular calcification in population-based studies of both the general population and chronic kidney disease (CKD) [4–9]. This mounting evidence suggests the possibility that lowering serum phosphate levels could reduce cardiovascular disease rates and thus, may be a future target of cardiovascular disease management. Therefore, understanding the mechanisms involved in the perturbed homeostasis of phosphorus observed in patients with kidney disease is of great importance. This review will summarize these key hormonal mechanisms.

Overview of Phosphate Homeostasis

The physiologic concentration of serum phosphorus in normal adults ranges from 2.5 to 4.5 mg/dL. A diurnal variation occurs in serum phosphorus of upto 1.0 mg/dL, the lowest concentration occurring between 8 am and 11 am. Of the phosphorus in the body, 80–85 % is found in the skeleton. The rest is widely distributed throughout the body in the form of organic phosphate compounds. In serum, more than 85 % of phosphorus is present as the free ion and less than 15 % is protein bound.

In healthy adults, the steady-state serum phosphorus concentration is determined by the balance between the intestinal absorption of phosphorus from the diet, the storage, and release of phosphorus in the bone, and the excretion of phosphorus through the urine. Abnormalities in any of these steps can result in either hypophosphatemia or hyperphosphatemia. Approximately 60–70 % of dietary phosphorus is thought to be absorbed in the intestine. The type II phosphate transporter NaPi-IIb has been proposed to be responsible for the sodium-dependent phosphate absorption by the small intestine [10, 11]. 1,25-Dihydroxyvitamin D ($1,25(OH)_2D$) stimulates gastrointestinal Na–Pi cotransport thereby increasing phosphate absorption, but only 30% of intestinal absorption is directly regulated by $1,25(OH)_2D$ [12]. The kidney plays a central role in the regulation of phosphorus homeostasis. Most of the inorganic phosphorus in serum is ultrafilterable at the glomerulus. Approximately, 6–7 g/day of phosphorus is filtered by the kidney. Of that amount, 80–90 % is reabsorbed by the renal tubules and the remainder is excreted in the urine. Most of the filtered phosphorus is reabsorbed in the proximal tubule by way of sodium-gradient-dependent process (Na-Pi cotransport) located on the apical brush border membrane. Most of the hormonal and metabolic factors that regulate renal tubular phosphate reabsorption, including alterations in dietary phosphate content, parathyroid hormone (PTH), and fibroblast growth factor 23 (FGF23), have been shown to modulate the proximal tubular apical membrane expression of type II Na-Pi cotransport proteins. Thus, the kidneys play a major role in controlling phosphate homeostasis [10]. The mechanism of these key hormonal regulators of phosphate metabolism including $1,25(OH)_2D$, PTH, and FGF23 is detailed in separate chapters in this text.

Metabolism of Phosphate in Chronic Kidney Disease

In patients with advanced kidney disease, hyperphosphatemia results from a decrease in functioning nephrons. With the initial fall in glomerular filtration rate (GFR) there is a decrease in the amount of filtered phosphate and subsequently excreted phosphate. This leads to an increase in ultrafiltered phosphate by the remaining nephrons and an increase in single nephron phosphate excretion, presumably due to the rise in serum concentrations of PTH and FGF23. PTH and FGF23 levels rise early in the course of kidney disease long before overt hyperphosphatemia occurs, and FGF23 rises earlier than PTH [13]. The increase in PTH and FGF23 results in decreased tubular reabsorption of phosphate and the fractional excretion of phosphate can reach as high as 90%. Thus, serum phosphate is maintained within normal limits until the GFR falls to less than approximately 30 mL/min [13]. Other hormones appear to contribute also to the regulation of phosphate homeostasis, but their actions are less well understood; these regulators include insulin and hormones of the somatotropic pituitary axis, and possibly fibroblast growth factor 7 (FGF7), matrix extracellular phosphoglycoprotein, and secreted frizzled-related protein 4 [10].

Hormonal Regulation

(a) *1, 25-dihydroxyvitamin D.* Vitamin D refers to two biologically inert precursors or prohormones: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_3 is the naturally occurring form in man, although vitamin D_2 has been widely used as the oral supplement. Humans derive vitamin D mostly from the exposure to sunlight and, to a lesser extent, from the diet and dietary supplements. During the exposure to sunlight, ultraviolet B radiation penetrates the skin where it is absorbed by 7-dehydrocholesterol that is present in the plasma membranes of epidermal keratinocytes and dermal fibroblasts [14], resulting in the formation of previtamin D_3. Once formed, the previtamin D_3 rapidly undergoes transformation to vitamin D_3 (cholecalciferol).

Vitamin D is incorporated into chylomicrons and transported by the lymphatic system into the venous circulation [15]. Vitamin D in the circulation is bound to the vitamin D-binding protein and transported to the liver, where it is converted by the 25-hydroxylase enzyme to 25-hydroxyvitamin D (25(OH)D) [14–16]. This form of vitamin D is biologically inactive and must be converted, largely in the kidneys, by 1-α-hydroxylase to the biologically active form $1,25(OH)_2D$ [14–16]. Hence, the vitamin D hormonal system consists of multiple forms, ranging from cutaneous precursors or dietary components, to the most active metabolite, $1,25(OH)_2D$, which acts upon the target organ receptors to maintain calcium and phosphorus homeostasis and bone health.

CYP27B1 encodes the enzyme responsible for 1-α-hydroxylation in the kidney and is induced by PTH, hypocalcemia, and hypophosphatemia [15]. FGF23,

hypercalcemia, and hyperphosphatemia reduce CYP27B1 expression. FGF23 and $1,25(OH)_2D$ also increase the activity of the renal CYP24 [15], which converts 25(OH)D and $1,25(OH)_2D$ into the inactive metabolites [15]. Upon ligand binding the $1,25(OH)_2D$/vitamin D receptor (VDR) complex forms in the nucleus heterodimers with RXR to activate vitamin D-responsive elements (VREs). In enterocytes of the intestinal tract $1,25(OH)_2D$ facilitates transcellular calcium uptake [15]. It also increases phosphate uptake from the diet [15]. $1,25(OH)_2D$ interacts with the VDR on bone preosteoblasts, resulting in their transformation into mature osteoclasts. Mature osteoclasts release calcium and phosphorus from the bone, maintaining the appropriate levels of these minerals in the plasma [14–16]. $1,25(OH)_2D$ also stimulates the synthesis and secretion of FGF23 by osteoblasts and osteocytes. At the level of the parathyroid gland, $1,25(OH)_2D$ acts to reduce PTH synthesis and secretion directly and it increases the calcium sensing receptor expression, thereby sensitizing the parathyroid gland to inhibition by calcium [17]. At the renal distal tubules, $1,25(OH)_2D$ enhances calcium absorption from the glomerular filtrate [17]. Overall the net effects of 1,25(OH)2D on the intestine, bone, kidney, and parathyroid gland are an increase in serum calcium and phosphorus levels. Thus, the endocrine effects of vitamin D are highly dependent on the synthesis of $1,25(OH)_2D$ by the kidneys.

When the GFR falls to 30 % of normal, the renal reserve of 1-α-hydroxylase becomes inadequate, resulting in reduced serum concentrations of $1,25(OH)_2D$ [15] and consequently decreased serum calcium and increased serum phosphorus levels; secondary hyperparathyroidism ensues. The compensatory increase in PTH maintains serum calcium by enhancing osteoclastic activity and serum phosphorus by its phosphaturic effects [15, 16]. Patients with advanced CKD often require the supplementation of $1,25(OH)_2D$ or its analogs to maintain calcium homeostasis and suppress PTH to avoid bone disease [15].

(b) *Parathyroid Hormone.* PTH is a polypeptide secreted from the parathyroid gland in response to a decrease in the plasma concentration of ionized calcium. Therefore, the major physiological role of the parathyroid gland is to regulate calcium homeostasis [17]. PTH acts to increase the plasma concentration of calcium in three ways: (a) It stimulates bone resorption; (b) it enhances intestinal calcium and phosphate absorption by promoting the synthesis of $1,25(OH)_2D$; and (c) it augments active renal calcium absorption. These effects are reversed by small changes in the serum calcium concentration which lower PTH secretion.

Parathyroid hormone secretion is tightly regulated on a transcriptional and post-transcriptional level dependent on the concentration of extracellular calcium. In fact, PTH gene transcription is increased by hypocalcemia, glucocorticoids, and estrogen. Hypercalcemia also can increase the intracellular degradation of PTH. PTH release is increased by hypocalcemia, adrenergic agonists, dopamine, and prostaglandin E2 [17]. This change is sensed by the calcium sensing receptor, which is a protein in the cell membrane of the parathyroid cells. The receptor

permits variations in the plasma calcium concentration to be sensed by the parathyroid gland, leading to desired changes in PTH secretion [17]. Decreased serum calcium was once thought to be central in the regulation of PTH synthesis and secretion, but it is now accepted that FGF23 also suppresses PTH mRNA synthesis and secretion in vitro and in vivo in a klotho-dependent fashion. End organ resistance to FGF23 at the parathyroid gland may be a key player in the excessive release of PTH during kidney disease [18].

In addition to calcium, PTH also regulates serum phosphate levels through its actions at several organs, and elevated serum phosphate concentration in turn stimulates PTH secretion, presumably by lowering extracellular calcium and increasing stability of the PTH mRNA.

PTH rises as the kidney function declines, although not in a significant way until GFR falls below 45 mL/min/1.73 m^2 [19]. This is initially an adaptive response to maintain calcium, phosphorus, and vitamin D homeostasis. Continuous exposure to PTH induces bone resorption by activation of osteoclasts indirectly through osteoblasts resulting in the release of calcium phosphate [20]. At the level of the proximal renal tubule, PTH acts via the PTHR1 expressed at the basolateral and apical membrane to activate a specific adenyl cyclase system. PTH diminishes the proximal reabsorption of phosphate by internalization of the sodium-phosphate cotransporters NaPi-IIa and NaPi-IIc, resulting in renal phosphate wasting and hypophosphatemia [21]. The net effect of PTH action on the kidney is a decrease in serum phosphorus concentration. This important phosphaturic effect is lost with advancing kidney disease, which is a key contributor of the abnormal phosphorus homeostasis seen in patients with declining kidney function.

(c) *Fibroblast Growth Factor 23.* For many years, perturbed phosphorus homeostasis in kidney disease was understood in terms of the trade-off hypothesis: decreased GFR and nephron mass result in phosphorus retention and a reduction in 1-α-hydroxylase, thus, decreasing $1,25(OH)_2D$, which, in turn, results in concomitant hypocalcemia and increased PTH. However, with the discovery of FGF23 [22], the understanding of mineral metabolism in patients with CKD has changed and FGF23 is now hypothesized to play a central role in phosphorus homeostasis (Fig. 13.1).

FGF23 was first discovered in autosomal dominant hypophosphatemic rickets (ADHR). Impairments in proteolytic inactivation cause high circulating levels of FGF23 and result in hypophosphatemia, rickets, osteomalacia, as well as bone pain and deformities. FGF23 is a 32-kDa protein made of 251 amino acids located on chromosome 12p13 [22]. It is produced in osteocytes and osteoblasts [23] and binds FGF receptors in the kidney and parathyroid gland along with its coreceptor Klotho, which increases its binding affinity [24]. FGF23 affects phosphorus metabolism in various ways: it induces phosphaturia by downregulating the sodium-phosphate cotransporter in the renal proximal tubule; it also inhibits renal 1-α-hydroxylase production and stimulates 24-hydroxylase to decrease $1,25(OH)_2D$; and, finally, inhibits PTH secretion from the parathyroid gland [25].

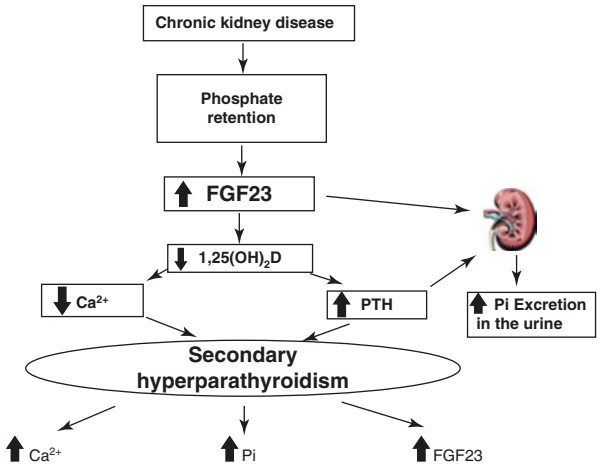

Fig. 13.1 Phosphorus homeostasis in chronic kidney disease

Studies in healthy individuals show a consistent relationship between dietary phosphate and circulating FGF23 levels. When subjects are fed a low phosphate diet for greater than 5 days, FGF23 is decreased along with a decrease in urinary phosphate excretion and increase in $1,25(OH)_2D$ levels. The reverse is observed when subjects are fed a diet high in phosphate [26–28]. However, the response of FGF23 to changes in dietary phosphorus is slow as demonstrated by subjects who were maintained on a phosphate-restricted diet for only 2 days and did not manifest any significant change in FGF23 levels [29]. It is not clear whether changes in serum phosphorus (as a result of dietary phosphorus intake) signal changes in FGF23. Indeed, in cultured osteoblasts, FGF23 is stimulated by $1,25(OH)_2D$ [30] and PTH [31] but not by phosphorus [30]. Furthermore, in a mouse model of progressive CKD, while dietary phosphorus restriction reduced fractional excretion of phosphorus, it did not reduce FGF23 levels, which remained elevated [32]. Similarly in a large cohort of subjects with CKD, dietary phosphorus intake did not correlate with FGF23 levels [13].

It is well accepted that FGF23 levels are elevated in CKD compared to healthy controls (Fig. 13.2). There is no consensus regarding the stage of CKD at which FGF23 becomes meaningfully elevated, however, there is a consistent relationship between increasing FGF23 levels and increased serum phosphorus and fractional excretion of phosphorus as well as decreased GFR and $1,25(OH)_2D$ (independent of GFR) [25]. The signaling mechanism for increased FGF23 in CKD is yet unknown. However, FGF23 is increased prior to PTH in stage III CKD (GFR 30–60 ml/min/1.73 m^2) [13]. In earlier stages of CKD (I and II), FGF23 seems to also induce urine phosphate wasting [13, 33].

Four different genes encode for the FGF receptors (FGFR1-FGFR4). All of these receptors share similar domain structure. Experimental studies using mice with kidney-specific deletion of FGFR1 suggest that the FGFR1 mediates the

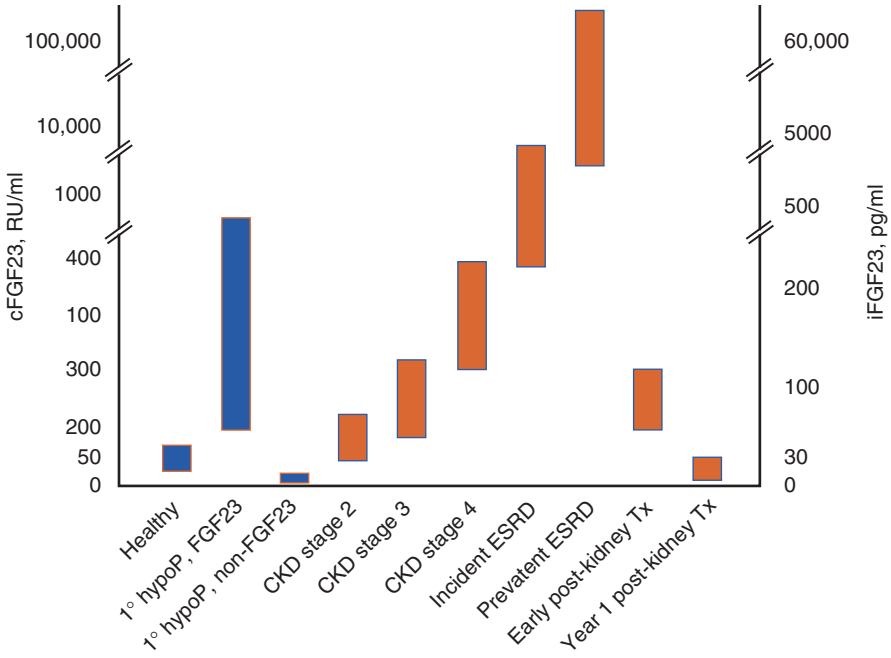

Fig. 13.2 Representative levels of FGF23 in health, various states of CKD (*orange bars*), and in primary hypophosphatemic disorders (*blue bars*). The dual *y*-axis presents FGF23 levels on the scales of the two commercially available assay platforms. The intact assay detects biologically intact FGF23 exclusively (*iFGF23*), whereas the C-terminus (*cFGF23*) assay is capable of detecting both the intact molecule and its C-terminal fragments. The gray area represents the presumed but incompletely defined normal ranges. "1° hypoP, FGF23" refers to hypophosphatemic disorders caused by primary FGF23 excess, for example, X-linked hypophosphatemia. "1° hypoP, non-FGF23" refers to hypophosphatemic disorders caused by mechanisms other than FGF23 excess, for example, hereditary hypophosphatemic rickets with hypercalciuria, in which FGF23 levels are secondarily suppressed

phosphaturic effects of FGF23 [34]. However, the specific site of action in the kidney is still controversial. Although FGF23 decreases expression of NaPi-IIa and NaPi-IIc and CYP27B1 activity in the proximal tubule [35], FGF23 coreceptor klotho is expressed mainly in the distal tubule suggesting other potential sites of action.

In summary, FGF23 acts through FGF receptors, with klotho as a coreceptor. It inhibits renal reabsorption of phosphorus and decreases circulating $1,25(OH)_2D$, and likely inhibits PTH secretion by the parathyroid gland. Its net effect is a reduction in serum phosphate and $1,25(OH)_2D$ levels. While elevated serum FGF23 functions as a phosphaturic hormone to maintain serum phosphate levels in patients with CKD through the course of kidney function decline, the supraphysiological levels may also be maladaptive and contribute to increased morbidity and mortality in patients with kidney disease as suggested in numerous epidemiological studies [36–41].

The Bone

The human skeleton has many essential functions. It not only provides structural support for locomotion and protection for important organs but also plays an important role in acid buffering and mineral metabolism. Bone contains 80–85% of the body's phosphorus. Therefore, it is not surprising that perturbed phosphorus homeostasis in CKD is related to changes that occur in the bone. Indeed, reduced bone mineral density is observed in early stages of CKD [42].

Renal osteodystrophy is a term that describes changes in bone turnover, mineralization, and volume in the setting of CKD. As described elsewhere in this chapter, $1,25(OH)_2D$ deficiency and elevated PTH in secondary hyperparathyroidism cause increased osteoclast activity and contribute to renal osteodystrophy. Bone histomorphometry studies in patients with end-stage renal disease (ESRD) confirm that high bone turnover is associated with higher serum phosphorus, which is due to release of phosphate from bone [43]. However, there are many other factors and bone cell-signaling changes that occur early in CKD and, thus, affect phosphorus homeostasis. Specifically, the osteocyte is emerging as the cell where changes in protein expression occur early in CKD and affect mineral metabolism.

Indeed, early in the course of CKD, osteocyte expression of FGF23 and dentin matrix protein 1 (DMP1) is increased [44]. Osteocyte expression of both FGF23 and DMP1 is increased as a result of hyperphosphatemia [44, 45]. While the phosphaturic effects of FGF23 are clear, the role of DMP1 is less well defined. It has been suggested that DMP1 serves as a negative regulator of FGF23 [46, 47], thus explaining why both increase. Alternatively, differences in post-translational protein cleavage may alter the function of DMP1 and its role in bone mineralization [48].

Osteocyte β-catenin-dependent canonical Wnt signaling is important in bone homeostasis and balances differentiation of osteoblasts and osteoclasts. β-Catenin deficient mice demonstrate a low bone-mass phenotype with increased osteoclast activity [49]. In a mouse model of progressive CKD that closely mirrors the biochemical changes of CKD in humans, bone histomorphometry demonstrates alterations associated with increased osteoclast activity and high-turnover bone disease along with repression of Wnt/β-catenin pathway and increased osteoclast activity. Furthermore, these changes in osteocyte cell signaling and bone histomorphometry occur prior to increases in PTH [50], thus confirming a further mechanism for perturbed phosphorus homeostasis in CKD.

Low-turnover bone disease, another component of renal osteodystrophy, is usually associated with suppressed levels of PTH and is characterized by decreased skeletal remodeling. In a model of CKD, where calcium, phosphate, and PTH levels were maintained within normal limits, adynamic bone disease developed (low-turnover bone disease) characterized by a decrease in osteoblasts, bone formation rate, and mineral apposition rate. Treatment with bone morphogenetic protein 7 (BMP-7), a promoter of osteoblast differentiation, reversed the abnormal skeletal

histomorphometry and also decreased serum phosphorus levels when compared to untreated controls [51]. BMP-7 is specifically made in the kidney and its production is decreased in CKD [52]. Even in CKD with high-turnover bone disease, administration of BMP-7 promotes bone mineralization and decreases bone resorption [53], affecting phosphorus homeostasis.

The Gut

Dietary phosphorus is absorbed as inorganic phosphate along the entire length of the gut with the majority of absorption occurring in the small intestine. Both active and passive transport are important for phosphate absorption, however, the contribution of each has varied among experimental conditions and likely depends on phosphate concentration. In passive transport, phosphate movement occurs via a paracellular pathway and is influenced by an electrochemical gradient across the epithelial layer. Sodium-dependent phosphate transporters, type II (SLCA34) and type III (SLC20), are responsible for active transport. Type III transporters, Pit1 and Pit2, are found on a variety of cells, including intestinal cells, and supply inorganic phosphate for cellular metabolism. The intestinal sodium-dependent phosphate transporter, Npt2b, is analogous to the renal sodium-dependent phosphate transporters, Npt2a and Npt2c, all of which are responsible for the inward phosphate movement. Studies discerning the regulation and control of intestinal Npt2b, especially in the setting of chronic kidney disease, are emerging [54].

Intestinal Npt2b is stimulated by two mechanisms: low phosphorus diet and $1,25(OH)_2D$ [55]. Unlike sodium-dependent phosphate transporters in the kidney, PTH does not regulate intestinal Npt2b [56]. Npt2b double-KO mice die in utero [57], indicating its importance in early embryonic development. However, adults with pulmonary alveolar microlithiasis caused by homozygous mutations in the Npt2b gene (SLC32A2) are normophosphatemic [58] suggesting that compensation exists elsewhere. Indeed, in Npt2b adult KO mice renal FGF23 was decreased and $1,25(OH)_2D$ increased, thus upregulating Npt2a and increasing phosphorus reabsorption in the kidney [59]. Intestinal Npt2b plays a role in systemic phosphate handling by interacting with the FGF23 and $1,25(OH)_2D$ axis making the gut, along with the kidney and bone, important mechanisms in phosphorus homeostasis.

In CKD, uremia does not seem to change intestinal phosphate absorption [60] but there is evidence that Npt2b plays a role in phosphorus homeostasis in CKD. Npt2b KO mice in both adenine-induced and 5/6th nephrectomy models of CKD have an attenuated rise in serum phosphate [61]. Treatment with the phosphate binder, sevelamer carbonate, which binds intestinal phosphate and limits passive absorption, further attenuated serum phosphorus in Npt2b KO mice with adenine-induced CKD, which also showed significantly lower levels of FGF23 and

reduced numbers of osteoclasts and rate of mineral apposition compared to wild-type mice with CKD [61]. Indeed, in both animal and human models of CKD, inhibiting Npt2b with nicotinamide reduces phosphate uptake in the intestine and decreases serum phosphorus [54]. Targeting intestinal Npt2b may have important implications for phosphorus control in CKD.

Conclusions

The knowledge about mechanisms involved in phosphorus homeostasis in CKD has significantly improved during the last years. The discovery of FGF23 has revolutionized the understanding of the links between serum phosphorus, vitamin D metabolism, and development of secondary hyperparathyroidism. FGF23 increases early in CKD and mediates processes that help restore serum phosphorus levels to the normal laboratory range. Increased levels of serum phosphorus have been related in epidemiological studies with adverse outcomes in patients with CKD. Recent studies suggest that increased serum FGF23 levels are associated with mortality, left ventricular hypertrophy, and progression of CKD independently of serum phosphorus levels. There is an ongoing debate about the "normal" or "desirable" levels of serum phosphorus in CKD and a new role of FGF23 as a marker of the disturbances of mineral metabolism in CKD is emerging. In this chapter, we have provided a review of the mechanism of abnormal phosphorus homeostasis in CKD. A discussion followed regarding the mechanism of phosphorus homeostasis, with a focus on the biochemical/hormonal factors vitamin D, PTH, and FGF23, as well as a brief consideration of the bone and the intestine.

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Hidden Forms of Phosphorus in the Diet: Impact in the General Population and in Individuals with Chronic Kidney Disease

14

Lisa Gutekunst

Key Points

- Phosphate-containing additives are prevalent within the food supply.
- Emerging evidence suggest that these additives may contribute to higher serum phosphorus levels in chronic kidney disease population and also the general population.
- More research is needed to evaluate correlations between phosphate intake from phosphate-containing additives with alteration in serum phosphate mediating hormones and cardiovascular disease to assess the full implications of excess phosphorus additive content in processed foods.

Introduction

In 2003, Drs. Jaime Uribarri and Mona Calvo [1] published an article concerning phosphate additives and its effects on individuals with chronic kidney disease (CKD). This article was the catalyst for subsequent research looking into dietary sources of hidden phosphorus, and on the prevalence and effects of additives in this population. It has now become standard practice to advise people with late stages of CKD to avoid foods containing such additives. Given observational data showing an association between serum phosphorus levels and cardiovascular disease (CVD) in the general population, this advice may be relevant to individuals beyond just those with kidney disease.

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Phosphate Additives in the Food Supply and the Effect of Poverty on Use Prevalence

The use of phosphate additives in the food supply has been readily expanding over the years. In 1990, it was estimated that phosphate additives contributed approximately 470 mg/day to the American diet. By 2003, the estimates had significantly increased, with Uribarri and Calvo suggesting that additives could contribute up to 1000 mg/day depending upon an individual's food choices [1].

To understand the vast infiltration of phosphate additives in the food supply, León et al. [2] obtained a Nielsen dataset of grocery sales in northeast Ohio for 12 months ending in February 2010. After excluding fresh produce, milk, pet food, candy, and paper products, they reviewed the nutrition labels for phosphate additives in 2400 top-selling food items delineated into 15 categories. They found phosphate additives in 44 % of the products reviewed with the prepared frozen foods (72 %), dry food mixes (70 %), packaged meat (65 %), and bread/baked good (57 %), soups (54 %), and yogurt (51 %) categories containing the most items with phosphate additives. Additionally, León created identical meals using additive-containing and comparable additive-free products to compare phosphate intake and cost per day to the consumer. Phosphate levels were 736 mg higher in the additive-containing meal (1788 mg vs. 1053 mg) and $2.00 cheaper ($7.76 vs. $9.76) in comparison to the additive-free meal.

The low cost of phosphate-additive containing foods make them attractive to people with financial constraints. Drs. Gutiérrez and Isakova have led two studies looking at the relationship between socioeconomic status and serum phosphate levels. Their first collaboration utilized data from the Chronic Renal Insufficiency Cohort (CRIC) Study [3]. CRIC large prospective study of individuals with CKD. Since its inception in 2003, CRIC has enrolled nearly 4000 individuals with CKD from cities around the United States. The results showed not only that the serum phosphate levels were higher in those whose income was less than $20,000 per year irrespective of race, but also that serum phosphate levels in blacks were significantly higher than those of whites at income levels > $20,001. Researchers speculated that cultural and environmental factors such as low cost convenience foods and fast foods which are disproportionally consumed by those in low socioeconomic settings may be major contributing factors to these findings, and pondered if biological differences, such as lower fibroblast growth factor 23 (FGF23) or a cultural food preference toward high phosphate-containing foods, could account for this difference [3] (Fig. 14.1).

In their second collaboration, Gutiérrez and Isakova highlight tendencies of impoverished Americans toward higher serum phosphate levels. The Third National Health and Nutrition Examination Survey (NHANES III) is a national study that gathered health and nutrition data between 1988 and 1994 on more than 16,000 noninstitutionalized adults in the United States. In addition to blood samples, participants provided information on diet and other lifestyle habits. Gutiérrez and Isakova compared serum phosphate levels with socioeconomic status and found that those in the lowest income ratio quartile (<100 %) had slightly higher phosphorus

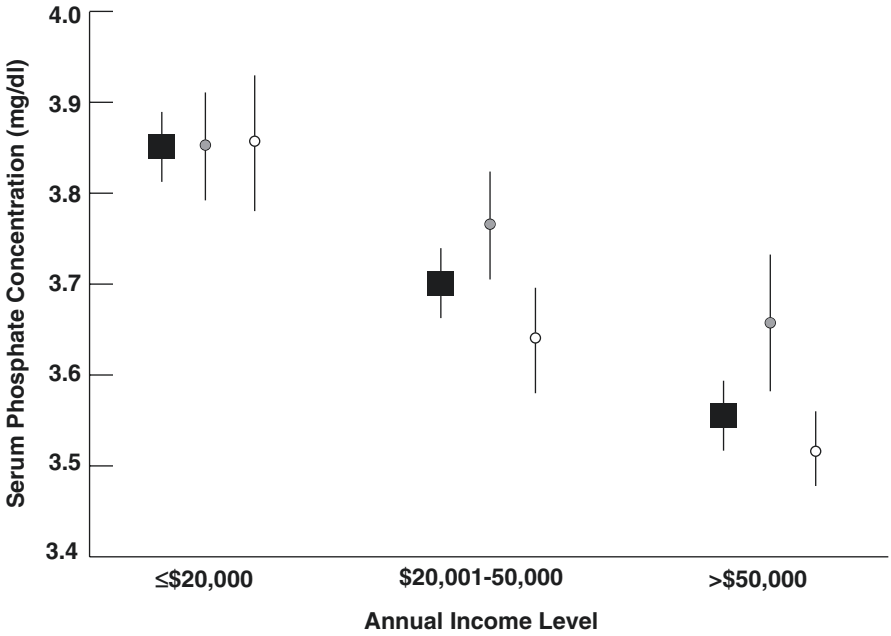

Fig. 14.1 Mean serum phosphate concentrations stratified by race and annual family income level. *Black boxes* represent mean values for the overall sample within each stratum of income, *filled circles* represent mean values for blacks, and *open circles* represent mean values for *whites*. *Vertical lines* represent SD

levels and a greater than twofold higher likelihood of hyperphosphatemia (>4.4 mg/dL) than those in better economic circumstances [4]. It should be noted that 94 % of the study participants had an eGFR of >60 mL/min/1.73 m^2 [4].

Low socioeconomic status within the CKD community is rampant. In the 2012 report of the US Renal Data System (USRDS), 73 % of hemodialysis patients and 63 % of peritoneal dialysis patients qualified for low income subsidy (LIS) benefits from Medicare Part D [5]. Americans in poverty are also increasing. The US Census Bureau reports a steady increase in the percentage of Americans living at or below the poverty line since 2000. In 2009, 14.3 % of the nation's population was considered to be poverty-stricken [6]. It is quite understandable that many would chose low cost phosphate additive-containing foods to feed their families.

Determining Phosphate Content of Food

Quantification of the phosphate content is not required on the Nutrition Facts label for manufactured foods. This is true not only in the United States but also in many other countries, therefore the consumer, as well as the professional turn to other tools to estimate phosphate content. These tools fall under two categories: commercial nutrient analysis programs and web-based data released by the food manufactures.

Most commercial nutrient analysis programs are based on data collected from the US Department of Agriculture (USDA) Nutrient Database and food manufactures. These programs offer professionals and consumers the ability to estimate nutrient content of individual foods as well as recipes and diets. However, these programs are flawed as they do not readily capture changes in manufacturing practices, are not able to update databases as quickly, and are slow to add information on new food products as they are introduced to the market. To compound matters, manufacturers are neither required to provide new data with each formulation change nor mandated to provide nutrient information to the USDA Nutrient Database. Therefore, when the consumer or the professional utilizes either the USDA Database or a commercial nutrient database, the information may be based upon formulations from years ago [7].

Alternately, those looking for nutrient analyses of manufactured foods can turn to the internet for information. Manufacturer websites, and websites dedicated to specific products, may glean some information regarding the phosphate content of a product. Unfortunately, this is not usually of much help if the product does not list the phosphate content on the Nutrition Facts label as the information typically found online is generally the same listed on the product's physical label. This approach can be very useful when searching for phosphate additives in products, as these must be included in the list of ingredients.

Even contacting the manufacturer directly poses problems. Information available by many customer service representatives include only that which is listed on the food label. Some manufacturers may have additional nutrient information available to customer service representatives, but the consumer or professional may have to request the customer representative to pass the information request to a different department. Even then, this information may not be available to the consumer or a healthcare professional [7].

Variance between expected and measured phosphate content of foods has been well documented over the years. Sullivan et al. used Elizabeth Stewart Hands Associates (ESHA) Food Processor SQL (version 9.8) to compare, reported with measured values of phosphate content of enhanced and unenhanced chicken products. ESHA prides itself on being the database of choice for many professionals and schools. They tout having data on 163 nutrient compounds on over 48,000 items. This food items include common foods (i.e., Red Delicious apple), brand name items, restaurant items, and baby food. They obtain nutrient information from the USDA Database, manufacturer's data, and data found in literature [8]. Despite this wealth of information, Sullivan found significant differences between the phosphate content reported by ESHA and the measured content. Of the 38 varieties of chicken products tested, the phosphate content of only 4 products was within a 10 % error window. The reported values on the remaining items were either overrepresented in phosphate content by 23–44 mg/100 g product or underrepresented in phosphate content by 12–165 mg/100 g product [9].

Sherman and Mehta examined the phosphate content of phosphate-additive enhanced and phosphate-additive free chicken and pork reported as milligrams of phosphate to grams of protein (mg PO4/g protein) [10]. As expected,

phosphate-additive free chicken and pork fell below the recommended ratio of ≤10–12 mg PO4/g protein. Phosphate-additive enhanced products had significantly higher ratios when compared with their additive-free counterpart. For example, an unenhanced pork chop had a 9.59 mg PO4/g protein, whereas the enhanced pork chop exhibited 17.35 mg PO4/g protein. Though some phosphate-additive enhanced products tested by Sherman and Mehta fell within the recommended guidelines for mg PO4/g protein, the bioavailability of phosphate from additives is much higher than phosphate found naturally in food. Sherman and Mehta also noted that phosphate additives were present even when they were not listed among the ingredients. Instead, they were "hidden" in terms such as "broth" and "solution added" [10].

These discrepancies have a huge impact on investigating the association between dietary phosphorus intake and serum phosphorus levels. In the two studies by Gutiérrez and Isakova, the reported dietary phosphate intake of the poorest participants was lower than or on par with more affluent participants [3, 4]. Though it is well recognized that databases used to determine phosphorus intake are flawed and do not capture diet intake from phosphate additives, it is this information that the US government uses to set nutrition policy, and the phosphate industry uses to justify the need and safety of their products.

Ramifications of Phosphate Additives in the Stage 5 CKD Population

Serum phosphorus levels in late stage CKD are managed by limiting dietary phosphate intake and using phosphate binding medications (PBMs). Education on dietary sources of phosphate is the key to maintaining serum phosphorus levels as PBMs have binding capacity limitations. The renal dietitian is the primary educator in the dialysis unit working with patients to identify and limit dietary phosphate intake and teaching patients how to titrate PBMs for the phosphate load at each meal. Unfortunately, over the years, the time allotted for face-to-face education between the dietitian and the patient has significantly declined. Recently, Wolfe looked at challenges facing the renal dietitian in the United States in implementing the National Kidney Foundation Disease Quality Initiative (KDOQI) clinical practice guidelines for nutrition in CKD. He reported that time demands placed on renal dietitians doubled compared to between 1983 and 1991/1992 due to an increase in the diabetic population in CKD. This increase in demands, reduced time spent with patients by 50 %. Similarly, by 1999 administrative responsibilities increased to a point that dietitians reported spending an average of 11 min of time with each patient each week [11].

With the implementation of the Conditions for Coverage (CfC) for dialysis facilities in the United States, additional documentation requirements have further reduced time spent on patient education. In an unpublished unadjusted survey conducted in 2012 by the National Kidney Foundation Council on Renal Nutrition and the Academy of Nutrition and Dietetics Renal Practice group, 54 and 53 % ($n = 512$) of respondents indicated that they spend less time with patients and less time

providing interactive learning activities for patients, respectively. Additionally, 32 % of respondents spend only 30 % of their time with patients and 30 % of respondents reported spending 40 % of their time with patients. Attending Quality Assessment Performance Improvement (QAPI) meetings, patient care meetings, and increased documentation requirements were cited by respondents as the biggest increase in their administrative responsibilities (71 %, 73 %, and 80 %, respectively) [12].

Intensive education can have a significant effect on reducing serum phosphate levels in patients with late stage CKD. In an exclusive study looking at the effects of diet education on serum phosphate levels, Sullivan et al. [13] were able to reduce serum phosphate levels in dialysis patients by 1.0 mg/dl (0.7–1.3 mg/dl) in comparison to 0.4 mg/dl in the control group (0.1–0.7 mg/dl) after a single 30-min face-to-face education session. This 3-month study included 279 patients from 26 hemodialysis units. The intervention group ($n = 145$) underwent a 30-min nutrition education session at which the effects of phosphate additives on the total phosphate content of food were discussed. Participants were armed with a small magnifying glass, list of common phosphate additives, and education materials on phosphate-containing foods found at fast-food restaurants. Participants were instructed to use these tools when shopping and to avoid purchasing foods containing these ingredients. At the end of the 3 months, phosphorus levels in the intervention group declined, however there was no significant increase in their overall food knowledge when compared to the control group. This suggests that 30 min of face-to-face education with a renal dietitian and simple tools can have a dramatic effect on the serum phosphorus levels without needing complete understanding of all of the foods that now contain phosphate additives [13].

Phosphate additives contribute significantly to the available phosphate load in meals. As discussed earlier, the bioavailability of phosphate from additives is close to 100 %. When including the phosphate additive-containing food into a meal, the overall bioavailable phosphate increases and this further increases the need for PBMs. Phosphate binding medications are not 100 % effective at binding bioavailable phosphate. Published in vivo phosphate binding capacity for the most common binders used in the United States is listed in Table 14.1.

Gutekunst and León demonstrated the need for significantly higher PBM than typically prescribed to be taken with common fast-food meals such as a meal with fried chicken. They used data compiled by Sarathy et al. [16] using a review of other studies and measurement of phosphate content of over 800 fast-food items from 15

Table 14.1 Phosphate binding capacity of phosphate binding medications (*in vivo*) [14, 15]

Phosphate binding medication	Binding capacity (*in vivo*)
–	–
Calcium carbonate	39 mg per 1 g
Calcium acetate	45 mg per 1 g
Magnesium	Unknown
Aluminum hydroxide	22.3 mg per 5 ml
Sevelamer	64 mg per 800 mg dose
Lanthanum carbonate	137 mg per 1 g

national fast-food chains. Gutekunst and León assumed 80 % bioavailability of phosphate as the food products contained 8 phosphate-containing additives. The meal included 3 strips of crispy fried chicken, a biscuit, and an individual serving of potato wedges. The total phosphate content of the meal was 824 mg with an assumed bioavailability of 659 mg. Given the published binding capacity data, this translated into needing 15 pills of calcium acetate, 11 sevelamer pills, and 5 grams of lanthanum carbonate to completely bind phosphate from this meal [17].

As the number of phosphate additive-containing foods increase, low phosphorus food choices decline. The diet restrictions for those on dialysis are daunting. "Restrictions" are perceived as "avoid" or "don't eat" by patients. Patients have very little control over their disease process, but they can control what they eat. Too often the patient hears "no," and attaches the label of "food police" to the very healthcare professional who is trying to give them control over his or her disease process. As a result, they ignore the advice of the dietitian, or just give up on looking for the "right" foods. Nonadherence with the low phosphorus diet is borne from frustration.

Effect of Dietary Phosphate Intake on Serum FGF23 in Early-Stage CKD

Current published guidelines for managing phosphate do not recommend implementing dietary phosphate restrictions or PBMs when serum phosphate concentrations are normal [18]. However, CVD associated with hyperphosphatemia does not begin when serum phosphate levels begin to rise. The process starts much earlier and many do not survive the process to reach the last stage of the disease. People with moderate CKD (eGFR <60 ml/min/1.73 m^2) show evidence of coronary calcification. The number of calcified sites increases as serum phosphorus levels increase (>3.0 mg/dl) with many people having four or more calcified sites when serum phosphate levels are greater than 4.0 mg/dl [19]. Even in early-stage CKD (creatinine levels >1.2 mg/dl for women and >1.5 mg/dl for men), mortality risk increases when serum phosphate levels are greater than 3.0 mg/dl. Evidence suggests that for each unit increase in serum phosphorus levels, there is a 35 % increased risk of an acute myocardial infarction (MI) and a 28 % increased risk for death and a nonfatal MI [20].

Understanding how cardiovascular disease begins in the presence of normal phosphorus levels is still not completely understood. Recently, there has been more research on the role of fibroblast growth factor 23 (FGF23) in CVD. FGF23 is a bone-derived hormone that regulates phosphorus and vitamin D metabolism. Increased levels have emerged as a risk factor for CVD events, kidney disease progression, and death among individuals with CKD as well as in the general public [21–24]. Elevated levels of FGF23 have been seen in people whose eGFR is <70 ml/min/1.73 m^2 [25]. At such an early level of CKD, serum phosphate levels are not yet elevated.

Studies examining the effect of dietary phosphate on serum FGF23 levels have shown mixed results. In one study, researchers looked at the effects of a low

phosphate diet (<700 mg/day) alone versus a low phosphate diet (<700 mg/day) with a PBM (lanthanum carbonate) on bone mineral markers. Participants were instructed on a low protein diet (0.80 gm/kg/day) and to avoid phosphate-rich foods. Serum levels of phosphate and FGF23, in addition to other bone markers, were taken at enrollment, at the end of the low phosphate diet only, and at the end of the low phosphate diet plus PBM arms. Results showed no decrease in FGF23 levels from enrollment to the end of the low phosphate diet only arm, but did show a significant decline in FGF23 levels when the low phosphate diet intervention was combined with the PBM. Weaknesses in this study include its sample size ($n = 18$), the unknown extent and content of the diet education, and unknown adherence with the low phosphate diet [26]. In a second study, researchers compared a high phosphate diet (2000 mg) with a low phosphate diet (750 mg), and with a low phosphate diet (750 mg) plus PBM (aluminum hydroxide) in both healthy controls and those with CKD. Results revealed a modest increase in FGF23 levels with the high phosphate diet and modest decrease in FGF23 levels with the low phosphate diet in both the control and CKD group (+2.89 pg/ml, + 7.17 pg/ml, −1.58 pg/ml, and −3.31 pg/ml, respectively). Declines in FGF23 levels were most dramatic in the CKD group when the low phosphate diet was combined with the PBM (−15.2 pg/ml). The diets for both the high phosphate arm and the low phosphate arm were constructed by registered dietitians using whole foods evaluated for phosphate content using Food Processor™ from ESHA research. Variations in phosphate content came from nonperishable manufactured food products. The authors contend that this process was used as these products "are known to contain extremely high and low phosphate content" [27]. However, as discussed earlier, there are significant flaws when using analyses programs.

These studies suggest that understanding phosphate content of foods, including the "hidden" sources from phosphate additives, has the potential to favorably modify the serum levels of hormone such as FGF23. The clinical implications of the magnitude of observed change, however, is uncertain at this time.

Serum Phosphate and FGF23 Levels in the Non-CKD Population Are Associated with an Increase in Cardiovascular Risk

Heart disease has been around since the dawn of humans. Diet and lifestyle modifications have been handed down through the centuries. In the 1970s, the American Heart Association developed a list of modifiable and nonmodifiable risk factors for heart disease. Findings from studies in the CKD world have opened up opportunities to better understand the development of CVD in the general population. And, once again, diet plays a big role.

Table 14.2 lists some of the studies that have been conducted looking at serum phosphate, dietary phosphate, and FGF23's influence on risk factors associated with the development or complications of CVD in the general population [28–37]. Observed serum phosphate levels in these populations fell within the "normal"

Table 14.2 Studies examining the association of serum phosphate and FGF23 in the general population

Study name	Author/year	Primary focus	*n*=	Follow-up period	Findings
Cholesterol and Recurrent Events Study (CARE)	Tonelli (2005) [28]	*Post hoc* analysis of data from randomized double-blind, controlled trial of pravastatin versus placebo in 4159 individuals with hyperlipidemia and history of myocardial infarction	4127	5 years	Participants with serum P levels >4.0 mg/dl were at significantly higher risk of developing heart failure (HR 1.43, 95 % CI, 0.95–2.14), experiencing myocardial infarction (HR 1.50, 95 % CI, 1.05–2.16), experiencing the composite outcome of coronary death, or nonfatal myocardial infarction (HR 1.32, 95 % CI, 0.95–1.84)
–	Kanbay (2007) [29]	Relation between serum calcium, phosphate, PTH, and "nondipper" circadian. Blood pressure variability profile in patients with normal renal function	190	NA	Nondippers had higher serum P (3.70 ± 0.61 vs. 3.35 ± 0.44 mg/dl, $p = 0.0001$) and PTH (75.7 ± 28.8 vs. 46.6 ± 17.1 pg/dl, $p = 0.000$) compared to dippers
Framingham Offspring Study	Dhingra (2007) [30]	Observational study of individuals without cardiovascular or renal disease looking at serum P and Ca to CVD incidence	3368	16.1 years	HR increased 1.31 (1.05–1.63) for each 1 mg/dl increase in P. HR for those in the highest P quartile (>3.5 mg/dL) was 1.55 (1.16–2.07) versus those in the lowest *p* quartile (1.6–2.8 mg/dL)
Atherosclerosis Risk in Communities (ARIC)	Onufrak (2007) [31]	Observational study of individuals without cardiovascular or renal disease comparing serum P with carotid intima-media thickness (cIMT)	13,340	Dataset gathered from 1987 to 1989 participants	In men, there was an increase in mean cIMT of 0.012 mm with each 0.48 mg/dl increase in serum P ($p < 0.007$)
Atherosclerosis Risk in Communities (ARIC)	Foley (2008) [32]	Observational study of individuals without cardiovascular or renal disease comparing serum P with coronary heart disease, stroke, and death	13,822	12.6 years	Risk increased as serum P levels increased among quartiles with the highest HR of 1.14 (1.09–1.20) with the highest serum P levels

(continued)

Table 14.2 (continued)

Study name	Author/year	Primary focus	*n*=	Follow-up period	Findings
Coronary Artery Risk Development in Young Adults (CARDIA)	Foley (2009) [33]	Prospective, multicenter, observational study of cardiovascular disease in healthy young adults (mean age at enrollment 25 years)	3042	Since 1985	Serum P levels >3.4 mg/dl were associated with an increase in coronary artery calcification scores with the highest likelihood of coronary artery calcification in participants having serum P levels >3.9 mg/dl
Appropriate Blood Pressure Control in Diabetes (ABCD)	Chonchol (2009) [34]	Evaluate serum P, Ca, Ca-P product with cardiovascular events and mortality in type 2 diabetics	950	4.8 years	HR 4.25 (1.16–16.65) for time averaged serum P levels >3.9 mg/dl
Uppsala Longitudinal Study of Adult Men	Larsson (2010) [35]	Prospective observation of men born between 1920 and 1924 in Uppsala, Sweden. Association between serum P and Ca with cardiovascular death	950	30 years	HR 1.10 (1.02–1.18) per SD serum P increase. HR highest in 3rd tertile (2.78–15.17 mg/dl): 1.31 (1.06–1.63)
Health Professionals Follow-up Study	Gutiérrez (2011) [36]	Ongoing prospective observation of male healthcare professionals. Dietary and nondietary parameters effect on serum FGF23 levels	1261	Participants enrolled in 1986. Serum samples obtained between 1993 and 1995	Each 500 mg higher dietary phosphate intake was associated with a 3.4 RU/ml higher FGF23
–	Yamamoto (2013) [37]	Subset of MESA participants comparing phosphate intake with left ventricular mass	4494	Unknown	With higher dietary phosphate, greater left ventricular mass. Risk highest among women with high phosphate intake

HR hazard Ratio, *P* serum phosphate, *Ca* calcium, *PTH* parathyroid hormone, *CVD* cardiovascular disease, *FGF23* fibroblast growth factor 23

range of 3.0–4.0 mg/dl and in many of the studies, the participants were free from kidney disease [29, 30, 32, 34]. Risks for developing CVD or experiencing a CVD event (myocardial infarction with or without death or stroke) increased in populations with and without known CVD with higher levels of these mineral metabolism factors, and persist with adjustments for other known risk factors.

Changes in cardiac morphology were not only seen in these general population studies, but were also a risk factor for CVD events. There is a positive correlation between dietary phosphate intake and left ventricular hypertrophy [37]. Earlier results from the Atherosclerosis Risk in Communities (ARIC) study showed an increase of 0.012 mm in carotid intima-media thickness (cIMT) for every 0.48 mg/dl increase in serum phosphate levels [31]. A second review of the ARIC study showed marked increases in hazard ratios of cardiovascular disease events as serum phosphate increased to levels >3.8 mg/dl [32].

Most disturbing are the results found in studies looking at the risk of developing CVD in the young and diabetic populations. Despite current public efforts to promote healthy diet choices and live a healthy lifestyle, increases in serum phosphate levels are associated with the development of CVD in both populations [33, 34]. It has to be asked what influence do poverty and the prevalence of phosphate-containing additives in low cost foods have on these populations.

Conclusions and Recommendations for Further Research

It is well established that phosphate-containing additives contribute to increases in serum phosphate levels in patients on dialysis to the point that those with late stage CKD are counseled to avoid or limit exposure. It has also been shown that intensive dietary counseling on the risks of additives, improves serum phosphate levels.

Emerging evidence also suggests that avoiding or limiting exposure to phosphate additives may be beneficial to the CKD population. However, the wide availability of additives in low cost food items and the lack of information concerning the phosphate content of these foods hamper efforts to keep dietary phosphate intakes low.

More studies are needed to fully understand the effects of different forms of dietary phosphate on phosphate mediating hormones and the risks for CVD in the general population. Required is a thorough examination of diets with equivalent amounts of total phosphate derived either from organic phosphate (that which is found naturally in foods) or inorganic phosphate (that which is found in phosphate-containing additives) to determine if exposure to phosphate is a modifiable risk factor for CVD.

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Dietary Phosphorus and Bone Disease

15

John J.B. Anderson and David W. Dempster

Key Points

- Too little phosphorus in the diet, especially a high Ca:P ratio (>1.5–1), may contribute to abnormal bone metabolism and structure.
- Too much phosphorus in the diet, especially a low Ca:P ratio (<0.5–1), may contribute to abnormal bone metabolism and structure.
- Parathyroid hormone and fibroblast growth factor-23 both increase to compensate for a low Ca:P ratio diet, i.e., high phosphate intake.
- A balanced intake of both phosphorus and calcium from foods and beverages is needed to support skeletal health, especially in adult life. Too little or too much phosphorus may induce abnormal bone tissue by altering homeostatic mechanisms that regulate serum phosphate and calcium concentrations.

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Introduction

Inorganic phosphate ions, HPO_4^{2-} (Pi), are required for the formation of hydroxyapatite in the skeleton, in teeth, and in ectopic calcifications. Both calcium (Ca) ions and Pi ions, along with other nonessential mineral cations and anions, form crystals via bone-forming cells, i.e., osteoblasts, or via bone-forming cells converted from smooth muscle cells in arterial walls and heart valves, or via tooth-forming cells, i.e., odontoblasts. Ectopic bone formation in arteries, heart valves, and other sites within the body results in hydroxyapatite crystals associated with an organic matrix of proteins, as found in normal bone formation (see Chap. 16). In addition, Pi ions in biological fluids are incorporated in many organic molecules that function intracellularly or extracellularly in the body. For example, Pi ions are used in the synthesis of nucleic acids, membrane lipids, and many intermediary molecules involved in energy metabolism, in additions to proteins at serine and threonine sites, and as intracellular messengers.

In general, the food supply of P, either in the mineral ionic phase or as part of organic structures of food constituents, is plentiful [1]. Practically all animal and plant foods have abundant amounts of P, and certain processed foods may contain the greatest amounts of P (see Chap. 4). Cola beverages, consumed by many, also supply large amounts of P. Of recent concern is the imbalance of intakes of Ca and P. A dietary molar ratio of Ca:P at approximately 1:1, or a mass ratio of Ca:P of 1.5:1, is considered optimal. In most diets in Western nations, such as the USA, the mass ratio approaches or even falls below 0.5:1 because of the consumption of too much processed food, snack foods, and other foods rich in P additives, which are used to enhance the appearance or otherwise improve the shelf life of these foods. In many parts of the world, the mass ratio of Ca:P may be as low as 0.2:1.

This chapter focuses on research findings relating chronic high P intakes to bone health and to the effects of Ca undernutrition on the bone, mainly from diets which are excessive with respect to P. Emphasis is placed on investigations of human adults with normal renal function, but considerable reference is made to studies of animal models. Adverse effects of a low-phosphorus diet, characterized as hypophosphatemic osteomalacia, are not covered herein except that one report [2] provided support for skeletal benefits in elderly osteoporotic women living in a nursing home in Lyon, France. Daily treatment with a calcium-phosphate supplement plus vitamin D over a minimal period of 6 months overcame the adverse skeletal damage resulting from the previously low Ca and P intakes. Supplementation of these older osteoporotic women with reasonably normal renal function improved their bone mass and density and also resulted in a significant reduction of hip fractures.

Homeostasis of Calcium and Phosphorus in Relation to the Bone

The homeostatic regulation of serum Ca and Pi intersects through the actions of PTH, FGF-23, and 1,25-dihydroxyvitamin D (calcitriol) which influence intestinal absorption, bone formation and resorption, and renal excretion [3]. Bone cells, i.e.,

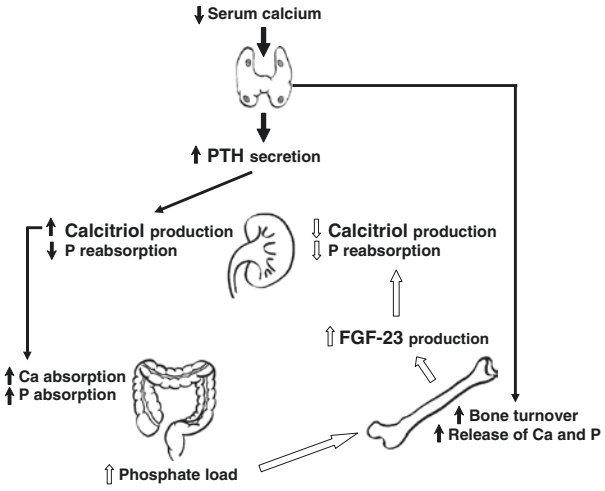

Fig. 15.1 Schematic figure of the effects of high P intake and absorption on serum PTH and FGF-23 concentrations; calcitriol effects are also shown. Postulated decreases in bone mass result from the action of PTH (From Wesseling et al. [53] with permission)

osteoblasts and osteocytes, synthesize FGF-23 when serum Pi concentrations become elevated. Osteocytes, which are the most abundant of the bone cells and are buried within bone tissue, produce FGF-23 in response to elevated serum Pi [4]. FGF-23 has three major functions, i.e., regulation of Pi excretion and suppression of calcitriol and PTH synthesis, and likely it has other roles that affect many cells of the body, with or without its cofactor Klotho. To exert its phosphaturic effect on the kidney, FGF-23 requires Klotho, which is a transmembrane protein expressed in renal tubules. Klotho acts as a co-receptor allowing FGF-23 to bind and activate the FGF receptor [5, 6]. The three major actions of PTH are bone resorption and the transfer of Ca and Pi from the bone to extracellular fluids and blood, the increase of Pi excretion by the kidneys, and the conversion of 25-hydroxyvitamin D (calcidiol) to 1,25-dihydroxyvitamin D (calcitriol) in the kidney. Figure 15.1 relates the hormonal changes to the bone.

The net effect of the hormonal actions is to maintain both serum Ca and Pi concentrations within normal ranges that support health. For serum Ca, the limits are narrow, and the total Ca concentration remains highly constant everyday for most of the adult lifetime, but for the fraction containing Ca ions, some flexibility in values is observed across a day in relation to the Ca and P content of meals [7]. For Pi, a circadian rhythm exists, and serum Pi concentration is modestly elevated when diets are high in P, such as following meals [8]. Figure 15.2 illustrates the circadian rhythm.

The normal serum concentrations of Ca and Pi are modestly perturbed by the absorption of P ions after the consumption of meals or cola beverages, but the perturbations are typically corrected by the abovementioned regulatory hormone actions within minutes to an hour after a meal or cola drink [7]. When these normal and fairly rapid adaptations occur, bone mass remains relatively constant, and any

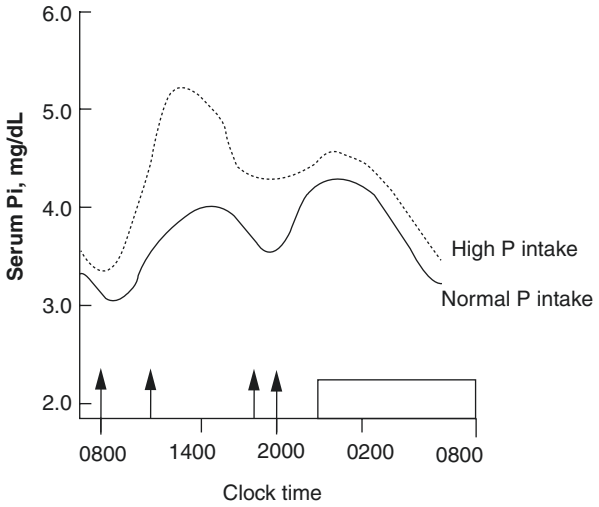

Fig. 15.2 The circadian rhythm of serum Pi concentration following a moderate intake of P compared to a high intake (From Anderson et al. [55] with permission)

amount of bone resorbed (lost) is replaced (zero balance) by an equivalent amount by bone formation [9]. Bone resorption, however, is much more rapid than bone formation, and so a lag time exists before restoration of bone mass is complete. These two processes, bone resorption and bone formation, occur in this order in adults, and they make the bone a dynamic tissue that for much of adult life adjusts to daily intakes of Ca and P. Bone mineral density (BMD) remains reasonably steady during much of adult life, but once peak bone mass is achieved by approximately age 30 years, BMD slowly declines over the next several decades. For postmenopausal women, the decline in BMD occurs at a considerably greater rate than for similarly aged men. In older adults when resorption exceeds formation, significant losses of bone mass contribute to a decline of BMD, which is accompanied by an increase in fracture risk, especially when diets are high in P [10]. Figure 15.3 shows these changes in BMD.

Adverse Skeletal Effects of High-Phosphorus Diets, Also Known as Phosphorus Loading

Usual Western diets are high in P and low in Ca, which translates to dietary Ca:P mass ratios of less than 0.5:1. An exception to this statement applies to vegan and many other types of vegetarian diets which typically have lower P content and healthier Ca:P ratios [11]. Low Ca:P diets stimulate both PTH and FGF-23 which increase in blood and then act on the kidneys to block Pi reabsorption, thereby increasing urinary excretion of Pi, but retaining Ca. PTH increases bone resorption, even when FGF-23 is elevated [12]. As far back as 1932, excessive P intake had been demonstrated to lower serum calcium concentration and stimulate PTH secretion [13]. This diet-induced disorder is also known as nutritional secondary hyperparathyroidism.

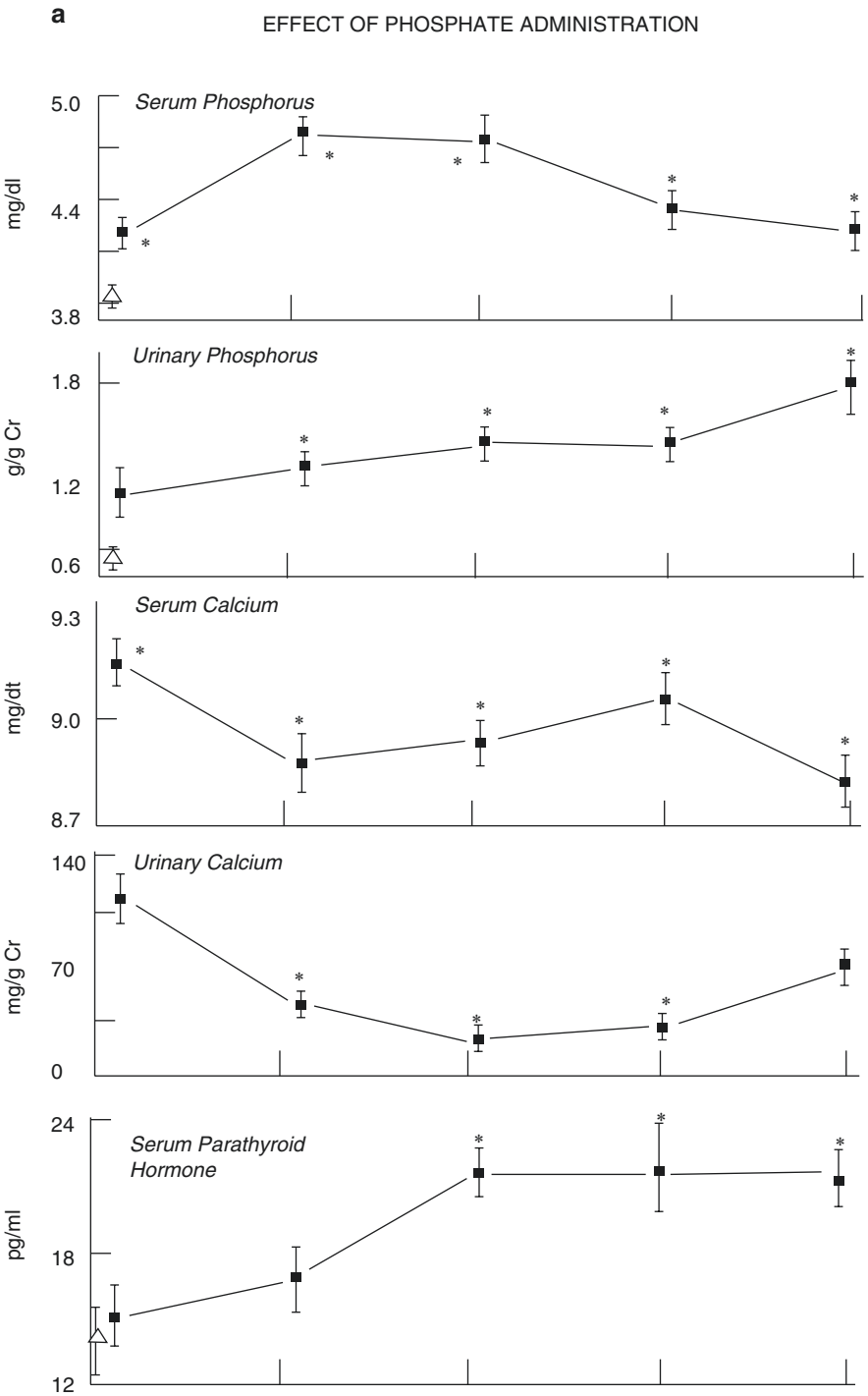

Fig. 15.3 Elevations of serum Pi and PTH and changes in other parameters after oral phosphate administration (From Silverberg et al. [10] with permission)

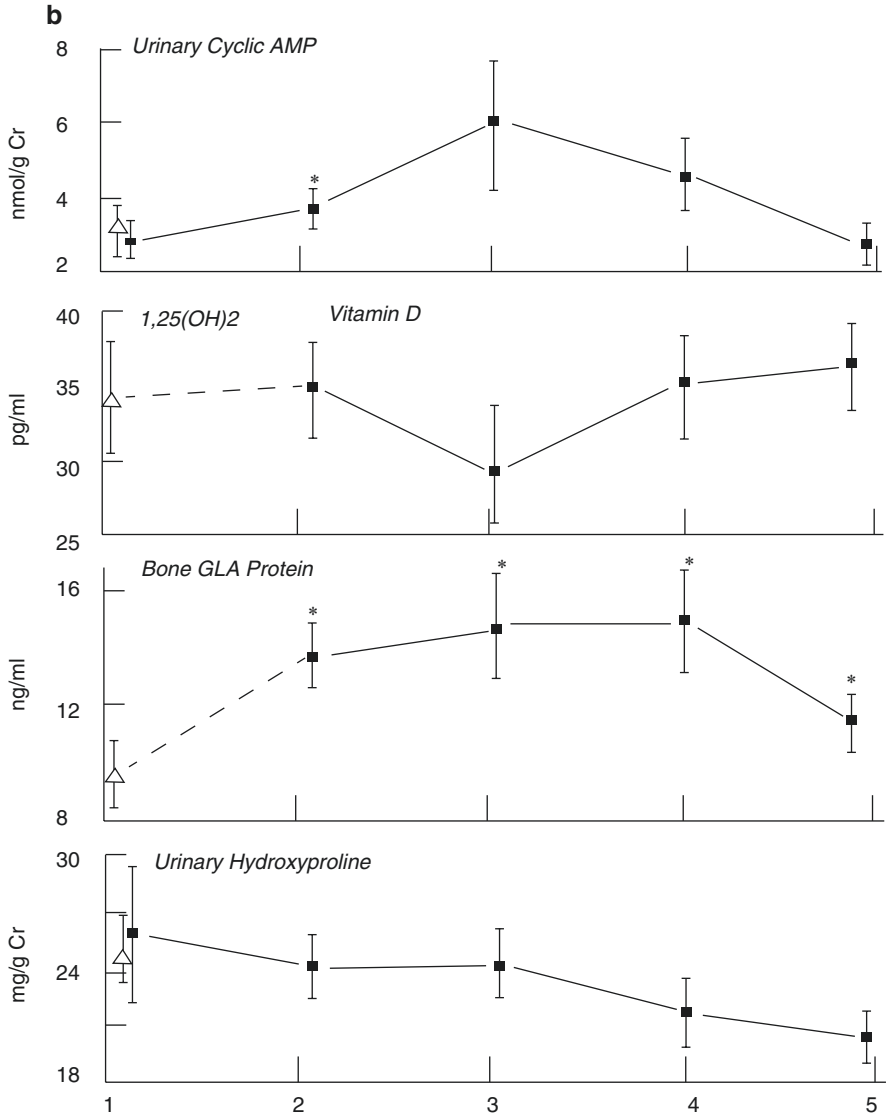

Fig. 15.3 (continued)

FGF-23 also feeds back on osteoblasts and osteocytes to inhibit further production of new bone. FGF-23 also feeds back on the parathyroid glands where it has been demonstrated to inhibit PTH synthesis and secretion [14]. In addition to reducing hyperphosphatemia after a P load, FGF-23 also inhibits renal calcitriol production and secretion which leads to a reduction in the intestinal absorption of both Ca and P [15]. Therefore, a modest elevation of dietary P results in several alterations of the normal homeostatic regulations of Ca and Pi, including a minor reduction in bone mass.

Phosphate Additives in Foods and Parathyroid Hormone Responses

Several reports over the last few decades suggest that US adults have been consuming increasing amounts of total P from foods, particularly from processed foods that contain additive phosphate minerals as functional ingredients [16–19]. The actual amounts of additives consumed, however, remain unknown because the Food and Drug Administration and similar agencies in other nations do not require food labels to state the total amounts of phosphate in processed foods, i.e., the naturally occurring amounts of P plus the quantity of additive phosphates. It remains unclear whether the P content of processed foods is currently increasing or has remained fairly constant. Worst-case scenarios for Ca:P mass ratios for the average diet of the US population most likely do not fall much below 0.4:1.

High phosphate intakes typically contribute to reductions in the dietary Ca:P ratio. In older adults with high phosphate food consumption, low Ca:P ratios were found in a cross-sectional population of US subjects studied in the NHANES 2005–2006 cohort [20]. Phosphate intakes of most subjects were greater than the current recommended dietary allowances for men and women over 50 years [21].

The increase in total dietary phosphate, especially from P additives, clearly has *acute* effects on calcium homeostasis by elevating serum PTH concentration in human subjects [22–30], but long-term *chronic* effects have been difficult to establish because of limited observational or experimental data from human investigations. Animal studies, however, have been very instructive in showing how severely damaging chronic low Ca:P mass ratios can be to the skeleton when the diets are inadequate in Ca [31]. Diet-induced hyperparathyroidism is a common theme among such reports.

Chronic high serum concentrations of Pi, presumably resulting from high P intakes, have been shown to be associated with an increased risk of mortality in individuals with or without chronic kidney disease [32]. The increase in P-related mortality is primarily from cardiovascular diseases, and a high serum Pi may be a critical factor in the mineralization of arterial walls [33].

Relationship of Dietary Calcium: Phosphorus Ratio to Bone

Sufficient intakes of Ca that provide a mass Ca:P ratio between 0.5:1 and 1:1 are needed to support bone health and, perhaps, reduce the risk of osteopenia and osteoporosis. Calcium-rich foods rather than Ca supplements are recommended to assure Ca adequacy in the face of an abundance of phosphate additives in the diet. Supplements are not recommended, except when prescribed by a physician for an established nutritional deficiency, because of the potential risk of arterial calcification [34], although this remains controversial [35].

Dietary phosphorus intakes in the USA typically exceed calcium intakes, except in users of calcium supplements, and the typical US adult intake by mass

Ca:P ratio falls close to 0.5:1 [16]. A more recent dietary assessment suggests that US adult intakes of calcium have increased [36]. Although phosphate salt supplements are not consumed orally, foods fortified with phosphate salts are commonly consumed in the USA [19]. Having a healthy dietary Ca:P ratio supports the maintenance of skeletal mass of adults if renal function remains close to optimal.

The question of whether the dietary Ca:P ratio has an important impact on bone mass and density remains [37]. A recent cross-sectional analysis of the Ca:P ratio assessed in the NHANES 2005–2006 data found no adverse effects of low ratios on BMD measurements of the hip (proximal femur) and lumbar spine of subjects 50 years of age and older [38]. The explanation for this somewhat surprising result is that, under chronic usual eating practices, both FGF-23 and PTH respond promptly to the elevated serum phosphate concentration after consumption of a meal is completed in individuals with normal renal function.

Bone Features of Nutritional Secondary Hyperparathyroidism

Evidence of the adverse skeletal effects of this disorder in human subjects has been limited because investigations have not lasted longer than 8 weeks, but long-term research results obtained using animal models support the deleterious changes of the bone from a chronic elevation of high-P-induced serum PTH, despite any effects of FGF-23 on phosphorus excretion which were not examined in these earlier reports. Diets consisting of low calcium and high phosphorus clearly stimulate the secretion of PTH which acts on the bone to increase resorption and on the kidney to reabsorb calcium while blocking phosphate reabsorption. The deleterious effects of a brief period of elevated PTH concentrations on mouse cancellous bone structure are illustrated in Fig. 15.4 [39].

In an early human investigation of seven postmenopausal osteoporotic women (63–75 years) given a phosphorus supplement for a period of 10–20 months, histomorphometric analysis of iliac crest bone biopsies revealed that bone-forming surfaces were decreased and bone-resorbing surfaces increased [24]. Serum PTH concentrations also were elevated in the study subjects. This report was the first one to demonstrate experimentally in human subjects that a high phosphate intake over periods approaching 1 year or longer contributes to elevated serum PTH and increased bone resorption.

The bone characteristics of nutritional secondary hyperparathyroidism, even under mild reductions in the Ca:P ratio (molar), are similar to osteitis fibrosa cystica and osteoporosis and, to a lesser extent, osteomalacia in adults. In a report on vitamin D-replete baboons, bone histomorphometry of iliac crest biopsies at 16 months featured increases in growth-plate thickness, osteoid volume, osteoid seam thickness, and mineralization lag time in the animals fed the low Ca-adequate P diet (ratio of 4 to 9 by weight) [40]. Also, at 16 months, osteoclast numbers, but not osteoblast numbers, were increased. After necropsy at 16 months, femur P content

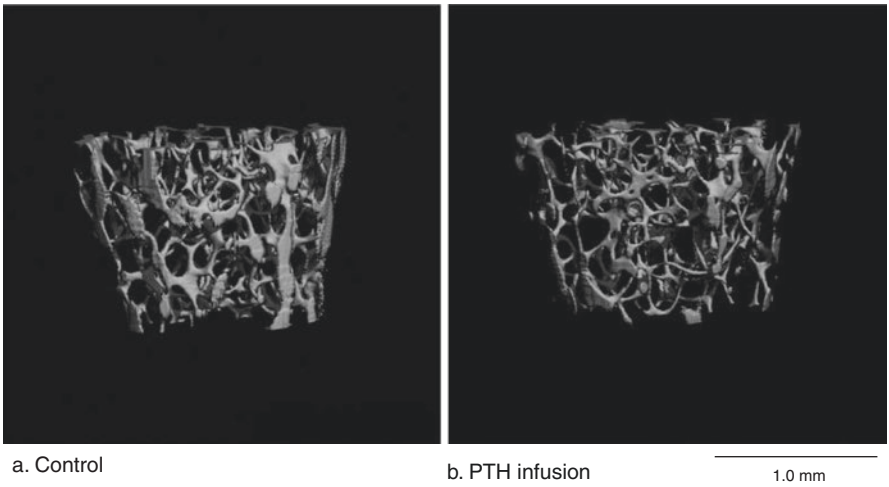

Fig. 15.4 Effect of PTH infusion on trabecular bone microarchitecture in mice (From Iida-Klein et al. [39] with permission)

was higher than in other groups fed moderate or high Ca (at constant P intakes), but the calcium content of this bone remained steady. Serum changes included increases in serum phosphate and alkaline phosphatase concentrations by the end of the study. The skeletal changes in the baboons were generally not apparent at 8 months, but they were clearly evident at 16 months. This long-term development of bone changes in the primates fed a mass Ca:P ratio of approximately 0.4–0.5 may serve as an experimental model for human skeletal changes in long-term consumers of moderately low Ca, but high P, intakes.

A follow-up study of nine calcium-deficient Gambian children (median age 8.5 years) consuming a molar Ca:P ratio of less than 0.33 demonstrated that the children had rickets-like leg deformities, but they did not have radiological signs of active rickets [41]. The persistent leg deformities of the calcium-deficient children occurred despite their higher serum 1,25-dihydroxyvitamin D concentrations that resulted from living near the equator with more than adequate exposure to ultraviolet B. Serum PTH measurements were not elevated in these African children, and no bone biopsies were taken for histomorphometric analysis. Renal phosphate wasting was attributed to the persistently elevated FGF-23 concentrations. The finding of the absence of a persistent rise in PTH in these children was not expected, and the adaptive elevation of FGF-23 to stimulate increased phosphate excretion on the low Ca:P diet is the first report on this phenomenon. A possible racial difference may exist in the adaptive mechanisms to low Ca:P ratio diets as racial differences in calcium and bone metabolism are well recognized [42, 43].

Short-term human studies [25, 26, 44] showed persistent elevations of serum PTH concentration when high-phosphate foods were added to the diet lowering the

overall Ca:P ratio to approximately 0.3 to 0.4 to 1. In addition, the report by Bell and colleagues in 1977 [44] included findings of significantly increased urinary hydroxyproline and cyclic AMP compared to subjects fed a control diet. Bone measurements were not performed because of the short duration of these studies.

Early studies of nutritional secondary hyperparathyroidism in animal models clearly demonstrated the chronic adverse skeletal effects of low Ca:P ratios [31]. Similar skeletal findings have been reported for several nonhuman species, including farm animals [45, 46]; dogs [23, 47, 48]; cats, especially zoo felines [49]; rodents [50, 51]; and simian apes [52] fed low-calcium/high-phosphate diets. Results of these earlier animal studies consistently support the hypothesis that a chronic low-mass ratio of Ca:P induces an increase in serum PTH concentration and resorption of the bone, although bone histomorphometry and bone mass or density measurements using DXA were not made.

Several subsequent animal reports provide biochemical and skeletal evidence of the progressive and long-term changes that result from low-mass Ca:P ratios, i.e., less than ~0.5–1. A few of these reports that included measurements of bone density or bone histomorphometry are highlighted. An investigation using adult dogs over a period of 40–60 weeks found a slight reduction in serum Ca and, surprisingly, small reductions in serum P concentrations in the face of an elevated serum PTH concentration [53]. In this same study, ulna cortical bone samples displayed significant increases in porosity that the authors attributed to the elevation of PTH. A study of growing rats demonstrated declines in bone mass and density of the femur, increased osteoclastic activity, and decreased bone strength following a low-Ca/high-P diet (mass Ca:P ratio of 0.5:1 or 0.3:1 in different experiments) [51].

If the dietary pattern of high P and low Ca becomes chronic, PTH is stimulated and acts to maintain serum calcium concentration within the normal range, but at the expense of the mass of both cortical and cancellous bone. Serum P concentration may rise, but in the animal experiments, this finding was not consistent, presumably as a result of the actions of both PTH and FGF-23-Klotho on the kidneys to increase P excretion. When this pattern of low Ca:P ratio diet becomes consistent, substantial bone loss contributes sequentially to osteopenia, osteoporosis, and fractures [7]. Prospective long-term observational human data, however, are needed to substantiate major losses of bone mass in response to P loading, in order to confirm studies in animal models. Only the long-term (16 months) investigation in baboons [41] and the 7-year study using monkeys [54] would be comparable to chronic high-P and low-Ca diets in human investigations. Research is clearly needed to rule in or out the potential adverse skeletal effects of P in individuals with normal kidney function and, definitely, in the many healthy elders with modestly reduced function, who may be adversely affected by high phosphate intakes or low calcium intakes or a combination of the two.

No human adult studies involving 4 or more weeks of participation on low Ca-high P intakes have been reported, and even the number of short-term human studies is limited. Reports of both long- and short-term human and primate studies are summarized in Table 15.1.

Table 15.1 The short- and long-term effects of high P intakes on calcium and Pi homeostasis, parathyroid concentration, and bone tissue

Report	Serum P	Serum Ca	Serum PTH	Bone changes
Long-term studies in primate (p) or human (h) subjects				
Goldsmith et al. [24] *h*	Elevated	Sl. decline	Increased	Modest bone loss
Pettifor et al. [40] *p*	Elevated	Sl. decline	Not measured	Features of NSH[a]
Braithwaite et al. [41] *h*	Normal	Normal	Lowered	Leg deformities (Rickets)
Anderson et al. [53] *p*	Elevated	Sl. decline	Increased	Modest bone loss
Short-term human studies				
Bell et al. [44]	Elevated	Declined	No change	Not assessed
Calvo et al. [25]	Elevated	Normal	Increased	Not assessed
Calvo et al. [26]	Elevated	Declined	Increased	Not assessed
Reiss et al. [22]	Elevated	Declined	Increased	Not assessed
Silverberg et al. [10]	Elevated	Declined	Increased	Not assessed

[a]Abbreviations: *NSH* nutritional secondary hyperparathyroidism including cortical bone loss, *Sl.* slight

Conclusions

Too much dietary P may have deleterious effects on bone mass and structure, and this dietary pattern may help contribute to the loss of bone mass observed with age and, hence, osteoporosis. A contrary report recently published, however, suggests that in older adults with normal renal function, hormonal homeostatic responses to high-phosphate loads respond appropriately to the excess dietary phosphate via renal excretion. Bone loss late in life is considered, at least in part, to occur because of the suboptimal adaptation of the homeostatic mechanisms to P loading from P-rich foods, especially additives, in the presence of inadequate Ca intakes, i.e., a Ca:P mass ratio below 0.5:1. The skeleton may respond to high P intakes by increasing PTH-directed resorption without sufficient new bone formation to replace the lost bone, especially in individuals with declining renal function. A decline in bone mass is accompanied by lower bone density and increasing porosity which may result in fractures of lumbar vertebrae, wrist bones (radius and ulna), ribs, and hips. This view of the effects of a pattern of excessive intake of P on the skeleton remains, however, incomplete because long-term, prospective human studies have not been undertaken.

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Phosphorus and Cardiovascular Disease

16

Markus Ketteler

Key Points

- Large epidemiological studies suggest that elevated serum phosphate concentrations are associated with all-cause and cardiovascular (CV) mortality, especially in patients on dialysis.
- Hyperphosphatemia is strongly linked to cardiovascular calcification by "osteochondrogenic transdifferentiation" of vascular smooth muscle cells (VSMC) leading to bone-like remodeling of the arterial vessel wall.
- Dietary phosphate restriction and phosphate binder treatments are recommended to lower serum phosphate and modify the risks associated with hyperphosphatemia, but randomized controlled trials are currently still missing, which prove the concept that phosphate lowering leads to improved longevity and prevents cardiovascular events.

Introduction

The global prevalence of chronic kidney disease (CKD) is currently estimated to be around 7 % in adults, with a higher prevalence (approx. 30 %) in people above 65 years [1]. CKD is a major determinant of poor outcomes among patients with major noncommunicable diseases and therefore represents a substantial worldwide health burden. Hyperphosphatemia is an unavoidable clinical consequence especially in

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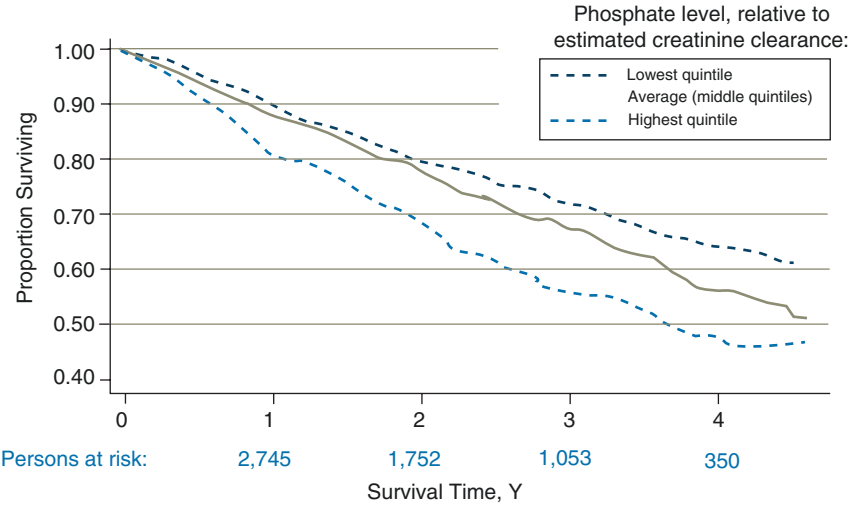

Fig. 16.1 Survival plots by serum phosphate group relative to estimated GFR in CKD patients not on dialysis ($n = 3490$; Ref. [4])

the advanced stages of CKD and linked with some serious clinical complications, including cardiovascular (CV) calcification, left ventricular hypertrophy and failure, as well as increased all-cause and cardiovascular (CV) mortality [2].

Large observational studies have shown a graded association between levels of serum phosphate and all-cause mortality in patients undergoing dialysis. For example, Block et al. studied a cohort of 40,538 hemodialysis patients and determined, after multivariate adjustment, that 12 % of the 10,015 observed deaths in this cohort were strictly associated with hyperphosphatemia [3]. Kestenbaum et al. subsequently published similar observations also in CKD patients not on dialysis. A total of 6730 CKD patients were first included for analysis, with 3490 of them having at least one serum phosphate measurement during the one and a half years prior to inclusion [4]. These data demonstrated that serum phosphate levels remained within the normal range until a GFR of 40 mL/min was reached, but from this point, mean phosphate levels started to increase rapidly with decreasing GFR in growing proportions of this cohort. Serum phosphate levels above 3.5 mg/dL and each subsequent 0.5 mg/dL increase in serum phosphate levels were found to be associated with an increased mortality risk (Fig. 16.1). Moreover, the association between high phosphate concentrations and higher mortality does not even seem to be restricted to patients with renal disease – it can also be observed in people with cardiovascular disease and even in the general population. High-normal serum phosphate concentrations are associated with coronary calcification in young, healthy men and were a significant predictor of cardiovascular events in the Framingham study [5]. Elevated mortality in association with high-normal serum phosphate concentrations was predominantly observed in persons with coexisting cardiovascular disease who had normal renal function. In the Framingham study, 375 of the 4127 subjects died

within 60 months; the adjusted mortality risk was 22 % for each 1 mg/dL increase in serum phosphate concentration. This chapter outlines the pathophysiological link between hyperphosphatemia and cardiovascular disease and reviews the currently available study evidence on phosphate management in different stages of CKD.

Phosphorus, Vascular Calcification, and Vessel Wall Remodeling

Strong and unidirectional associations exist between the severity of cardiovascular calcifications and mortality in patients with advanced chronic kidney disease (CKD) [6]. In the past 10 years, a wealth of experimental and clinical information has been published focusing on the key pathophysiological events that might trigger the development and progression of vascular and soft-tissue calcifications. These processes involve a sensitive balance of calcification inhibition, induction, and removal, including the loss of endogenous calcification inhibitors, deficient clearance, actions of vitamin K and vitamin D, and active inducers such as phosphate- and calcium-triggered osteogenic transdifferentiation [7–9]. The latter process is strongly linked to increases in extracellular phosphate concentrations.

In 2000, Jono et al. published the groundbreaking experimental observation that increasing phosphate concentrations in vitro trigger a reprogramming of cultured human arterial vascular smooth muscle cells (VSMC) into an osteoblastic phenotype [10]. The higher the extracellular phosphate concentration, the more phosphate was found to enter the cell (actively transported via the sodium-dependent phosphate transporter PiT-1), with the consequence of downregulating VSMC-specific genes and inducing the osteoblast transcription factor RUNX2 (cbfa-1). This so-called osteochondrogenic transdifferentiation of VSMC was followed by the expression of several bone-specific proteins (osteopontin, alkaline phosphatase, osteocalcin, and collagen-1) and by releasing precalcified matrix vesicles into the vessel wall (Fig. 16.2).

A later study using epigastric arteries surgically obtained from adult patients on dialysis demonstrated that this VSMC phenotype change indeed occurs in humans, since RUNX2 and alkaline phosphatase expression could be detected by immunohistochemistry and in situ hybridization strictly limited to areas of vascular calcification [11]. A uremic environment seemed to accelerate this process, whereas the calcification inhibitor fetuin-A effectively antagonized the production and secretion of such matrix vesicles in vitro. More recently, Shroff et al. again studied epigastric as well as omental arteries, but this time these vessels were obtained from three groups of children during indicated surgical procedures [12]: the first group had normal renal function, the second group included children in predialysis CKD stages, and the third group were children on dialysis. These atherosclerosis-free arterial samples were subsequently cultured as intact rings for a total 3 weeks and exposed to both normal and elevated concentrations of phosphate and calcium, respectively. The key findings of this approach were that the more advanced the CKD stage, the more severe was the calcification deposition in the vessel wall, and that phosphate and calcium synergistically potentiated each other's effects.

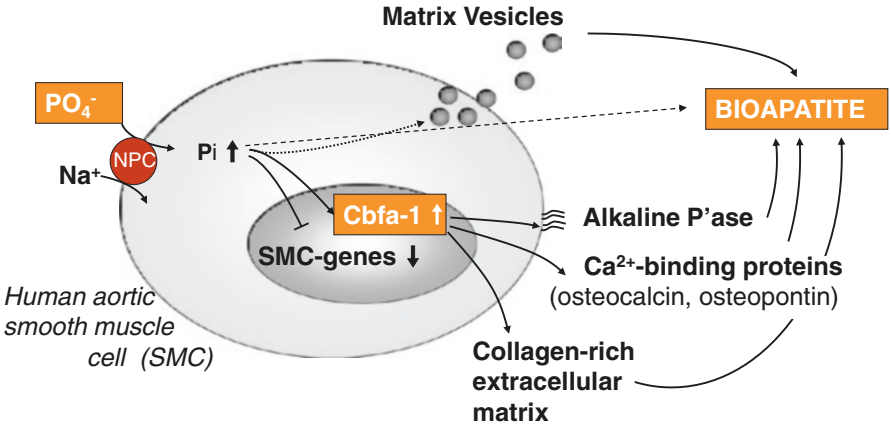

Fig. 16.2 Hyperphosphatemia is a prerequisite for an active uptake of phosphate into vascular smooth muscle cells (VSCM). The consecutive intracellular increase of phosphate concentrations leads to a reprogramming of the VSMC into an osteoblast-like phenotype. This process is termed osteochondrogenic transdifferentiation (Ref. [7])

Independent of the deleterious effect of high phosphate on VSMC, recent studies have shown that high phosphate levels directly affect endothelial function both in vitro and in vivo. For example, Shuto et al. showed that endothelial-derived nitric oxide production may be directly impaired with dietary phosphate loads, leading to compromised forearm dilatation when performed under standardized conditions [13]. Di Marco et al. demonstrated annexin II downregulation and inhibition of endothelial-dependent angiogenesis following phosphate exposure in vitro [14]. The results of these two studies would potentially put phosphate into a similar corner as smoking and LDL-cholesterol, with regard to its vasculotoxic effects. Further concepts argue that phosphate is among the key signaling molecules of aging, connected to the fibroblast growth factor-23 (FGF23)/klotho system, as reviewed elsewhere in this text, and CKD may indeed be a process of premature aging [15].

Phosphorus and Calciprotein Particles (CPP)

Our general perception is that extraosseous vascular calcification is irreversible. However, extraosseous calcification may be a more dynamic process than previously anticipated. In a prospective study, small iliac artery samples were harvested from patients on dialysis at the time, when they were receiving a kidney transplant [16]. In these arterial samples, precalcified matrix vesicles could be visualized as they were thought to be induced by local osteochondrogenic

transdifferentiation. Such vesicles showed a core-shell structure (diameter 20–500 nm) and were composed of hydroxyapatite and whitlockite, thus representing calcium phosphate nanocrystals. However, these structures also contained proteins such as fetuin-A, albumin, and other acidic proteins and were later termed calciprotein particles (CPP) [8]. Heiss et al. were then the first to study the properties of CPP in ascitic fluid from a patient with peritoneal dialysis-related calcifying peritonitis [17]. Hamano et al. by using ultracentrifugation for harvesting such particles from the sera of patients on dialysis and from predialysis patients with diabetes mellitus found them to be composed of fetuin-A, fibrinogen, fibronectin 1, and calcium phosphate nanocrystals [18]. Stimulated by these insights, Pasch et al. recently developed a nanoparticle-based assay detecting, in the presence of artificially elevated calcium and phosphate concentrations, the spontaneous transformation of spherical colloidal primary CPP to elongate crystalline secondary CPP [19]. While primary CPP may be a beneficial mineral chaperone removing and transporting calcification debris within the body into the bone or the reticulohistiocyte system, secondary CPP may be irreversible and toxic compounds, which damage vessels and organs. Phosphate accelerates the transformation of primary into secondary CPP potentially representing another culprit with regard to the cardiovascular risk pattern associated with hyperphosphatemia.

Phosphorus Management According to Guidelines

Current KDIGO ("Kidney Disease – Improving Global Outcomes" initiative) guidelines suggest maintaining serum phosphate levels in the normal range in CKD stages 3–5 and lowering serum phosphate levels toward the normal range in dialysis patients [20]. These recommendations are not based on data from randomized controlled trials, since those are currently not available, but on the biological plausibility of phosphate's cardiovascular toxicity, as outlined above, and on a large amount of observational studies unanimously pointing to a strong cardiovascular and mortality association with hyperphosphatemia.

Dietary restriction of phosphate has long been a cornerstone of therapy for disordered mineral metabolism and should still be implemented in the management of hyperphosphatemic CKD patients according to guidelines [20]. Because dietary measurement on its own is usually not fully efficient to control and restore a neutral phosphate balance, and further carries a risk toward protein malnutrition, phosphate binders are considered the most important tool in the treatment of hyperphosphatemia. While selective exclusion of phosphate additive-rich food items may become a more powerful dietary approach than general phosphate restriction in the future, adjunctive therapy with oral phosphate binders is recommended in the current guidelines for most patients with advanced CKD and elevated serum phosphate levels [20, 21]. Caution is advised with regard to calcium-containing phosphate binders in situations of hypercalcemia, preexisting cardiovascular calcifications, low parathyroid hormone serum levels, and proven adynamic bone disease [20]. This is

because these conditions favor the development and progression of cardiovascular calcifications and because calcium may be a synergistic pathophysiological cofactor to hyperphosphatemia in this process.

Types of Phosphate Binders and Outcomes

Numerous studies have investigated the safety and efficacy of oral phosphate binder therapy in patients on dialysis, but definite evidence from a randomized controlled trial, however, is still not available. However, and especially in dialysis patients, such hard outcome trials with, for example, targeting elevated versus much lower serum phosphate levels over prolonged periods of time are thought to be of borderline or even no ethical justification, given the strong risk associations between hyperphosphatemia and death. Nevertheless, similar expectations were present when studies were initiated testing hemoglobin normalization by erythropoiesis-stimulating agents (ESA), with the surprising results that no sustainable benefit (and even some danger signals) arose from such intervention [22–24].

Despite investigating hard outcomes, the majority of prospective studies targeted the surrogate outcome cardiovascular calcification in a design in which calcium-free vs. calcium-containing binders were compared. The Treat-to-Goal (TTG; prevalent dialysis patients) and the Renagel in New Dialysis (RIND; incident dialysis patients) showed that treatment with the calcium-free phosphate binder sevelamer slowed the progression of coronary artery calcification versus calcium-containing binders, but other studies were less equivocal of these findings [25]. Meanwhile, three studies primarily reported on the survival associated with these two treatment options in hemodialysis patients. The Dialysis Clinical Outcomes Revisited (DCOR, prevalent dialysis patients) trial was negative and could not demonstrate a survival advantage for patients treated with sevelamer, with the exception of the subgroup of patients above the age of 65 years [26]. A predefined secondary analysis of the RIND trial did find a survival benefit for patients treated with sevelamer in a post-hoc open label extension of the study [27]. More recently, di Iorio et al. (the INDEPENDENT investigators) published prospective study results from incident hemodialysis patients ($n=466$ observed for 24 months) demonstrating improved survival with sevelamer treatment when compared to calcium carbonate [28]. In this study, however, phosphate control turned out to be superior with sevelamer, which may have significantly and positively affected this comparison.

Jamal et al. performed a meta-analysis merging together all available all-cause mortality data from prospective controlled trials comparing calcium-containing (calcium acetate, calcium carbonate) versus calcium-free phosphate binders (sevelamer-HCl and -carbonate, lanthanum carbonate) [29]. A total of 11 trials could be identified involving a total of 4622 patients. This systematic review showed a significant 22 % all-cause survival advantage in favor of the calcium-free compounds. Due to the nature of the raw data, no selective analysis on cardiovascular mortality could be performed, and it remained unclear, whether there were any more detailed differences between the potency of the different phosphate-binding agents. Further,

in these studies relatively high monotherapy doses had been used, so it remains unclear, if there may be a dosage threshold or if binder combinations may impact on endpoints.

Phosphate Lowering in Patients Not on Dialysis

The TTG, RIND, and DCOR trials exclusively included patients on hemodialysis, and the majority of patient data entered into the meta-analysis by Jamal et al. were also derived from dialysis patients. The situation in CKD patients was just recently approached in more depth and may be more complex than anticipated.

As phosphate metabolism is disrupted early in the course of CKD, a rationale may exist for treatment of this disorder as promptly as possible. While Russo et al. had already shown benefits of the calcium-free phosphate binder sevelamer on calcification progression in CKD patients not on dialysis vs. calcium-containing binder treatment or no binder treatment, di Iorio et al. recently published the first randomized hard outcome trial in CKD patients not on dialysis ($n=212$ observed for 36 months, serum phosphate concentration 3.5–5.5 mg/dL at baseline) comparing sevelamer vs. calcium carbonate [30, 31]. A significant survival advantage was observed with sevelamer, associated with ameliorated calcification progression. Due to its relatively small cohort size, this study may require confirmation by a larger trial approach.

Despite such suggestive and impressive data, there are also some adverse signals with regard to phosphate binder use in CKD patients not on dialysis. Block et al. performed a prospective, randomized, placebo-controlled pilot study of 148 patients in CKD stages 3b and 4 assigned to sevelamer carbonate, lanthanum carbonate or calcium acetate, respectively, with an observation period of 9 months [32]. Patients were not overtly hyperphosphatemic, but had serum phosphate levels within or just above the normal range. There were only minor decreases of serum phosphate concentrations, but a 22 % drop in urinary phosphate excretion due to binder treatment. Unexpectedly, patients on phosphate binders developed significantly higher progression rates in coronary artery (80.6 % versus 18.1 %, $p=0.05$) and abdominal aorta (15.4 % versus 3.4 %; $p=0.03$) calcification scores than the placebo cohort. When separating the three different phosphate binder groups, most of this calcification progression was found in the calcium acetate subgroup, but both calcium-free treatment groups were by no means better than placebo.

In a similar line, Hill et al. investigated calcium and phosphate balance in a small cohort of CKD stage 3b–4 patients under metabolic ward conditions [33]. Patients received prepared diets, which contained approximately 1 g calcium and 1.4 g phosphate per day, distributed over three daily meals. In a crossover design, these meals were then served with or without 500 mg calcium carbonate, each of these two periods lasting for 1 week. The main result of this study was that these patients were found to be in a neutral calcium and phosphate balance when calcium carbonate was not added to the diet. However, addition of the phosphate binder caused a positive calcium balance while phosphate balance even remained unchanged. These

results would be in complete accordance with the observations by Block et al. concerning the association between calcium-containing phosphate binder treatment and calcification risk in CKD patients not on dialysis. From this current background, differentiated recommendations with regard to the use of phosphate binders in early CKD remain uncertain at this time. Theoretically, fibroblast growth factor-23 (FGF23)-guided therapeutic decision making may help us out of this dilemma to identify those CKD patients who are at risk in the future [15]; however, we again first need to be provided with convincing prospective data on such an approach.

Phosphate Binder Treatment and Survival

Upto now, no prospective, placebo-controlled, randomized trial evaluating phosphate binder therapy is available. As already pointed out above, with regard to the situation in dialysis patients, it is widely thought that such a protocol may be unethical, because of the strong biological plausibility that hyperphosphatemia causes progressive cardiovascular calcification and because of the unanimous mortality association seen in observational trials. However, experiences in the field of renal anemia demonstrate that observational data may be misleading and that therapeutic approaches could be less powerful than initially anticipated [22–24].

Isakova et al. were the first to report on a mortality association of phosphate binder treatment based on a nested analysis of a cohort of 10,044 incident dialysis patients from the Accelerated Mortality on Renal Replacement (ArMORR) study [34]. The comparison was made between patients who were started on phosphate binders within the first 3 months after the start of dialysis and those who were not. Phosphate binder-treated patients showed a superior outcome (1-year mortality risk reduction between 18 % and 30 % in the as-treated population, dependent on adjustments). This effect was not dependent on the presence of hyperphosphatemia, but strongest in patients with higher phosphate levels. This study was not able to discriminate the efficacy of single binders. The second report came from the Dialysis Outcomes and Practice Patterns Study (DOPPS) on prevalent dialysis patients ($n = 23{,}898$) [35]. Again, a 25 % survival advantage could be documented with phosphate binder therapy vs. without such treatment for patients with phosphate levels above 3.5 mg/dL, and after adjustment for nutritional parameters (serum albumin level, nPCR, creatinine, BMI, cachectic appearance), this effect decreased to 12 % but still remained significant.

Finally, data from the Current management Of Secondary hyperparathyroidism: A Multicenter Observational Study (COSMOS) trial recently indicated into the same direction (Fig. 16.3) [36]. The COSMOS study is a multicenter, open-cohort, noninterventional prospective study based on a random sample of the European dialysis population ($n = 6797$; carried out in 227 dialysis centers from 20 European countries). Following multivariate analysis, patients prescribed phosphate-binding agents showed a 29 % and 22 % lower all-cause and cardiovascular mortality risk, respectively, and all single and combined therapies with phosphate-binding agents, including calcium-containing binders, except aluminum salts, showed a beneficial

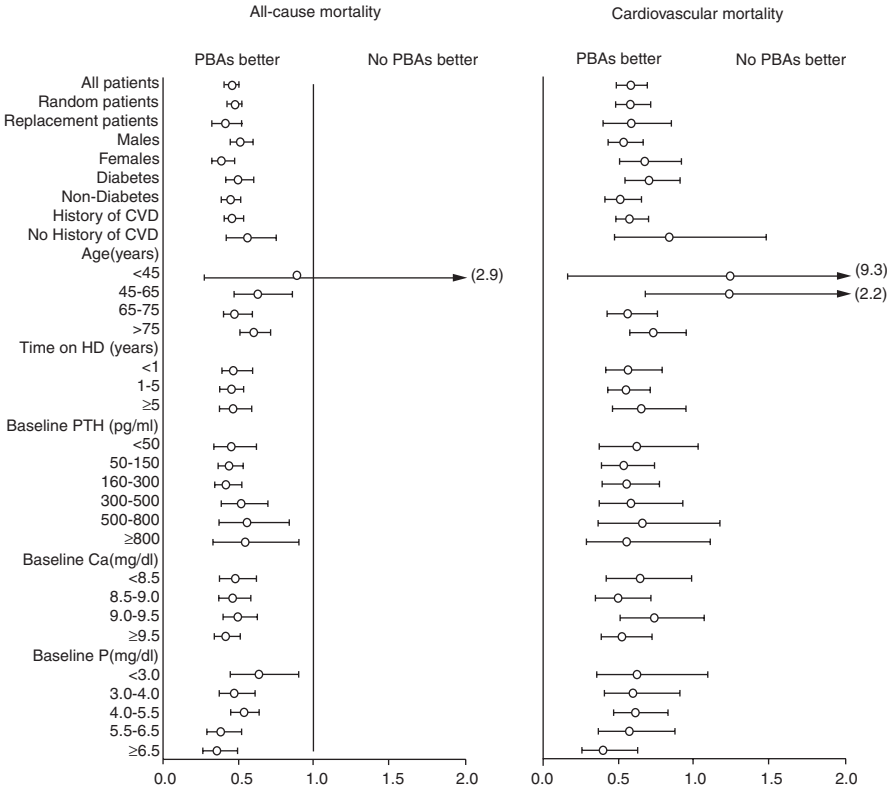

Fig. 16.3 The COSMOS trial was a prospective noninterventional study based on a random sample of the prevalent European dialysis population ($n = 6797$) with a follow-up of 3 years. Clinical practice of CKD-MBD treatment was recorded. The use of phosphate binders vs. no binder treatment was found to be associated with significantly improved outcomes, largely independent of baseline phosphate levels (Ref. [36])

association with survival. So the epidemiological evidence is unidirectionally and unequivocally in favor of phosphate-lowering therapies in dialysis patients with regard to hard outcomes, but since randomized controlled trials are missing, this evidence remains weak.

It may indeed be considered unethical maintaining hyperphosphatemic dialysis patients without binder therapies for months to years in order to perform an appropriate prospective trial on all-cause mortality and cardiovascular events. However, this situation is different in CKD patients not on dialysis. Although we have reasons to believe that patients with high normal serum phosphate levels or only modest hyperphosphatemia (or elevated FGF23 serum concentrations?) may benefit from phosphate-lowering approaches, we have no proof for such a concept and even a few adverse signals [32, 33]. Such a prospective, randomized placebo-controlled trial on the effects of phosphate binders in CKD patients not on dialysis could potentially look at a composite endpoint of all-cause mortality, progression

of CKD, cardiovascular events, and hospitalizations. Concerning the design of the "active arm," it would possibly be optimal to compare a calcium-free with calcium-containing phosphate binding.

Conclusions

More than any other population, patients with CKD often develop severe and progressive extraosseous and particularly cardiovascular calcifications. In this regard, the magnitude of calcification in patients with CKD quite strictly correlates with impaired outcomes, including mortality and cardiovascular events. Thus, preventive and therapeutic strategies slowing or even regressing calcification play a key role in the management of this population. We clearly see signals that hyperphosphatemia may be one of the key culprits in the pathophysiology of progressive cardiovascular calcification and may further be related to endothelial damage and adversely interfere clearance mechanisms of early crystal deposition (via calciprotein particles; CPP). In this context, there is now also growing evidence that exposure to calcium loads may be harmful, in both the dialysis population and in CKD patients not on dialysis. Observational data suggest that phosphate binder treatment generally seems to be related to improved outcomes, at least in the dialysis population and relatively independent on baseline serum phosphate levels. However, no randomized controlled trials are currently available providing definite proof that phosphate binders or reaching some serum phosphate target levels may improve longevity and protect from cardiovascular events. While such studies may not be feasible anymore in the dialysis population, they seem more than justified in patients not on dialysis, and significant efforts should be done in order to design and realize such protocols.

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Phosphorus and Malignancies

17

George R. Beck Jr.

Key Points

- Phosphorus, usually in the form of inorganic phosphate (Pi), is abundant in the Western diet and represents a dietary element that has the potential to influence multiple facets of cancer etiology and progression.
- High serum Pi levels and dietary Pi consumption has direct cellular and molecular functions as well as the potential effects of Pi-responsive endocrine/paracrine/autocrine factors that promote noncancerous cell growth and cancerous transformation to malignancy.
- The possibility that Pi acts as an important systemic signaling molecule capable of altering cell behavior through changes in transcription, protein modifications, and general energy balance suggests a novel target for cancer prevention, however these concepts have only begun to be addressed.

Introduction

Traditionally, inorganic phosphate (Pi) has been thought of as a required but passive ion for critical cell functions; however, recent studies suggest a more active role in the regulation of cell growth processes. Cell-based studies have revealed that excess Pi availability alters growth properties associated with cancer through specific

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signal transduction pathways and gene expression. Recent studies in animal models of cancer concur with the cell-based studies and identify dietary Pi consumption as capable of modulating tumorigenesis. Although the mechanisms remain to be fully elucidated, a number of circulating factors are known to respond to changes in dietary Pi consumption and may act in concert with cell autonomous effects. Collectively, results suggest that increased consumption or changes in serum Pi levels may be a predisposing risk factor to increased cell growth potential leading to malignancy.

Dietary and Serum Phosphorus and Carcinogenesis

Carcinogenesis encompasses the processes by which a normal cell becomes cancerous and is often considered to comprise three basic steps, proliferation, promotion and progression (transformation), and ultimately malignancy or metastatic spread. Each of these steps might require multiple somatic mutations as well as changes in the microenvironment to promote cancerous cell growth. Key functional events include self-sustained proliferation with lack of senescence control, enhanced survival or resistance to apoptosis, angiogenesis, invasion through surrounding connective tissue, and finally growth at distant sites (malignancy). In addition to somatic events, the influence of the tumor microenvironment, including the influence of factors secreted from noncancerous neighboring cells on cancer progression has recently gained appreciation. A general estimate of the potential contribution of diet to cancer ranges from 10 to 70% [1]. However, in most cases a sufficient understanding of how individual nutrients affect tissue function does not exist to generate consensus dietary recommendations for prevention of cancer. The events leading to malignancy can often take multiple decades making the study of how diet and individual nutrients influence cancer challenging. Phosphorus, usually in the form of inorganic phosphate (both forms referred to as Pi for ease of use), is critical to cell function and changes in systemic Pi levels result in changes in endocrine/paracrine/autocrine factors. Therefore, Pi might influence both the somatic as well as the microenvironment aspects of carcinogenesis. Because of the challenges associated with study of the nutritional influences on cancer in humans, mouse models are commonly used. Two recent studies in mice have investigated the possibility that altering dietary Pi consumption would alter carcinogenesis.

Effects of Dietary Pi on Carcinogen-Induced Skin Cancer

To investigate the potential effect(s) of altered dietary Pi on cancer initiation and promotion, a model of carcinogen-induced cancer was used. The two-stage skin carcinogenesis model induces papilloma formation by treating the skin with a carcinogen (DMBA) followed by weekly treatment with a tumor promoter (the phorbol ester TPA); papilloma formation occurs between 10 and 20 weeks after initiation of treatment. This cancer model was used in combination with diets that contained

high Pi (1.2 %) or low Pi (0.2 %) levels, approximating 1800 mg/day or 500 mg/day intake, respectively, in humans. The diets were designed to be isocaloric and contained equal amounts of calcium (0.6 %). Nineteen weeks after carcinogen initiation, papilloma number, multiplicity, and size were recorded [2]. The mice on the high Pi diet exhibited no difference in weight compared to the low Pi diet but a significant increase in serum Pi and decrease in serum calcium. These mice also had significantly elevated serum parathyroid hormone (PTH) and osteopontin (OPN), a secreted cytokine-like factor (discussed in more detail below). The mice fed the high Pi diet developed twice as many papillomas as mice fed the low Pi diet and these papillomas developed at an earlier time point and were initially larger [2]. The data therefore suggests that a high Pi diet alters both initiation and progression of tumorigenesis.

Effects of Dietary Pi on Oncogene and Carcinogen-Induced Cancer

A second commonly used model to study cancer in mice is the K-*ras*^{LA1} model of spontaneous lung cancer [3]. These mice develop lung cancer due to a latent transgene that through spontaneous recombination results in an activate K-ras (G12D) allele and production of a constitutively active protein. The model represents an example of oncogene-driven tumorigenesis. In this study, K-*ras*^{LA1} mice, at 5 weeks of age, were fed a normal (0.5 %) or high Pi diet (1.0 %) (~750 and 1500 mg/day intake) for 4 weeks. Assessment of animal health suggested no significant change in animal weight and a significant increase in serum Pi. Analyses of lung tumors revealed a significant increase in both tumor number and diameter in mice on the high Pi diet [4]. Histological analyses of the lungs identified significantly increased proliferation as measured by PCNA labeling index in mice on the high Pi diet. Interestingly, this group also identified an increase in lung tumor number in K-*ras*^{LA1} mice in mice fed a low very Pi diet (0.1 %) compared to the normal Pi diet (0.5 %) [5]. The results raise the possibility of a U-shaped curve regarding Pi intake and risk of tumorigenesis. Taken with the data from the skin cancer model, the results suggest that dietary Pi consumption is a potential risk factor for cancer initiation and progression, modulating both carcinogen and oncogene-induced tumorigenesis.

Dietary and Serum Pi and Cancer Risk in Humans

To date, few epidemiological studies have investigated Pi consumption or serum Pi as primary outcomes for cancer risk. Investigations surrounding a potential nutritional link to prostate cancer have focused on calcium and vitamin D with Pi intake being measured as a secondary parameter in a number of these studies. These cohort and case control studies had a relatively small sample size and produced conflicting results with some finding modest increased risk correlated with Pi consumption and others finding a modest decreased risk (reviewed in [6]). It should be

noted that most of these studies were based on estimated Pi consumption from dietary questionnaires which are thought to underestimate actual Pi intake [7, 8]. Further, Pi consumption does not necessarily reflect serum Pi levels which are not commonly measured. Recently, the association between serum Pi and risk of cancer was analyzed in a population-based observational assessment of the Swedish Apolipoprotein Mortality Risk (AMORIS) study [9]. Multivariate Cox proportional hazard regression analyses were used to assess the relationship between serum Pi and cancer risk at specific sites in 397,292 study participants followed for 12.75 years (mean). The analyses were adjusted for age, gender, and socioeconomic status and additional factors used in the multivariable models included creatinine, lung disease, and season at time of baseline measurement. The report found a statistical correlation with increasing serum Pi quartiles in men and increased risk of pancreatic, lung, thyroid, bone, and "other" cancers, and an association with liver and gallbladder only in the highest quartile [9]. In women high serum Pi was correlated with increased risk of esophageal, lung, and non-melanoma skin cancer and an association with stomach and bone cancer only in the highest quartile. Interestingly, an inverse relationship between serum Pi and cancer risk was found for breast, endometrial, and "other" endocrine cancers with the highest serum Pi levels associated with reduced risk. The results suggest a possible association with estrogen and, in fact, estrogen has been demonstrated to negatively regulate Pi transport in the kidney reducing Pi reabsorption in humans and rodents [10–12]. The decline in estrogen levels induced by menopause results in increased serum Pi levels in healthy women [13, 14]. Therefore, one could speculate that the inverse relationship between serum Pi and endocrine cancers in women may be reflective of estrogen levels (not analyzed in this study). In summary, these epidemiological links between Pi and cancer are associative and more studies are needed before causality can be established.

Pi-Induced Cellular and Molecular Effects; Cell Growth, Proliferation, and Transformation

It is becoming increasingly apparent that diet can have profound effects on functional genomics and represents an area of research that has yet to be exploited for potential health benefits. Pi represents a common dietary element that may directly alter cell phenotype by changes in cellular and molecular processes. In early life, a period of rapid growth, dietary Pi is critical for bone and mineral formation and energy metabolism. Weanling rats fed a low Pi diet (0.2 %) have reduced bone mineralization rates [15] and severely restricted young rats (0.02 %) die within 8 weeks [16]. Interestingly, in adult animals a high Pi diet is thought to be detrimental to bone [17]. The results suggest a differing and complex relationship between cell/tissue Pi needs during periods of organismal growth and senescence. Studies using drosophila as a model to investigate Pi sensing pathways also found a dependence of early development on sufficient Pi. However reduced cellular uptake of Pi by

adults favored an extended life span similar to mouse models and humans with kidney disease [18], suggesting an evolutionarily conserved significance of Pi requirements associated with age. The fact that Pi is tightly linked to cell functions associated with growth and energy metabolism has led to the growth rate hypothesis which proposes that tumors, or any fast-growing organism, have an increased demand for Pi associated with accelerated proliferation [19]. The consequences of Pi availability on cell and tissue function, beyond the requirements of the rapid growth phase associated with youth are being investigated using cell culture models.

Requirements of Pi for Cell Function

Pi is critical to cell function as a required component of energy metabolism in the form of adenosine triphosphate (ATP), enzymatic reactions such as kinase signaling, and the formation and function of DNA, RNA, and lipids. Intracellular levels of free Pi are approximately 1–2mM although certain cell types and cellular processes can cause large changes; for example, Pi levels in muscle can reach 30mM during exercise. Levels of intracellular Pi generally vary with changes in extracellular Pi. Emerging data suggest that extracellular Pi plays an active role as a signaling molecule and is capable of regulating cell function and altering cell phenotype (reviewed in [20]). Cell culture studies have linked elevated extracellular Pi to osteoblast mineralization [20, 21], chondrocytes differentiation [22–24], cementoblast formation [25], odontoblast differentiation [26], osteoclast differentiation [27, 28], parathyroid proliferation [29], as well as pathological calcification of osteoarthritic cartilage [30] and vascular smooth muscle [31, 32], and altered kinetics of transport in the kidney [33]. As most of these studies were performed in cell culture models in the absence of changes in Pi-responsive endocrine factors, the results suggest direct stimulatory effects of Pi on cell function.

Effects of Pi on Cell Growth, Proliferation, and Stimulation of Transformation

It was noted almost four decades ago that contact-inhibited murine fibroblast cells respond to serum stimulation with a rapid increase in Pi transport [34–36]. Other studies have noted that Pi availability is a regulating factor in the proliferation of swiss 3T3 fibroblast cells [37–39] and can actively alter cell growth properties [40]. In addition to mammalian cells, studies in yeast have detailed a network for sensing Pi and describe a dependence of proliferation on nutritional Pi [41], identifying Pi as an evolutionary conserved element necessary for cell growth. More recently, increasing medium Pi has been demonstrated not only to be a required element but to actually increase proliferation or [^{3}H]thymidine incorporation in less transformation sensitive cell types including: human parathyroid cells [29, 42],

human bronchial epithelial cells [43], murine osteoblasts [44], and keratinocytes [2]. In these studies Pi was added in addition to standard medium Pi and therefore is acting as a mitogen. However, not all studies agree. Two recent studies using osteosarcoma (U2OS) and breast cancer (MDA-MB-231) cells found decreased growth in response to elevated Pi [45, 46]. The differences may be due to cell type specific responses, specific mutations within certain cancer cells lines, or technical issues associated with cell culture conditions including the concentrations of Pi used. An increase in cell proliferation is one of the early stages of cancer initiation and progression but additional events are required to transform a cell allowing for survival in a nutrient and oxygen challenged microenvironment of a growing tumor. Cell models of transformation have further demonstrated a role for Pi beyond proliferation. Increasing extracellular Pi increased focus formation, a measure of early stage cancer, in the NIH3T3 transformation model [47, 48] as well as growth of JB6 keratinocytes in soft agar, another assay for cell transformation [2]. Taken together, these cell culture studies suggest Pi is not only necessary for cell growth but that abundance can drive proliferation and transformation, at least in certain cell types.

Phosphate Sensing

The effects of elevated Pi on cell behavior have been demonstrated to be the result of the cells' ability to sense changes in extracellular Pi. The main mechanism by which cells handle the distribution of Pi is through a family of sodium-dependent Pi transporters [49]. The family is divided into three subclasses, type 1, 2, and 3. Type 1 transporters (current nomenclature Slc17a1-7) are not thought to be phosphate specific and may serve as general anion channels (reviewed in [50]). Type 2 transporters (current nomenclature Slc34a1-3) are thought to be responsible mainly for absorption in the intestine and resorption in the kidney (reviewed in [51]), although recent data suggests the possibility of a more diverse function [52]. Type 3 transporters (current nomenclature Slc20a1-2) are expressed more ubiquitously but evidence suggests important roles in calcifying tissues (reviewed in [53]). In addition to changes in extracellular Pi levels, a number of factors have been identified that will directly facilitate the transport of Pi into cells including parathyroid hormone (PTH), insulin-like growth factor-I (IGF-1), platelet-derived growth factor (PDGF) [54], calcium [55], IL-8 [30], insulin [56], and the phorbol ester, 12-*O*-tetradecanocylphorbol-13-acetate (TPA) [57, 58]. These findings suggest that cells might be affected by changes in cellular Pi uptake in the absence of changes in extracellular and serum levels. Interestingly, two recent studies have identified the coordinated requirement of fibroblast growth factor (FGF) receptor signaling for both extracellular Pi-induced cell signaling and gene expression [59, 60] (Fig. 17.1). These cell culture studies also identified the synergistic effect of FGF23 in the cellular response to Pi. The results suggest an interesting potential regulatory mechanism by which the net result of FGF23 signaling on cell function may be modulated by the amount of extracellular/serum Pi.

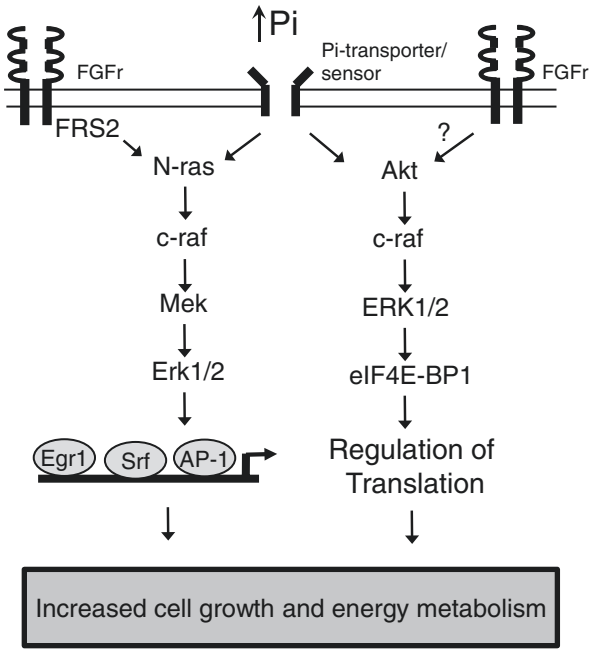

Fig. 17.1 Membrane and signaling events associated with extracellular Pi-induced altered cell function. A number of cell culture studies in both cancerous and noncancerous cells have defined specific cellular events activated and/or required for Pi-induced changes in cell function. Two membrane events include the requirement of sodium-dependent Pi transporter/sensor as well as FGF receptor signaling. Downstream signaling proteins include the FGF receptor associated factor FRS2 as well as N-ras, c-raf , Mek, and ERK1/2 leading to activation of transcription factors such as AP-1, Srf, and Egr-1 and expression of genes associated with cell growth and metabolism. A second pathway identified as Pi responsive includes Akt, c-raf, and ERK1/2 ultimately resulting in the regulation of eIF4E-BP1, a key component of the protein translation machinery. Both pathways alter cell functions related to increased cell growth and metabolism

Slc20a1 and Slc34a2

Two particular Pi transporters have been the focus of investigations related to Pi transport and proliferation and cancer: Slc20a1 (*Pit-1*, *Glvr-1*) and Slc34a2 (*NPT2b*, *NaPi-IIb*). Recent studies have demonstrated the requirement of Slc20a1 for extracellular Pi-induced changes in cell behavior [61–64]. Knockdown of Slc20a1 in Hela cells with siRNA decreased tumor growth in a xenograft implantation model [65]. Overexpression of the sodium-dependent Pi-transporter Slc20a1 gene was demonstrated to increase transformation in the NIH3T3 focus formation model [66] suggesting a mechanistic role for this particular Pi transporter in the response and emphasizing the potentially important role of Pi transport (or sensing) in regulating the initiation and progression of tumorigenesis. Observational gene expression profiling studies have identified Slc20a1 to be more highly expressed in cervical cancer patients that do not respond to therapy [67], pancreatic cancer cell lines [68], and

associated with BRCA2 mutations in breast cancer [69]. Slc34a2 has also been linked to carcinogenesis. A study investigating proteins associated with ovarian cancer by antibody generation identified MX35 [70] as a marker of ovarian cancer and this antibody was later determined to recognize Slc34a2 [71]. Gene expression studies have identified overexpression of Slc34a2 in ovarian cancer, particularly well differentiated tumors [72, 73]. Other gene profiling studies identified Slc34a2 overexpressed in papillary thyroid cancers [74] and breast cancer samples, although an association with overall survival was not apparent [75]. These results support the notion that Pi transporters represent putative therapeutic targets as well as biomarkers of cancer stage.

Effects of Pi on Gene Expression in Cell Culture

The idea that changes in extracellular Pi could directly and actively alter cell behavior is supported by studies identifying specific genes responsive to elevated extracellular Pi. Some of the original studies focused on calcifying cells such as osteoblasts and vascular smooth muscle cells with osteopontin being the first Pi-responsive genes identified [21, 31, 76]. Subsequent studies identified elevated extracellular Pi as capable of regulating gene expression in cell types ranging from osteoblasts and cementoblasts [25, 77] to keratinocytes [2], vascular smooth muscle [31, 78] and bronchial epithelial cells [43]. Together, these studies, and others, have identified hundreds of genes temporally upregulated or downregulated by increased extracellular Pi. Among the different cell types, many of the same genes have been identified as Pi responsive suggesting common regulatory networks. The functions of these high Pi-responsive genes/proteins generally fall into two categories, regulation of calcification or cell proliferation/cancer [44]. Examples of genes tightly linked to cell proliferation/cancer include, among others, osteopontin, cyclin D1, Fra-1, Egr1, c-fos, and vegfα. The mechanism by which these genes are regulated by changes in extracellular Pi has also been investigated. Although a number of transcriptional regulators of the Pi-response have been identified, the activator protein 1 (AP-1) dimer appears to be a common factor across different cell types and time frames [2, 59]. AP-1 proteins have been demonstrated to respond to changes in extracellular Pi with rapid posttranslational modifications, DNA binding, and transcriptional activation [2, 59] (Fig. 17.1). AP-1 is known to regulate genes associated with proliferation, differentiation, and apoptosis and inhibiting activation of AP-1 is considered a therapeutic target for cancer prevention [79]. The potential that serum Pi levels directly affect gene expression in vivo is more difficult to interpret because of the accompanying changes in endocrine factors. A recent microarray analysis of kidneys from mice on high (1.2 %) and low (0.3 %) Pi diets for 24 days identified increased expression of genes such as osteopontin (*spp1*), Timp1, c-fos (*Fos*), and Egr1 [80], all previously identified as Pi-induced in cell culture studies [59, 77, 81] supporting the possibility of direct Pi-regulated gene expression in vivo.

Effects of Pi on Cell Signaling in Cell Culture and In Vivo

The signaling mechanisms by which elevated extracellular Pi might alter gene expression have also been investigated in cell culture. Although not fully elucidated, data suggest that elevated extracellular Pi generates a complex, temporally controlled series of specific signaling events likely as specific as many traditional signal molecules. Two signaling proteins that have been identified as responsive to elevated Pi are the mitogen-activated protein kinase (MAPK)-ERK1/2 [76] and Akt [43] (Fig. 17.1). These proteins become phosphorylated in response to elevated Pi and inhibition of these proteins by either pharmacological or siRNA knockdown results in elimination of downstream Pi-induced effects on gene expression [43, 76]. Both signaling kinases are known to be involved in cell growth and transformation. The response of the MAPK pathway to elevated Pi has also been identified in drosophila suggesting evolutionary conservation [82]. Additional signaling proteins identified as Pi responsive include N-ras and c-raf [2, 43, 63], as well as PKC [76] and nitric oxide [83] (Fig. 17.1). A second Pi-responsive pathway has been identified using human lung cancer cells consisting of Akt, c-raf, and ERK1/2 which results in the phosphorylation of eIF4E-BP1 and increased cap-dependent protein translation [43] (Fig. 17.1). These results were supported by in vivo studies of from 2 week old mice on a high Pi diet for 4 weeks [84] supporting the relevance of the cell culture studies to mammalian physiology. Further, a cell culture study found Pi-induced posttranscriptional regulation of the AP-1 factor Fra-1 resulting in increased protein levels [44] identifying multiple cellular and molecular mechanisms by which Pi might influence cell function. Collectively these studies identify changes in extracellular Pi as an initiator of specific signal transduction pathways resulting in changes in behavior.

Pi-Responsive Endocrine Factors and Cancer: PTH, Vitamin D, FGF23, Klotho, and Osteopontin

There are a number of endocrine factors known to be influenced by Pi consumption, serum Pi levels, and chronic kidney disease (CKD), or associated changes in calcium including parathyroid hormone (PTH), Vitamin D, FGF23, Klotho, and osteopontin, among others. The role of these factors in Pi homeostasis and the mechanisms of action are discussed in detail in other chapters and will therefore only be briefly summarized here. An increase in Pi consumption, serum Pi, and CKD results in increased serum levels FGF23, PTH, and the cytokine-like osteopontin (discussed below) and decreased Pi consumption or serum Pi levels results in increased 1,25-dihydroxyvitamin D and α-klotho. It is therefore possible that these endocrine factors could influence cancer initiation and/or progression in response to changes in Pi (Fig. 17.2). As discussed below, most of these circulating factors have correlative links to cancer or have been identified as putative biomarkers but to date have not been functionally implicated in the etiology of cancer related to changes in Pi levels.

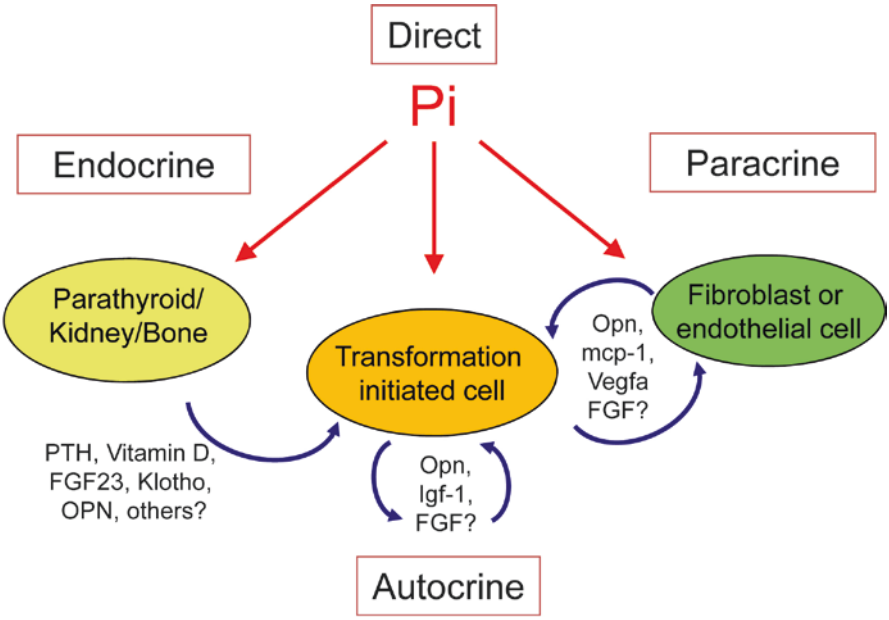

Fig. 17.2 Schematic of potential mechanisms by which changes in Pi consumption or serum levels might influence cancer initiation and/or progression. Based on a combination of in vitro and in vivo studies, Pi might alter cell behavior by four general mechanisms: (1) direct changes in cellular signaling pathways and gene expression, (2) through changes in endocrine factors such as PTH, Vitamin D, Klotho, FGF23, and osteopontin, (3) through changes in autocrine, or (4) paracrine factors. The changes in expression and secretion of these factors and likely others yet to be identified (?) could be due to either the direct effects of Pi on target cells, cells of the microenvironment, or distant endocrine tissues

Vitamin D

Vitamin D has physiological functions in addition to calcium regulation and bone homeostasis and has been investigated for a potential role in cancer prevention for well over two decades [85]. A number of in vitro and in vivo studies have demonstrated the property of vitamin D to promote growth inhibition and differentiation in numerous cell types and tissues as well as cancerous cells (reviewed in [86]). Vitamin D insufficiency is common and many epidemiological studies have investigated the possible inverse correlation of serum vitamin D and cancer risk with inconsistent outcomes [87]. A recent analysis of the Third National Health and Nutritional Examination Survey (NHANES III) participants did not support the hypothesis that serum 25(OH) D levels are associated with reduced cancer mortality [88]. The conflicting results may be due to the differences in units given as well as confounding effects of vitamin D supplementation on calcium and phosphorus as well as other endocrine factors. Although still controversial, some studies have suggested a U-shaped curve for the cancer prevention benefits associated with serum 25-hydroxyvitamin D levels. Taken together, the putative cancer preventative

properties of vitamin D and the link to dietary Pi suggest that increased levels of vitamin D in response to a low Pi diet may be a strategy to reduce cancer formation and/or progression or conversely that the decrease in vitamin D in response to a high Pi diet might be cancer permissive.

Parathyroid Hormone (PTH)

PTH is an endocrine factor secreted by the parathyroid gland in response to decreased serum calcium or increased serum Pi. To date, there is little evidence that PTH is directly involved in regulating aspects of tumorigenesis or malignancy in vivo. One study has identified increased proliferation and chemotaxis of certain prostate cancer cell lines in response to PTH treatment in vitro [89] and corresponding increased expression of the PTH type-I receptor [90] leading to a hypothesis that elevated PTH might enhance osteoblastic prostate cancer metastasis in bone [91]. Intermittent low dose recombinant human PTH (1-34) is currently being used as an effective therapy for osteoporosis and although links to cancers have not been detected in humans, early studies in rodents did detect an increase in osteosarcoma [92]. There are a number of secreted factors that are stimulated by PTH that might influence cancer progression such as IL-6, MCP-1, and IGF-I [93–95]. These factors, among others, are known to influence cancer progression although a direct link with PTH, Pi homeostasis, and cancer has yet to be established. A protein that shares aminoterminal sequence homology to PTH is parathyroid hormone-related peptide (PTHrP). PTHrP binds the same cell surface type 1 PTH/PTHrP receptor and has been associated with breast cancer cell growth although in vivo studies have not been as conclusive [96]. Collectively, the studies published to date do not support a substantial direct role for PTH and cancer initiation or progression although evidence suggests a potential role for the PTH receptor.

Fibroblast Growth Factor 23 (FGF23)

FGF23 is one of three endocrine FGFs and is now widely accepted as critical for Pi homeostasis. There is strong evidence that FGF signaling, in general, plays a role in cancer initiation and progression. FGF signaling has been demonstrated to increase cell proliferation, survival, migration, and angiogenesis. The mechanisms include activating mutations and gene amplification of the receptors as well as and through autocrine/paracrine effects. There is also evidence that FGF signaling, in certain circumstances, can have tumor suppressive effects on cancer (reviewed in [97]). Pharmacologically targeting FGF signaling is an ongoing therapeutic endeavor for the treatment of cancer. However, a complication is the inhibition of FGF23 signaling which results in hyperphosphatemia and subsequent tissue calcification [97]. Although there is strong evidence for a role of FGF signaling and FGF receptors in cancer initiation and progression, to date there is little evidence for a causal association of FGF23 with cancer. However, two recent studies have correlated circulating

FGF-23 levels with advancing stages of malignant ovarian cancer [98] and increased risk for metachronous colorectal adenoma [99] identifying the possibility that FGF23 is a biomarker for certain cancers.

Klotho

Klotho is a membrane-bound protein predominantly expressed in the kidney and parathyroid gland that functions as a coreceptor for FGF23 increasing affinity and therefore signaling [100]. The extracellular domain of Klotho can be cleaved resulting in a secreted soluble form of the protein capable of acting on other tissues. A number of studies have recently linked this secreted form of Klotho with cancer in both mice and humans. Klotho has been suggested to function as a tumor suppressor gene through inhibition of IGF-1 in breast cancer [101]. Through similar mechanisms Klotho has also been associated with inhibition of pancreatic cancer cell growth [102] and melanoma [103]. Loss of Klotho has been linked to epigenetic silencing in human cervical carcinoma [104], gastric [105], and colorectal cancers [106], and low Klotho levels were accompanied by increased epithelial to mesenchyme transition in renal cell carcinoma [107]. Klotho has also been demonstrated to function as a secreted antagonist to Wnt signaling [108] and to inhibit transforming growth factor β1 signaling induced EMT and cancer metastasis in mice [109]. Collectively, these recent results identify Klotho as an exciting and novel factor in suppressing cancer initiation and progression.

Osteopontin (OPN)

OPN (*spp1*, *2ar*) is a highly posttranslationally modified secreted factor that circulates in the serum. OPN was one of the first Pi-responsive genes originally identified in cell culture experiments [21] and strong Pi-induced expression has since been demonstrated in numerous cell types. Serum OPN has been described as increased in response to changes in Pi consumption and serum Pi levels in mice [2, 110]. OPN is ubiquitously expressed and therefore the source tissue is not clear although expression in the kidney of rats was increased in response to a high Pi diet (1.5 %) [111]. OPN was identified as a marker of neoplastic transformation in 1979 by Senger and colleagues and as a protein elevated in the blood in patients with advanced metastatic cancers [112]. The protein influences cell functions by acting as a cytokine through its ability to bind multiple integrin receptors as well as CD44 and as such can act in an endocrine, paracrine, or autocrine manner. Numerous subsequent studies have demonstrated the tight association of OPN with various cancers (overexpressed relative to normal tissue) and arising from most tissue types (reviewed in [113, 114]). OPN plays a key role in malignancy and metastasis and as a mediator of the inflammatory response often associated with tumorigenesis (reviewed in [115]). Taken together, reports strongly support a role for OPN in transformation, invasion, and metastasis in numerous cell and

tissue types. Of all the circulating factors responsive to changes in Pi consumption and serum Pi levels OPN is most strongly linked to cancer initiation and progression to malignancy however, to date, the necessary studies to determine any functional relationship between Pi, OPN, and malignancy in vivo have not been reported.

Conclusions

Cancer initiation is generally a somatic event with progression strongly influenced by environmental factors including diet. A more complete understanding of how individual dietary components interact with the cells of the body to alter tissue function either directly or by autocrine, paracrine, or endocrine mechanisms will greatly enhance the ability to manipulate diet for cancer prevention and general health benefits. The possibility that Pi acts as an important systemic signaling molecule capable of altering cell behavior through changes in transcription, protein modifications and general energy balance suggests a novel target for cancer prevention, however these concepts have only begun to be addressed. Cell culture studies identify extracellular Pi levels as capable of influencing cell behavior through stimulation of specific signal pathways and gene expression. Results from mice and cell culture suggest endocrine/paracrine/autocrine as well as direct cellular and molecular mechanisms by which high and low Pi availability might influence cancer initiation and or progression to malignancy (Fig. 17.2). Further, that a moderately low Pi diet may represent a novel cancer prevention strategy. Whether these findings correlate to humans is still mostly unknown. Studies directly addressing the possible functional role of dietary Pi, serum Pi, cellular Pi transport, and Pi-responsive endocrine factors and cancer initiation and progression to malignancy are clearly needed.

Key Points

- Phosphate can directly alter cell function through stimulation of specific cell signaling pathways and gene expression or through systemic changes in endocrine/paracrine/autocrine factors.
- Changes in extracellular phosphate can influence cell growth, proliferation, and transformation in cell culture models.
- High dietary phosphate intake has been linked to increased tumor formation in mice.
- Phosphate consumption and cellular phosphate transport represent novel targets for modulating cancer initiation and progression to malignancy.

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