Nutrition and Health
Series Editors: Adrianne Bendich · Connie W. Bales

Nathan S. Bryan
Joseph Loscalzo *Editors*

Nitrite and Nitrate in Human Health and Disease

Second Edition

Humana Press

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Foreword

The short-lived, free radical molecule nitric oxide (NO) has emerged as one of the most versatile cell signaling transmitters produced by mammalian biological systems. NO, identified as "endothelium-derived relaxing factor" and proclaimed "Molecule of the Year" in 1992, functions critically in physiology, neuroscience, and immunology. The vascular effects of NO alone include vasodilatation, inhibition of platelet aggregation and leukocyte adhesion to the endothelium, scavenging of superoxide anions, and inhibition of smooth muscle cell hyperplasia. Early studies on NO stemmed from work with nitroglycerin in an attempt to elucidate the mechanism through which it relieved pain due to angina pectoris. It was discovered that the formation of NO from nitroglycerin accounts for its therapeutic efficacy for angina by dilating constricted and diseased blood vessels in the heart. Not surprisingly, some of the most prevalent diseases result, at least in part, from decreased NO availability, for example, hypertension, atherosclerosis, diabetes mellitus, and hypercholesterolemia.

The discovery of the formation of NO from the semi-essential amino acid L-arginine through one of three isoforms of nitric oxide synthase provided a key therapeutic target, which is still the focus of much research today. Dietary supplementation of L-arginine has been shown to enhance NO production in healthy individuals (despite already saturated extracellular concentrations), and this may both provide cardiovascular protective effects and enhance athletic performance. Indeed, endothelial dysfunction, an early sign of cardiovascular disease, has been reversed through enhanced NO production. This observation leads us to believe that intervention through the NO pathway is a viable route for treatment and prevention of vascular dysfunction.

Recently, the oxidative "waste" products of nitric oxide, nitrite and nitrate, have been evaluated in a new context, due to their ability to form NO independent of nitric oxide synthase enzymes, through reductive electron exchanges. Since nitrate (as well as nitrite) is primarily ingested in the form of fruits and vegetables, which have been known for some time to protect against diseases from atherosclerosis to cancer, a new paradigm has emerged regarding the role of these once feared nitrogen oxides. Both public and scientific perception of nitrite and nitrate still revolve around fears of nitrosamine formation and carcinogenesis. What has not been considered, however, is the fact that consumption of antioxidants with nitrite and nitrite (both significant components of fruits and vegetables) inhibits the formation of nitrosamines in the gastric milieu. Furthermore, a human nitrogen cycle consisting of commensal bacteria in the oral cavity, which serve a reductive role in the conversion of approximately 20% of ingested nitrate to nitrite, now appears to provide a significant NOS-independent source of NO generation.

This body of work may have revolutionary implications in terms of developing strategies to combat heart disease and many other contemporary diseases associated with a NO deficiency. Furthermore we may finally have an explanation for the many known and undisputed benefits of the Mediterranean diet. Perhaps now we should consider nitrite and nitrate as the bioactive food components that account

for the protective benefits of certain foods and diets. Numerous clinical trials of supplementation with various antioxidants borrowed from heart-healthy diets, such as those typical of Mediterranean countries, have consistently failed to replicate the protective effects of the foods themselves. Consistently absent, but the primary human source, is dietary nitrate and nitrite. Recent work has shown various cardioprotective effects from modest supplementation of nitrite and nitrate. Nitrite, in particular, has been shown to prevent hypercholesterolemic microvascular inflammation and protect against injury from ischemic events.

The broader context of research regarding nitrate, nitrite, and nitric oxide suggests these simple nitrogen oxides serve as a critical dietary component for protection against various chronic diseases. Currently, heart disease and cancer lead the nation in cause of deaths. Concurrently, the dietary patterns of the West have transitioned towards heavily processed foods and lack significant quantities of fruits and vegetables. The explanations have been varied but overlook simple molecules known to play critical roles in multiple organ systems through the chemical messenger NO. The dietary contributions to normal NO homeostasis would not only help explain significantly lower rates of cardiovascular disease in those who regularly consume fruits and vegetables but also arm scientists and physicians with a relatively simple and inexpensive therapeutic intervention.

This text effectively overviews the important role nitrite and nitrate play in biological systems and NO homeostasis. A risk benefit analysis has shown nitrite and nitrate present no danger when consumed in modest quantities and preferably with antioxidants. In fact, research appears to suggest nitrite acts as a redundant NO reservoir when NOS activity is insufficient or stress requires a secondary source. The future use of nitrite/nitrate in dietary considerations will likely have a significant impact on current public health policy. This book brings the NO-story full circle and presents novel thought on the future treatment for many of the country's most pressing health issues. This is a relatively new area of nitric oxide research but a very exciting one. The L-arginine pathway for NO synthesis may turn out to be only part of the story. The symbiosis between humans and the bacteria that reside in and on our body may be just as important in terms of utilizing nitrate and nitrite to make NO under conditions when the oxidation of L-arginine is dysfunctional. Drs. Nathan S. Bryan and Joseph Loscalzo have assembled the world's experts to present a first of its kind, comprehensive work on nitrite and nitrate in human health and disease, carefully examining the context for a risk benefit assessment.

Louis J. Ignarro

Preface

Our major objective and driving force in developing the second edition of *Nitrite and Nitrate in Human Health and Disease* is to consolidate and update all the key research and new knowledge in one volume in order to establish a framework based on the totality of evidence for nitrite and nitrate and the effects of these two anions on human health and disease. Since the publication of the first edition in 2011, more evidence has been published in human trials demonstrating the safety and efficacy of nitrite and nitrate in human disease, but also more insight has been gained into mechanisms of action. These new data are now included in the second edition. Although the biomedical science community is excited and optimistic about the potential for developing new therapeutics and perhaps regimens of disease prevention based on their ability to generate nitric oxide under appropriate conditions, epidemiologists, nutritionists, and cancer biologists have historically had cause for concern owing to the inherent nitrosative chemistry of nitrite and NO that could form potentially carcinogenic N-nitrosamines. The new data on nitrite and nitrate have even shone new light on this minimal potential risk. As a result, a risk benefit analysis for nitrite and nitrate can now be evaluated where it appears that at the right doses and routes of administration, the potential benefits far outweigh any potential risks. This is the second edition of the first book in the Springer/Humana Nutrition Series dedicated to understanding the nutritional aspects of nitrite and nitrate for human health. It is our intent to deliver a comprehensive review of nitrite and nitrate, from basic biochemistry to the complex physiology and metabolism of these two naturally occurring molecules in the human body.

Overall, the book contains well-organized and well-referenced chapters by respected scientists and physicians that covers the rich history of nitrite and nitrate, sources of exposure and physiological effects when consumed through foods containing nitrite and nitrate. The first portion of the book describes the biochemistry, metabolism, and physiology of nitrite and nitrate, and how these molecules are incorporated into the foods we eat and subsequently how they are systematically metabolized to bioactive nitric oxide. This biochemistry involves the environmental processes of nitrogen fixation and the presence of a human nitrogen cycle involving symbiotic bacteria that reside in and on the human body. The book then shifts focus to the sources of exposure to nitrite and nitrate, both environmental and dietary, as a means to quantify exposure estimates and what this may mean for human health. We also discuss the epidemiology and dietary effects on the nitric oxide pathway. This portion of the book also examines systems in nature which this pathway is exploited, including the breast milk of nursing mothers. Finally, the last section of the book discusses nitric oxide-based therapeutics and how nitrite and nitrate biochemistry can be harnessed safely and efficaciously to improve human health through the production of nitric oxide. We end with a summary of the collective body of knowledge presented in the book and what we might expect going forward. Each chapter begins with an abstract and Key Points that outline the concepts presented to assist the reader in understanding the fundamental principles presented in each chapter.

It is undisputed in the biomedical community that NO is one of the most important molecules the human body produces. If NO is such an important molecule in practically every organ system in our body, why, then, is there only a singular pathway for its production, i.e., the complex oxidation of L-arginine? Phosphorylation is another fundamental cellular process that is just as important in cell signaling, with over 500 recognized kinases and many phosphatases to regulate this biochemical process; by contrast, there is only one class of enzymes, the nitric oxide synthases, to produce NO. Most physiological systems are rich in redundancy, allowing backup systems to support the primary system. The provision of nitrate and nitrite as sources of NO may then be viewed as a system of redundancy. After all, a one-electron reduction is energetically and kinetically favorable to a five-electron oxidation.

According to the World Health Organization, cardiovascular disease is the number one killer of both men and women in the United States. These deaths represent a staggering 40 % of all deaths. Close to one million people die each year and more than six million are hospitalized due to cardiovascular disease. Therefore, developing new strategies to correct NO insufficiency and replete NO availability is of paramount importance and could potentially save millions of lives worldwide and lessen the burden on the health care system. We now appreciate that reduced or insufficient NO production or activity is a hallmark of a number of disorders, including many complex, chronic cardiovascular diseases and even Alzheimer's and diabetes mellitus. Therefore, developing new strategies to restore and replete bioactive NO is of paramount importance and could potentially lessen the burden of disease for society. Thus, understanding the biological activity of nitrite and nitrate may not only lead to novel treatments for disease but may lead to strategies to prevent disease development or progression and even the physiological basis for the benefits of certain diets such as the Mediterranean diet. To achieve this laudable goal, we must first establish the context for potential benefit while preventing unwanted risks or harm. We hope the information provided in this text will begin to help define that context, be a source of valuable information, and be useful for anyone who wants the most important and updated information about nitrate and nitrite.

We have invited the world's leading experts to share their research and perspectives, which we hope will help define the context for benefits vs. any potential risks associated with nitrite and nitrate, through either dietary ingestion or therapeutic dosing. This diverse collection of authors includes muscle biologists, physiologists, physicians, epidemiologist, cancer biologist, registered dietician, chemist, and public health experts from five countries around the world in both academia and government. This approach will provide a fair and balanced view of nitric oxide biochemistry, and nitrite and nitrate biochemistry in physiology and in the food sciences. As a result, we are indebted to these many individuals. We feel extremely honored and grateful to have many of the world's experts contribute their knowledge and perspective. We realize the time and dedication it takes to compose a book chapter on the latest body of knowledge, so we appreciate these authors' taking the time to help develop this volume. Without their creative contributions, this book would not have been possible. We also sincerely thank Springer-Humana Press for including this body of work in the Nutrition and Health Series with Dr. Adrianne Bendich as Series Editor. The Editors also wish to thank Stephanie Tribuna for her time and effort in organizing and managing this project with the Editors. This is an exciting time in NO and nitrite-based research. This has been—and we predict will continue to be—an area of intense research in the future. It is our hope that the information contained here will educate and inform scientists, physicians, health care professionals, nutritionists, dieticians, and even the general public on the effects of nitrite and nitrate in human health and disease.

Houston, TX — Nathan S. Bryan
Boston, MA — Joseph Loscalzo

Series Editor Page

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

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

"**Nitrite and Nitrate in Human Health and Disease, 2nd Edition,**" edited by **Nathan S. Bryan, Ph.D., and Joseph Loscalzo, M.D., Ph.D.,** is a welcome addition to the Nutrition and Health Series and fully exemplifies the Series' goals. This unique volume is a very timely update of the first edition that was published in 2011. Over the past 6 years, there has been a significant increase in the understanding of the huge role of nitrate and nitrite in health and disease. This comprehensive review of the science behind active nitrogen-containing molecules and their effects on humans is of great importance to the nutrition community as well as for health professionals who have to answer client questions about this expanding area of clinical research. The editors strive to inform scientists, physicians, related health care professionals, nutritionists, dieticians, advanced students, and those who have an interest in objective, data-driven analyses of the health effects of nitrite and nitrate. Thus, with these critical issues in mind, the editors have decided to not only update the relevant chapters from the first edition but also add nine new, timely chapters that are of great value to clinicians, nutritional health professionals, and advanced students. The importance of this updated volume is captured in the Foreword, written by Dr. Louis Ignarro, 1998 recipient of the Nobel Prize in Medicine, who writes: "This book brings the NO-story full circle and presents novel thought on the future treatment for many of the country's most pressing health issues. This is a relatively new area of nitric oxide research but a very exciting one."

The internationally recognized clinical researchers who serve as the editors of this as well as the first edition of *Nitrite and Nitrate in Human Health and Disease* are highly qualified in nitrate and nitrite investigations. Dr. Nathan S. Bryan, Ph.D., is currently Adjunct Assistant Professor at Baylor College of Medicine in the Department of Molecular and Human Genetics in Houston, TX. He is a pioneer in recognizing the important effects of dietary nitrite and nitrate and elucidating the novel metabolism of nitrate and nitrite in humans. Dr. Bryan has been involved in nitric oxide research for more than 16 years and has made many seminal discoveries in the field that have resulted in seven patents. Specifically, Dr. Bryan was the first to describe nitrite and nitrate as indispensable nutrients required for optimal cardiovascular health. Dr. Bryan's lab was the first to demonstrate and discovered an endocrine function of nitric oxide via the formation of S-nitrosoglutathione and inorganic nitrite. Through the drug discovery program in natural products chemistry, Dr. Bryan discovered unique compositions of matter that can be used to safely and effectively generate and restore nitric oxide in humans. This technology is now validated in many published clinical trials. These discoveries and findings have unveiled many beneficial effects of nitrite in the treatment and prevention of human disease and may provide the basis for new preventive or therapeutic strategies in diseases associated with NO insufficiency and new guidelines for optimal health. He is also a successful entrepreneur who has commercialized his nitric oxide technology through the formation of Human to the Power of N (formerly Neogenis Labs, Inc.) where he is Co-founder and Chief Science Officer. Dr. Bryan's most recent work has focused on the role of oral nitrate reducing bacteria in control of endogenous nitric oxide production and hypertension. He has published a number of highly cited papers and authored or edited six books. Dr. Bryan is a member of the Society for Free Radical Biology and Medicine, American Physiological Society, Nitric Oxide Society, American Heart Association, and the American Association for the Advancement of Science.

Joseph Loscalzo, M.D., Ph.D., is the Hersey Professor of the Theory and Practice of Medicine at Harvard Medical School and serves as Chairman of the Department of Medicine and Physician-in-Chief at Brigham and Women's Hospital in Boston, Massachusetts. Dr. Loscalzo is recognized as an outstanding cardiovascular scientist, clinician, and teacher. Dr. Loscalzo is one of the early pioneers in the nitric oxide field with many of his seminal publications and discoveries dating from the early 1980s. He was the first to demonstrate the formation of S-nitrosoproteins and to elucidate the anti-thrombotic and antiplatelet mechanisms of NO, as well as many other contributions to this field. He has received many awards, including the Clinician-Scientist Award, the Distinguished Scientist Award, the Research Achievement Award, and the Paul Dudley White Award from the American Heart Association; a Research Career Development Award, a Specialized Center of Research in Ischemic Heart Disease Award, and a MERIT Award from the National Institutes of Health; the George W. Thorn Award for Excellence in Teaching at Brigham and Women's Hospital, and Educator of the Year Award in Clinical Medicine from Boston University; the Glaxo Cardiovascular Research Award, and the Outstanding Investigator Prize from the International Society for Heart Research; and election to the American Society for Clinical Investigation, the Association of American Physicians, and the Institute of Medicine of the National Academy of Sciences. He has served on several NIH study sections and editorial boards and has chaired the Gordon Conference on Thrombolysis. He served as an associate editor of the *New England Journal of Medicine* for 9 years, Editor-in-Chief of *Circulation* for 12 years, Chair of the Cardiovascular Board of the American Board of Internal Medicine, Chair of the Research Committee of the American Heart Association, Chair of the Scientific Board of the Stanley J. Sarnoff Society of Fellows for Research in the Cardiovascular Sciences, and Chair of the Board of Scientific Counselors of the National Heart, Lung, and Blood Institute of the National Institutes of Health. He is a former member of the Advisory Council of the National Heart, Lung, and Blood Institute and a former member of the Council of Councils of the National Institutes of Health. He is currently Director of the NIH-funded Center for Accelerated Innovation (the Boston Biomedical Innovation Center), the NIH-funded Harvard Undiagnosed Disease Network program, and a senior editor of *Harrison's Principles of Internal Medicine*. His most recent work has

established the field of network medicine, a paradigm-changing discipline that seeks to redefine disease and therapeutics from an integrated perspective using systems biology and network science. Moreover, these two eminent editors have chosen the most respected and knowledgeable authors for the volume's 22 insightful chapters. Thus, this volume addresses the critical issues of the roles of nitrite and nitrate in human health and disease with objective, data-driven chapters that reflect the goals of clinical nutrition today. The chapters contain Key Points as well as integrated Conclusions as it is the intention of the editors that this volume serve as an important resource for educators and students.

This comprehensive volume contains 22 chapters that are organized in three broad parts: Biochemistry, Molecular Biology, Metabolism and Physiology; Food and Environmental Exposures to Nitrite and Nitrate; and Nitrite and Nitrate in Therapeutics and Disease.

Part I: Biochemistry, Molecular Biology, Metabolism and Physiology

The six chapters that comprise the first, introductory part of the volume provide a grounding in the chemistry, biochemistry, and functions of the nitrogen oxides, nitrite and nitrate. The first chapter, written by the volume's editors, describes the discovery of the gas, nitric oxide (NO), and its formation in the body. We learn that the L-arginine-NOS (nitric oxide synthase) pathway was the first NO production system discovered and is responsible for about 50 % of endogenous nitrate and nitrite in humans. Nitrite can also form directly in blood and tissues via NO autoxidation, which involves the reaction of two molecules of NO with oxygen, a reaction that is catalyzed by the plasma protein ceruloplasmin. Nitrate is by far the dominant final NO oxidation product; the levels in blood and tissues exceed those of nitrite by at least two orders of magnitude. The half-life of nitrite is about 110 s when added to venous blood. Nitrate, by contrast, has a circulating half-life of 5–8 h. The interactions between oral bacteria, stomach acid, and dietary antioxidants mainly from fruits and vegetables are reviewed. The importance of NO for cardiovascular health is also discussed. The second chapter examines the natural nitrogen cycle and its role in provision of sources of nitrite and nitrate in our diets. Nitrogen (N_2) gas makes up approximately 78 % of the atmosphere. It is highly inert, being bound together by a triple bond that requires considerable energy to break, and higher organisms, including humans, cannot convert nitrogen to biologically active forms. The conversion of nitrogen from inert N_2 to biologically available nitrogen (called fixation) requires multistep enzymatic pathways; vast amounts of energy in the case of lightning; or a combination of heat, pressure, and metallic catalysts. The conversion of atmospheric nitrogen to a fixed, biologically available nitrogen species is only accomplished by prokaryotes, eubacteria, and archaea. These organisms are known as diazotrophs and they have three modes of survival: free-living, symbiotic (within plant roots), and those that are associated mainly with plant roots. The formation and use of fixed nitrogen by plants is described. In humans, nitrogen is principally ingested as protein. The average US young adult consumes approximately 90 g of protein daily which equates to about 14.5 g of nitrogen, nearly all of which is excreted in the urine as urea, creatinine, uric acid, and ammonia. Only a tiny proportion of this nitrogen is converted to nitric oxide via the nitric oxide synthase pathway and some is also generated from L-arginine. The importance of the oral commensal bacteria in salivary glands in the generation of nitrite is reviewed.

Chapter 3, coauthored by the volume's coeditor, discusses the importance of human oral nitrate reducing bacteria as humans do not have the enzymes to reduce nitrate to nitrite which is then swallowed and further reduced to NO in the acidic stomach. The chapter reviews the enterosalivary nitrate-nitrite-nitric oxide pathway that represents a potential symbiotic relationship between oral bacteria and their human hosts. The oral commensal bacteria provide an important metabolic function in human physiology by contributing an NOS-independent source of NO. The chapter stresses the

importance of these bacteria in maintaining adequate concentrations of NO that are associated with reduced risk of cardiovascular disease. Conversely, the chapter explores the consequences of disruption of nitrite and NO production in the oral cavity that may contribute to the oral-systemic link between oral hygiene and cardiovascular risk and disease. The new emerging area of epigenetics and how these changes to DNA's environment can affect NO production are the focus of Chap. 4 that is coedited by one of the volume's editors. Epigenetics involves chromatin-based modifications to DNA or histone proteins that influence patterns of gene expression. NOS genes and other genes involved in NO production are targets for epigenetic modification. Of relevance, prenatal exposure to pharmacological agents or special diets can promote epigenetic changes that affect gene expression and physiology in offspring. Maternal diet can affect epigenetic regulation of gene expression and modulate disease outcomes. This detailed chapter, containing over 100 relevant references and six helpful figures, reviews numerous targets for epigenetic alterations in methylation reactions as well as changes in NOS expression. There is a discussion of the direct and indirect epigenetic mechanisms that regulate NOS gene expression and NO production.

Chapter 5 examines the role of mitochondria and the link to nitrogen-containing molecules. Nitrite has been shown to be an endogenous signaling molecule that regulates mitochondrial number, morphology, and function. The chapter, containing over 125 references, provides an in-depth examination of the functions of the mitochondria as these have expanded greatly in the past decade. Included are reviews of the importance of the mitochondrion as a physiological subcellular target for nitrite; regulation of mitochondrial respiration, reactive oxygen species generation and apoptotic signaling, as well as nitrite's control over mitochondrial number and dynamics. The last chapter in this part, Chap. 6, outlines the major endogenous and exogenous sources of exposure to nitrogen oxides. Nitrate and nitrite are found in the human diet, are present in drinking water, and in many vegetables as well as part of food preservation systems in cured meats. Exposure is dependent on geographic location and diet choices. Endogenously produced nitrate and nitrite are derived from nitric oxide metabolism and approximately 25 % of plasma nitrate is retained in the body via an enterosalivary pathway discussed in detail in Chap. 3. The enterosalivary recycling involves the oral reduction of nitrate to nitrite by commensal bacteria. There is a discussion of the discovery of the endogenous formation of NO that is synthesized by mammalian cells from L-arginine: NO was shown to be a potent vasodilator, inhibitor of platelet aggregation, and active species of nitroglycerin. The importance of maintaining a safe level of nitrate and nitrite in drinking water, especially in areas of high use in plant fertilizers, is reviewed. Also outlined are the major environmental exposures to nitrogen oxides including cigarette smoke and air pollution and the potential exposure to nitrosamines.

Part II: Food and Environmental Exposures to Nitrite

Part II contains six chapters that examine the sources of nitrite and nitrate from food and environmental exposure and their biological impacts. Chapter 7 reviews the long history of use of salting meats as a means of preservation and indicates that, in ancient times, salt was obtained from crystalline deposits by mining directly from the earth, or evaporating water from brine pools or seawater. As a consequence, it often contained natural contaminants such as sodium or potassium nitrate (saltpeter or "niter") that contributed directly to the curing reaction and the preservation process. The contaminants were actually the primary components in curing reactions. Importantly, nitrite inhibits the growth of a number of aerobic and anaerobic microorganisms and especially suppresses the growth of *Clostridium botulinum* spores. Nitrite in combination with salt and other curing factors may also control the growth of other pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens*. Nitrite also reacts with myoglobin to produce the reddish-pink cured meat color, inhibits lipid oxidation (and reduces oxidative rancidity), and contributes to the cured flavor of meat products.

The chapter includes an overview of the regulatory limits of nitrite and nitrate in cured foods as established by the United States Department of Agriculture—Food Safety Inspection Service (USDA-FSIS) or Food and Drug Administration (FDA), the scientific research to determine the limit of consumption based upon the formation of nitrosamines that are considered as carcinogenic agents, and tables that describe the concentrations of naturally occurring nitrates in selected vegetables and an extensive discussion of the regulatory history of establishing safe levels of nitrate/nitrite in municipal water supplies in the United States and many other countries.

Chapter 8 provides an overview of the field of nutritional epidemiology and specifically examines the critical issues involved in determining dietary intakes of nitrogen-containing compounds in food and water. Nitrite and nitrite oxide are biologically active compounds involved in vasodilatation, inhibition of endothelial inflammation, and platelet aggregation. Nitrates and nitrites in the diet are the relevant compounds for studying the nutritional components of cardiovascular disease. However, compared to other nutrients, quantifying nitrate and nitrite intake is challenging. There are large within-food variation in nitrate and nitrite concentrations and large geographical and seasonal differences in the nitrate content in drinking water. Conclusions include that to date, the epidemiologic evidence for the relationship between dietary nitrate and nitrite and bladder or gastric cancers is limited and weak. There is no direct epidemiological evidence relating dietary nitrite and nitrate to risk of cardiovascular disease. However, the primary source of dietary nitrate, green leafy vegetables, has been associated with lower risk of cardiovascular disease. Processed meats are important contributors of nitrite intake. However, the increased risks for cardiovascular disease are also observed for red meat to which no preservatives are added. A recent finding links dietary nitrate with a reduced risk of a particular type of glaucoma, and this finding needs to be confirmed.

Although the nutritional epidemiology does not find a strong link between dietary sources of nitrite/nitrate and reduction in cardiovascular disease risk, Chaps. 9 and 10 examine the physiological effects at the cellular level of NO and how these effects could affect cardiovascular disease risk. NO relaxes the underlying vascular smooth muscle to improve vascular compliance and to reduce vascular resistance. In addition, endothelium-derived NO inhibits platelet adhesion and aggregation, suppresses leukocyte adhesion and vascular inflammation, and limits the proliferation of the underlying vascular smooth muscle cells. The chapter objectively reviews the data indicating that short-term effects of NO may not result in overall reduction in cardiac vessel disease or its consequences. The list of substances examined include, but are not limited to, arginine, B vitamins, antioxidants, fiber, vegetable protein, lipids, soy, and phytoestrogens and several diets including the DASH diet and the Mediterranean Diet, and the chapter includes over 150 relevant references. Chapter 10 reviews the importance of NO for optimal vascular functions and suggests that disturbed NO homeostasis is a hallmark of the development of chronic cardiovascular disease and affects the level of risk for acute ischemic events such as an acute myocardial infarction. Additionally, the flavonoids that are often found in nitrate-containing foods are reviewed as important bioactive molecules that enhance NO vascular activity. Several of the key dietary components that have high flavonoid content are green tea, red wine, and products made from cocoa beans; these are described in detail.

In addition to plants, human milk contains relatively high concentrations of nitrate and nitrite. Chapter 11, coauthored by one of the volume's editors, examines the importance of these molecules in infant development. The chapter reviews the evidence that these anions are necessary for growth and development as well as immunological support in the newborn infant. Emerging data suggest that the difference between the nitrite and nitrate content of breast milk compared to formula milk may account for some of the health factors that have been shown to differ in breast-fed versus formula-fed infants. The chapter includes a detailed explanation of "blue-baby syndrome" that is linked to excessive nitrate in ground water, and not from breast feeding. Methemoglobinemia, or blue-baby syndrome, has been seen in infants less than 6 months of age that are exposed to excess nitrates in bacterially contaminated well water. Infants who are younger than 6 months old are particularly susceptible to nitrate-induced methemoglobinemia because of the lower than needed stomach acid

production, large concentration of nitrite-reducing bacteria, the relatively easy oxidation of fetal hemoglobin, and immaturity of the methemoglobin reductase system. There is a detailed description of the nutritional and physiological importance of colostrum as well as breast milk and the changes in composition that occur during 6 months of breastfeeding. The final chapter in this part, Chap. 12, describes the dilemma currently that has arisen because of the known adverse effects of overexposure to environmental nitrate pollution in the face of relatively new data on the importance of nitrite and nitrate and NO for cardiovascular function, immune function, and other clinical areas that are discussed in detail in subsequent chapters. The authors suggest that there be a reappraisal of all of the clinical data to better understand the risk/benefit ratio and provide data-driven guidance to consumers and health professionals.

Part III: Nitrite and Nitrate in Therapeutics and Disease

Part III addresses the recent uses of nitrogen-containing molecules as therapeutics and reviews the association of NO status and disease risk in ten clinically focused chapters. Chapter 13 provides an overview of the section and is authored by one of the volume's coeditors. The authors enumerate some of the major disease conditions that have been researched and published in over 140,000 publications in the last 12 years. NO has been shown to be involved in and affect neurotransmission, memory, stroke, glaucoma and neural degeneration, pulmonary hypertension, penile erection, angiogenesis, wound healing, atherogenesis, inflammation such as seen in rheumatoid arthritis, nephritis, colitis, other autoimmune diseases, destruction of pathogens and tumors, asthma, tissue transplantation, septic shock, platelet aggregation and blood coagulation, sickle cell disease, gastrointestinal motility, hormone secretion, gene regulation, hemoglobin delivery of oxygen, stem cell proliferation and differentiation, and bronchodilation. The chapter focuses on the cardiovascular and nervous systems and the functions of NO in the immune system. There are detailed discussions of the biochemistry of the three enzymes involved in the synthesis of NO within cells: neuronal NOS, inducible NOS, and endothelial NOS and the reactions that follow the formation of NO within cells.

Chapters 14–17 are practice-oriented chapters that examine the therapeutic usefulness of nitrogen-containing molecules in patients with cardiovascular disease (CVD) and/or pulmonary diseases that require dilation of vessels and organs such as the lungs and heart. Chapter 14, containing more than 100 useful references, describes the use of inhaled NO as a therapeutic agent for the treatment of pulmonary vasodilation. There is an historic review of the use of nitric oxide-generating drugs, including nitroglycerin and sodium nitroprusside, that have been used by clinicians to treat patients with a broad spectrum of cardiovascular diseases including angina pectoris and systemic hypertension. In addition to reviewing the animal model data on the development of inhaled NO for a number of clinical indications, the chapter highlights the use of inhaled NO to treat hypoxia in the newborn, to prevent the development of bronchopulmonary dysplasia in premature infants, and several other new therapies. One example is the use of inhaled NO in adults and children undergoing cardiac surgery and cardiac transplantation to treat pulmonary hypertension and improve cardiac output in the perioperative period. Of great value to clinicians, the safe use of inhaled NO is clearly described. Chapter 15 provides detailed explanations of the biochemical reactions and therapeutic value of the vasodilation that result from using NO-generating therapies. The chapter, containing over 150 relevant references, also explains the negative phenomena of nitrate tolerance and the induction of endothelial dysfunction: these side effects are described and new data, including epigenetic effects of organic nitrates, as well as methods to avoid the appearance of these adverse effects, are outlined. Chapter 16 examines the potential for nitrite to improve recovery from ischemia-reperfusion injury. This injury, which occurs following a myocardial infarct, ischemic stroke, or other cause of blood flow disruption, is characterized by numerous detrimental cellular events including oxidative damage to proteins and

lipids, enzyme release, and inflammatory responses, which ultimately lead to necrosis and apoptosis of affected tissues. The chapter includes an extensive review and tabulation of the clinical studies that have used low dose nitrite or NO donor molecules in patients with ischemia/reperfusion injuries. Chapter 17 reviews the importance of nitrogen-containing molecules for hypertensive patients. NO is unique in its capacity to maintain blood pressure homeostasis because its vasodilatory action affects both arterial and venous sides of the circulation. The chapter includes an historical perspective on the use of inorganic nitrite as a vasodilator and the recent epidemiological data linking consumption of green leafy vegetables (natural sources of nitrate) with reduced risk of hypertension has encouraged the development of small clinical studies using beet juice compared to juice that does not contain nitrate in normal and hypertensive patients. Preliminary data are promising, but further work is warranted. Also discussed are the potentially detrimental effects of antimicrobial mouth washes that adversely affect oral bacteria involved in the generation of nitrite from food nitrate. In total, these four clinically interrelated chapters provide the reader with over 650 targeted references as well as 16 valuable tables and figures.

The unique chapter, Chap. 18, examines the historical significance of Chinese and other traditional herbal medicines and supplements that have been in use for centuries to treat cardiovascular and other diseases. A number of these herbs have the potential to increase intakes of nitrite and nitrate directly and may also provide compounds that can alter synthesis of nitrogen-containing molecules. The author reminds us that plants require nitrogen for growth. In addition, we learn that many herbal medicines are made from the roots and leaves of certain plants that contain abundant nitrate/nitrite or related compounds. In these plants, NO can be produced by several routes via plant L-arginine-dependent or independent NO-synthesizing enzymes; by the action of plasma membrane-bound nitrate/nitrite reductase; as an end product of the mitochondrial electron transport chain; and through nonenzymatic reactions as the roots of certain plants may contain high levels of unstable nitro compounds, which may release NO spontaneously. Moreover, plants exposed to nitro-types of fertilizers typically generate more NO than those grown in the wild. The recent interest in the plants and plant parts used to affect cardiovascular symptoms is reviewed and findings are tabulated as well as illustrated in informative figures.

The final four chapters in this comprehensive volume examine the effects of aging on NO availability, the role of nitrite and nitrate in exercise, and the association of NO status and the risk of cancer, followed by a critically important chapter by the editors on future research areas. Chapter 19 provides the rationale for the decline in NO with aging and evidence that enhancement of NO status during the senior years may reduce the risk of chronic, noninfectious diseases of aging. NO bioavailability decreases with age. The reasons for the age-associated decrease in NO bioavailability are multifactorial, but evidence suggests that an age-related increase in oxidative stress is the primary cause. Oxidative stress is defined as an imbalance in reactive oxygen species relative to antioxidant defenses and results in the destruction in cellular concentrations of NO. Additionally, chronic, low-grade inflammation also develops with advancing age and this also reinforces oxidative stress; low-grade inflammation has also been seen in obesity, diabetes, rheumatoid arthritis, and certain other diseases. The well-accepted function of NO as a vasodilator is especially important in the aging population. The authors remind us that NO is produced within the vascular endothelium and diffuses to the vascular smooth muscle, providing a powerful vasodilatory signal that adjusts the diameter of the vessel to accommodate changes in blood flow. In aging as well as pathological states in which NO bioavailability is low, this signaling pattern is disrupted, creating an environment conducive to endothelial dysfunction and stiffening of the large elastic arteries, two primary contributors to the increased risk of CVD in middle-aged and older adults. There are also data on cognitive and muscle function that are adversely affected by lower concentrations of NO seen with aging. Short-term clinical studies using diet and/or supplements are reviewed and the chapter includes over 180 clinically relevant references.

Chapter 20 examines the physiology of muscle function during exercise and the role of NO in muscle function. We learn that nitrite is a precursor of nitric oxide synthesis in hypoxic and acidic conditions. Skeletal muscles become acidic and hypoxic during exercise, increasing the potential for NO synthesis in the presence of nitrite. In fact, nitrate supplementation has been shown to improve exercise economy and endurance performance in moderately trained, but not well-trained, subjects and improve short-duration, high-intensity exercise performance. The chapter, containing over 130 relevant references, summarizes nitrate$^-$ supplementation procedures, often with beet juice, exercise settings, and the current status of supplementation studies in several sports and exercise routines. Chapter 21 critically reviews the early in vitro and laboratory animal studies using very high doses of nitrite that linked their formation into carcinogenic nitrosamines and increased the risk of gastric cancer. Newer, more controlled laboratory studies have failed to replicate the earlier findings and human epidemiological studies have also not consistently found a positive association between dietary intake of nitrite/nitrate and cancer risk. Additionally, we have learned that vegetables are the major source of dietary nitrogen-containing compounds and that cured meats now have antioxidants added to prevent nitrosamine formation. The chapter carefully reviews the potential points of error that can be found in epidemiological studies, such as when the exposure to the potential carcinogen is not known—this is the case of nitrate, nitrite, and other compounds that can be involved in nitrosamine synthesis. It is difficult to accurately determine exposure because of growing conditions and environmental stressors to plants; also, it appears that nitrosamines may be carcinogenic in animal models but may not be carcinogenic in humans. This balanced, data-based chapter corroborates the independently arrived at similar conclusions found in Chap. 8. The authors of both chapters confirm the lack of consistent data linking dietary intake of nitrite and nitrate with increased risk of gastric cancer. The authors provide excellent examples of the importance of objective evaluation of epidemiological studies.

The final chapter, written by the volume's excellent editors, objectively identifies the key areas where future research is needed in order to move clinical research in the functions of nitrogen-containing compounds forward. Two key areas identified are the determination of optimal ranges of intake and the establishment of national government—recommended dietary guidelines that are associated with maximum benefit and assurance of reduced risk of overexposure. Once this information is accepted by the clinical nutrition community and related health professionals, future research using safe and effective doses of nitrite and nitrate in long-term clinical trials will become possible.

Conclusions

The above description of the volume's 22 chapters attests to the depth of information provided by the 39 well-recognized and respected chapter authors. Each chapter includes complete definitions of terms with the abbreviations fully defined and consistent use of terms between chapters. Key features of this comprehensive volume include over 60 detailed tables and informative figures, an extensive, detailed index and more than 2200 up-to-date references that provide readers with excellent sources of worthwhile information that is of great value to clinicians, health researchers and providers, government food and nutrition policy leaders as well as graduate and medical students.

In conclusion, "**Nitrite and Nitrate in Human Health and Disease, 2nd Edition,**" edited by **Nathan S. Bryan, Ph.D., and Joseph Loscalzo, M.D., Ph.D.,** provides health professionals in many areas of research and practice with the most up-to-date, well-referenced volume on the importance of nitrite and nitrate, precursors of NO as key molecules in the maintenance of cardiovascular health and optimal functioning of the nervous and immune systems and other critical cells and tissues in the body. The value of this volume is captured in the Foreword, written by Dr. Louis Ignarro who was awarded the Nobel Prize in Medicine for his seminal research on the effects of NO in blood vessels.

The chapters review the role of diet, food, salivary bacterial nitrite synthesis, essential and nonessential nutrients, water, and other components of the diet in maintaining overall health as these provide sources of nitrite and nitrate as well as complementary molecules, such as antioxidants, that enhance the actions of NO with cells, mitochondria, and other cellular components. There is an overriding goal of the editors to evaluate the multiple effects of NO, within populations that suffer from undernutrition to obesity throughout the lifecycle, so that targeted progress can be made in improving the health of those at greatest risk of deficits of dietary and supplemental sources of nitrogen-containing compounds. Areas emphasized include new research on the importance of NO for maternal and infant health, especially in preterm infants, adolescent health and exercise strategies, and the health of older individuals at risk of increased oxidative stress. The importance of optimal dietary intakes of vegetables that are excellent sources of nitrate and the safe use of supplements (such as arginine and other compound that increase circulating levels of NO) are reviewed in detail in populations with increased risk of noncommunicable diseases, especially cardiovascular disease, diabetes, and obesity. The broad base of knowledge in this volume is evidenced by the contrasting, well-referenced chapters that present differing perspectives on the historic use of certain products versus the reliance on randomized, controlled clinical studies. Unique chapters review the implications of applying an evidence-based medicine approach to the use of NO-enhancing therapies, and in contrast, there is an equally informative chapter that describes the use of Chinese herbal medicine products for the same purpose. The volume serves the reader as the benchmark in this complex area of interrelationships between molecules that, at high concentrations, are air pollutants and perhaps carcinogens, and yet at low doses, within our bodies, are critically important in maintaining the movement of blood throughout the body. The editors, Dr. Nathan S. Bryan, Ph.D., and Dr. Joseph Loscalzo, M.D., Ph.D., and the excellent chapter authors are applauded for their efforts to develop the most authoritative and unique resource on the importance of nitrite and nitrate in health and disease, and this excellent text is a very welcome addition to the Nutrition and Health Series.

Morristown, NJ, USA

Adrianne Bendich, Ph.D., FACN, FASN
Series Editor

About the Series Editors

Adrianne Bendich, Ph.D., F.A.S.N., F.A.C.N. 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 "Nitrite and Nitrate in Human Health and Disease, 2nd Edition," edited by Nathan S. Bryan, Ph.D., and Joseph Loscalzo, M.D., Ph.D., major new editions published from 2012 to 2017 include:

1. Nutrition and Health in a Developing World, 3rd Edition edited by Dr. Saskia de Pee, PhD, Dr. Douglas Taren, PhD, and Dr. Martin W Bloem, MD, PhD, 2017
2. Nutrition in Lifestyle Medicine edited by James M. Rippe, MD, 2016
3. L-Arginine in Clinical Nutrition edited by Vinood B. Patel, Victor R. Preedy, and Rajkumar Rajendram, 2016
4. Mediterranean Diet: Impact on Health and Disease edited by Donato F. Romagnolo, Ph.D., and Ornella Selmin, Ph.D., 2016
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6. Nutrition in Cystic Fibrosis: A Guide for Clinicians edited by Elizabeth H. Yen, M.D., and Amanda R. Leonard, MPH, RD, CDE, 2016
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8. Glutamine in Clinical Nutrition edited by Rajkumar Rajendram, Victor R. Preedy, and Vinood B. Patel, 2015
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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 Physicians" edited by Dr. Edward Wilson, Dr. George A. Bray, Dr. Norman Temple, and Dr. Mary Struble; "Nutrition and

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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," coedited 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 *Nutrition*, the International Journal; 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. Dr. Bendich 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. Dr. Bendich has coauthored over 100 major clinical research studies in the area of preventive nutrition. She is recognized as a leading authority on antioxidants, nutrition and immunity, pregnancy outcomes, vitamin safety, and the cost-effectiveness of vitamin/mineral supplementation.

Dr. Bendich received the Roche Research Award, is a *Tribute to Women and Industry* Awardee, and was a recipient of the Burroughs Wellcome Visiting Professorship in Basic Medical Sciences. Dr. Bendich 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, Ph.D., R.D. 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.*

About Volume Editors

Nathan S. Bryan, Ph.D. earned his undergraduate Bachelor of Science degree in Biochemistry from the University of Texas at Austin and his doctoral degree from Louisiana State University School of Medicine in Shreveport where he was the recipient of the Dean's Award for Excellence in Research. He pursued his postdoctoral training as a Kirschstein Fellow at Boston University School of Medicine in the Whitaker Cardiovascular Institute.

After a 2-year postdoctoral fellowship, in 2006 Dr. Bryan was recruited to join the faculty at the University of Texas Health Science Center at Houston by Ferid Murad, M.D., Ph.D., 1998 Nobel Laureate in Medicine or Physiology. During his tenure as faculty and principle investigator at UT, his research focused on drug discovery through screening natural product libraries for compounds and extracts that could generate authentic NO gas or stimulate NO release from endothelial cells. His 9 years at UT led to several discoveries which have resulted in seven currently issued patents.

Dr. Bryan has been involved in nitric oxide research for more than 16 years and has made many seminal discoveries in the field. Specifically, Dr. Bryan was the first to describe nitrite and nitrate as vitamins and indispensable nutrients required for optimal cardiovascular health. Dr. Bryan's lab was the first to demonstrate and discover an endocrine function of nitric oxide via the formation of S-nitrosoglutathione and inorganic nitrite. Through the drug discovery program in natural product chemistry, Dr. Bryan discovered unique compositions of matter that can be used to safely and effectively generate and restore nitric oxide in humans. This technology is now validated in many published clinical trials. These discoveries and findings have unveiled many beneficial effects of nitrite in the treatment and prevention of human disease and may provide the basis for new preventive or therapeutic strategies in diseases associated with NO insufficiency and new guidelines for optimal health.

He is also a successful entrepreneur who has commercialized his nitric oxide technology through the formation of Human to the Power of N (formerly Neogenis Labs, Inc) where he is Co-founder and Chief Science Officer.

Dr. Bryan's most recent work has focused on the role of oral nitrate reducing bacteria in control of endogenous nitric oxide production and hypertension. He has published a number of highly cited papers and authored or edited six books.

Joseph Loscalzo, M.D., Ph.D. is Hersey Professor of the Theory and Practice of Medicine at Harvard Medical School, Chairman of the Department of Medicine, and Physician-in-Chief at Brigham and Women's Hospital. Dr. Loscalzo received his A.B. degree, *summa cum laude*, his Ph.D. in biochemistry, and his M.D. from the University of Pennsylvania. His clinical training was completed at Brigham and Women's Hospital and Harvard Medical School, where he served as Resident and Chief Resident in medicine and Fellow in cardiovascular medicine.

After completing his training, Dr. Loscalzo joined the Harvard faculty and staff at Brigham and Women's Hospital in 1984. He rose to the rank of Associate Professor of Medicine, Chief of Cardiology at the West Roxbury Veterans Administration Medical Center, and Director of the Center for Research in Thrombolysis at Brigham and Women's Hospital. He joined the faculty of Boston University in 1994, first as Chief of Cardiology and, in 1997, Wade Professor and Chair of Medicine, Professor of Biochemistry, and Director of the Whitaker Cardiovascular Institute. He returned to Harvard and Brigham and Women's Hospital in 2005.

Dr. Loscalzo is recognized as an outstanding cardiovascular scientist, clinician, and teacher. He has received many awards, including the Clinician-Scientist Award, the Distinguished Scientist Award, the Research Achievement Award, and the Paul Dudley White Award from the American Heart Association; a Research Career Development Award, a Specialized Center of Research in Ischemic Heart Disease Award, and a MERIT Award from the National Institutes of Health; the George W. Thorn Award for Excellence in Teaching at Brigham and Women's Hospital, the Educator of the Year Award in Clinical Medicine from Boston University, and the William Silen Lifetime Achievement in Mentorship Award from Harvard Medical School; the Glaxo Cardiovascular Research Award, and the Outstanding Investigator Prize from the International Society for Heart Research; election to fellowship in the American Association for the Advancement of Science, and in the American Academy of Arts and Sciences; and election to the American Society for Clinical Investigation, the Association of American Physicians, and the Institute of Medicine of the National Academy of Sciences (National Academy of Medicine). He has served on several NIH study sections and editorial boards and has chaired the Gordon Conference on Thrombolysis. He served as an associate editor of the *New England*

Journal of Medicine for 9 years, Editor-in-Chief of *Circulation* for 12 years, Chair of the Cardiovascular Board of the American Board of Internal Medicine, Chair of the Research Committee of the American Heart Association, Chair of the Scientific Board of the Stanley J. Sarnoff Society of Fellows for Research in the Cardiovascular Sciences, and Chair of the Board of Scientific Counselors of the National Heart, Lung, and Blood Institute of the National Institutes of Health. He is a former member of the Advisory Council of the National Heart, Lung, and Blood Institute and a former member of the Council of Councils of the National Institutes of Health. He is currently Director of the NIH-funded Center for Accelerated Innovation (the Boston Biomedical Innovation Center), the NIH-funded Harvard Undiagnosed Disease Network program, and a senior editor of **Harrison's Principles of Internal Medicine**.

Dr. Loscalzo has been a visiting professor at many institutions, holds two honorary degrees, has authored or coauthored more than 800 scientific publications, has authored or edited 41 books, and holds 31 patents for his work in the field of nitric oxide and redox biology. He is also the recipient of many grants from the NIH and industry for his work in the areas of vascular biology, thrombosis, atherosclerosis, and, more recently, systems biology over the past 30 years. He founded three companies, one of which became a publically traded company, and sits on the scientific advisory and corporate boards of several biotechnology and pharma companies. His most recent work has established the field of network medicine, a paradigm-changing discipline that seeks to redefine disease and therapeutics from an integrated perspective using systems biology and network science.

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Part I
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