

Rutherford's

VASCULAR SURGERY AND ENDOVASCULAR THERAPY

10TH EDITION

VOLUME 1

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Chapters done in 9e version - blue tick

- 1 Epidemiology and Research Methodology
- 2 Embryology and Developmental Anatomy
- 3 Vessel Wall Biology
- 4 Atherosclerosis
- 5 Intimal Hyperplasia

- 11 Atherosclerotic Risk Factors: Smoking
- 12 Atherosclerotic Risk Factors: Diabetes
- 13 Atherosclerotic Risk Factors: Hyperlipidemia
- 14 Atherosclerotic Risk Factors: Hypertension

24 Radiation Safety

67 Nonaortic Stents and Stent Grafts

- 69 Arterial Aneurysms: Etiology, Epidemiology, and Natural History
- 70 Aortoiliac Aneurysms: Evaluation, Decision Making, and Medical Management
- 71 Abdominal Aortic Aneurysms: Open Surgical Treatment
- 73 Endovascular Aneurysm Repair Techniques
- 74 Ruptured Aortoiliac Aneurysms and Their Management
- 75 Isolated Iliac Artery Aneurysms and Their Management

81 Aortic Dissection: Epidemiology, Pathophysiology, Clinical Presentation, and Medical and Surgical Management

- 86 Cerebrovascular Disease: Epidemiology and Natural History
- 87 Cerebrovascular Disease: The Unstable Carotid Plaque
- 88 Cerebrovascular Disease: Diagnostic Evaluation
- 89 Cerebrovascular Disease: Decision Making Including Medical Therapy
- 95 Carotid Body Tumors

100 Acute Limb Ischemia: Evaluation and Decision Making

101 Acute Ischemia: Treatment

- 125 Renovascular Disease: Pathophysiology, Epidemiology, Clinical Presentation, and Medical Management
- 126 Renovascular Disease: Open Surgical Treatment
- 127 Renovascular Disease: Endovascular Treatment

131 Mesenteric Arterial Disease: Epidemiology, Pathophysiology, and Clinical Evaluation

132 Chronic Mesenteric Arterial Disease: Clinical Evaluation, Open Surgical and Endovascular Treatment

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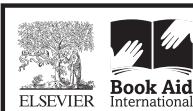
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To Michelle and Nicholas, my pride and joy. I have watched you grow into accomplished professionals with exemplary personal attributes and wonderful family and friendship values. Your ability to balance life and overcome challenges has been simply inspiring. To Mary, your selflessness, hard work, and dedication to family and career have been our north star that guided us all through good times and life's challenges.

Tony

To Rachel and Mason, who through your character and ethical conduct of your lives, and your unique professional talents and pursuit of your own individual career goals, your amazing accomplishments, and your wisdom beyond your years, make me so proud every day, and to Patti, whose selfless nurturing helps make you who you are.

Bruce

We also dedicate this contribution to our vascular surgical colleagues, and all caregivers across the healthcare field, who have given unparalleled and selfless dedicated service during the COVID pandemic – we consider it an honor to have you as our colleagues.

In particular, to our friend, colleague, and contributor to this book, Dr. Robyn Macsata who suddenly left us, we miss your bright outlook and passion for our specialty.

Tony and Bruce

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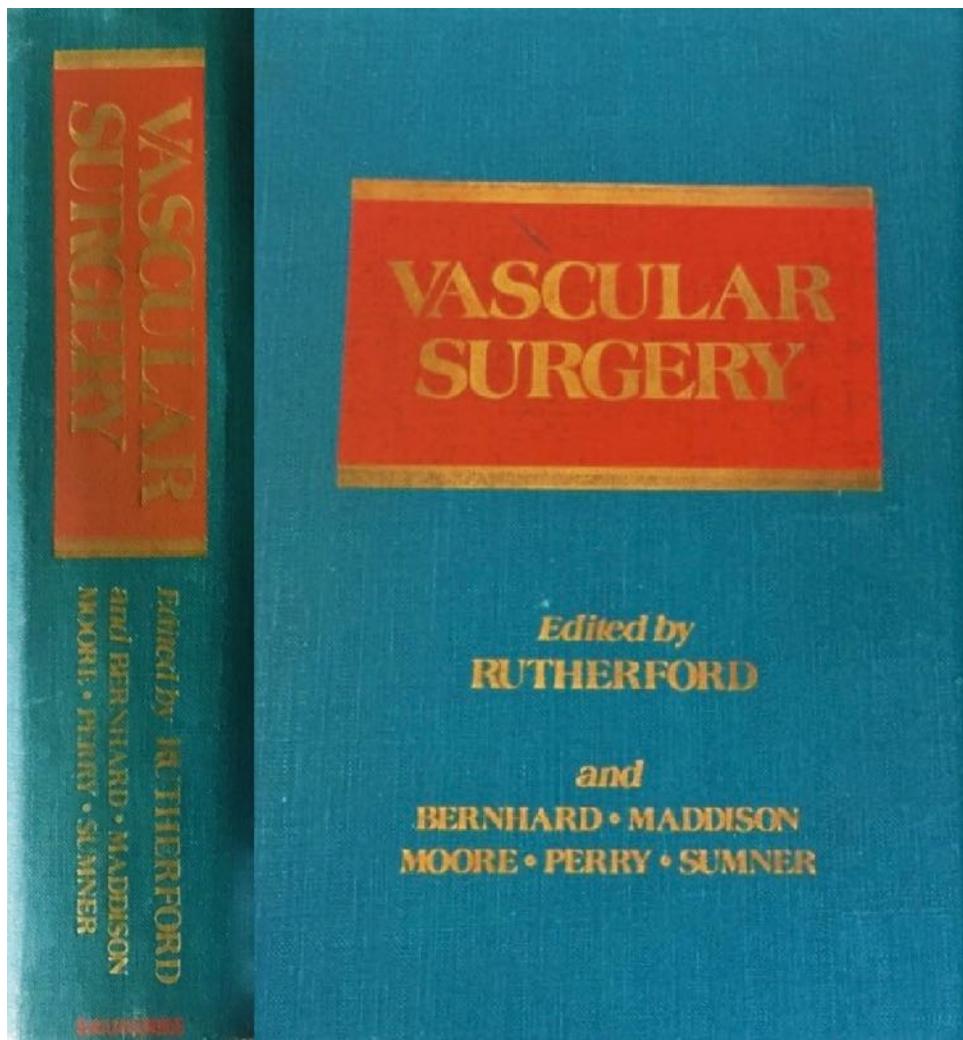
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PREFACE

This year with the publication of the tenth edition of this text, we commemorate the 45th anniversary of the

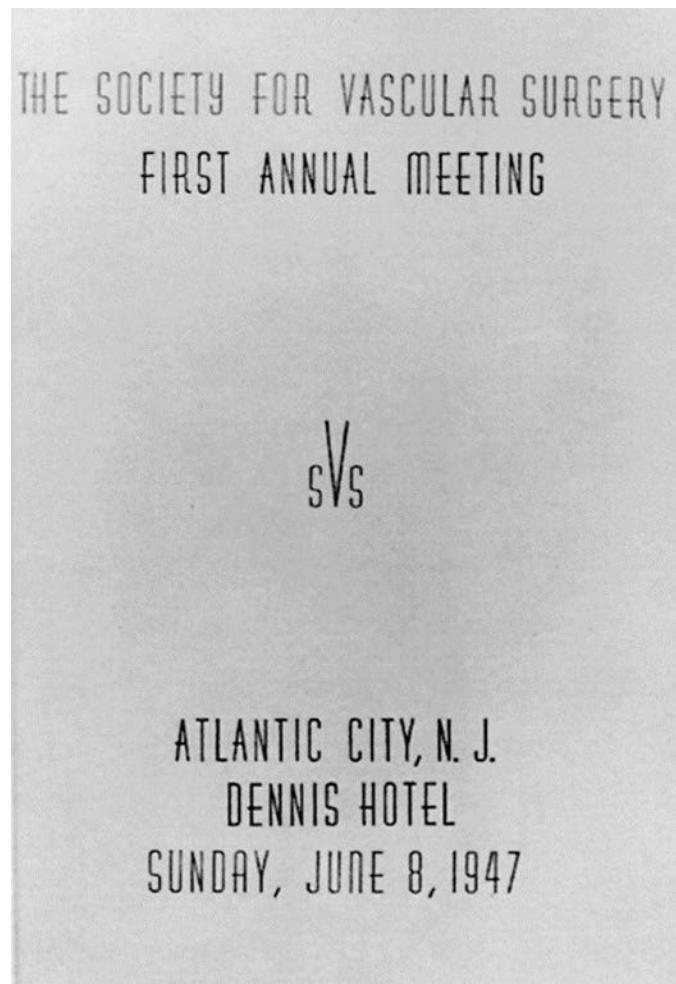
publication of the first edition of *Rutherford's Vascular Surgery* in 1977.



Dr. Robert Rutherford was an internationally recognized iconic leader in vascular surgery, a respected colleague, and a mentor and a dear personal friend to us both, whose impact on the education of students, residents, fellows, and practicing clinicians has been immeasurable. However, we suspect that even Dr. Rutherford could not have predicted the monumental impact that his textbook would have on the field.

This year also commemorates the 75th anniversary of the founding of the Society for Vascular Surgery (SVS) to which Dr. Rutherford entrusted future publication of his textbook. Since its inception in 1946, the SVS and its members have

seen many transformational changes in the diagnosis, management, and prevention of arterial and venous disease as documented in the programs of its annual meetings starting with the first meeting on June 8, 1947 at the Dennis Hotel in Atlantic City, NJ, when a total of nine presentations, including Dr. Alton Ochsner's Presidential Address "Venous Thrombosis," were made, and mainly centered on venous disease. The entire print program fit on one page compared to hundreds of presentations in the SVS Vascular Annual Meeting in 2021 accessed by an interactive format through smart mobile device application.



The transformation of the content of the programs of the annual meetings over the last 75 years chronicles the advancements and evolution in the diagnosis and management of vascular disease; an evolution that we aimed to emphasize in this edition of this text by including the most up-to-date information available.

It was with a sense of deep professional pride and responsibility that we assumed the editorship of the ninth edition of the book, and with that edition we changed the title to *Rutherford's Vascular Surgery and Endovascular Therapy*, to reflect the dramatic evolution in the evaluation and treatment of patients with circulatory disease. It is with an equal level of dedication and enthusiasm that we have devoted the past three years to produce the tenth edition of this work, which we believe is an unparalleled resource to support clinicians in our mission to optimize the care of patients with vascular disease, and to continue as a living testimonial to Dr. Rutherford's memory and legacy.

From its first edition, in the nearly half century of its existence, this textbook has always been, and without question, we believe, remains today, *the definitive* reference text in the field. We are indebted to Dr. Rutherford for his vision and commitment, and to our colleagues, Drs. Jack Cronenwett and Wayne Johnston, who edited the seventh and eighth editions,

for handing over to us a superb text to build on; a book that is without question the *bible* of vascular surgery.

When we assumed the editorship of the ninth edition, it was our core belief that we should commit ourselves to continually improving the textbook. While we were deeply gratified by the overwhelmingly positive book reviews of the ninth edition and the response of our readership, we rededicated ourselves to make the tenth edition even better through significant improvements that will quickly be apparent to the reader.

Technology is advancing and new medical information is developing at a faster rate than at any time in our history with respect to the care of patients with vascular disease. In addition, the practice of medicine in general, and vascular surgery in particular, is becoming increasingly complex and challenging. Indeed, the content of these two volumes reflects the totality of care delivered by vascular surgeons and other specialists devoted to the evaluation and management of the vascular patient in contemporary practice – namely, open surgery, endovascular therapy, and medical management of patients with the entire spectrum of circulatory disease – as well as presenting the most valuable diagnostic modalities; the basic science foundation of vascular disease and its treatment; and, the delivery of vascular care within the context of what is today the business of medicine.

This tenth edition contains 207 chapters organized in 32 focused sections. This is the first edition of this textbook in which we have included in the majority of the chapters concise yet very comprehensive diagnostic and therapeutic algorithms vital to the diagnosis and management of the condition addressed in that particular chapter. These algorithms provide for the reader a practical and succinct summary of the substance of the information in that chapter. The number of chapters has increased not only by adding new chapters in evolving areas of practice, but also by creating more directed presentations of the subject matter in shorter and more focused chapters to allow easier access to the desired information; having that in mind, we also included at the beginning of every chapter a listing of the topics discussed in that chapter.

The authors in this text were carefully selected to represent a roster of multidisciplinary national and international innovative thought leaders in the field, and whose contributions represent collectively the most up-to-date advances in the scientific basis for, and the management of, vascular disease to provide an unparalleled insight into the most appropriate contemporary and future treatment of these conditions. No other text can match the level of expertise assembled in this one book. Optimal patient outcomes increasingly are achieved through multidisciplinary care; therefore, we believe a strength of this text results from the insights of a unique group, these most respected experts from the entire spectrum of medical specialties, as well as vascular surgery and basic science. Likewise, in an increasingly global healthcare system, the international authorship is a strength of this book.

As noted in the ninth edition, we increased the number of chapters in the book not only to present the material in a more concise and focused manner, but also by adding new chapters for this edition. In fact, every chapter in this book, previously published, has been revised for the tenth edition and in some cases the authorship changed. We felt that the expansion in the number of chapters was necessary to incorporate new topics, reflect the rapid generation of new information, and reorganize information on topics that gained more relevance over the years, or add topics that have not been included in past editions. This book covers the totality of commonplace vascular problems as well as esoteric challenges infrequently seen by the vascular specialist, such as vascular oncologic conditions and pediatric vascular aneurysms and tumors. It presents the most up-to-date information on the endovascular management of vascular disease, including complex aneurysms such as aortic arch and thoracoabdominal aortic aneurysms. Recognizing the increasing influence of economic considerations in the delivery of care, we completely revised the section on the business of medicine for the tenth edition, including chapters on the operation of multidisciplinary cardiovascular centers, outpatient vascular centers, and outpatient dialysis care. Further, an entirely new section on the use of technology platforms and social media in vascular surgery includes the most up-to-date information on the marketing of a vascular practice and branding of vascular surgery, including focused chapters on the use of the

internet and social media in vascular practice, and the evolving strategy of telemedicine for the vascular surgeon. Furthermore, we continue in the tenth edition to provide the latest information with respect to managing less common but still occasionally encountered, and especially challenging clinical problems such as medial arcuate ligament syndrome and its contemporary management, vascular reconstructions in oncologic surgery, management of complex regional pain syndrome, and management of chronic compartment syndrome, among others. With the increasing performance of endovascular interventions and consequent reduced exposure to open surgery, we believe a text that devotes significant space to open vascular surgical exposure is more important than ever. This text directly addresses that need with chapters devoted to open surgical exposure in various vascular beds and operative techniques with extensive illustrations and videos.

It was our vision when we began this project to produce a book that would be of enormous value to not only practicing vascular surgeons, as well as interventional radiologists and interventional cardiologists treating patients with circulatory disease, but of equal if not greater value to residents and fellows in these disciplines and medical students and other allied healthcare professionals treating patients with vascular disease, and we believe we have achieved that vision. We also believe this text will be especially valuable to vascular surgical trainees as they prepare for in-service and board certification examinations.

Finally, we have all been functioning in a healthcare system, and in our own communities and personal lives, at a time of unprecedented stress related to the COVID pandemic in this country and globally. Despite all the challenges that this has created, the work of producing this book continued unabated. In that light we are especially indebted to our twelve associate editors, international leaders in the field, who were each responsible for editing specific sections of the book. Words cannot express our deep appreciation to Drs. AbuRahma, Aulivola, Brown, Duncan, Eidt, Forbes, Harris, Henke, Hoballah, Rowe, Upchurch, and Velazquez. Their diligence in working with the contributors to control the size and direct the focus of each chapter was instrumental in allowing us to execute our goal of increasing the number of chapters in the book while meeting our page allotment. We would like to thank our contributors who managed to produce the most up-to-date information available and produce algorithms that summarize such information and put it in a practical format for the readers to benefit from it in the care of their patients; they are the ones who did the majority of the work while following our sometimes burdensome instructions to make the book look and feel as one entity despite the participation of over 350 authors. They did this work mostly in the middle of the COVID-19 pandemic while working hard in untoward and mostly unsafe circumstances risking themselves and their families and loved ones, taking care of infected patients. We also greatly appreciate the hard work and attention to details by the production team at Elsevier, in particular, Joanie Milnes, Senior Content Development

Specialist; Jessica McCool, Content Strategist, Books; and Joanna Souch, Production Manager.

Finally, we would like to again thank the Society for Vascular Surgery and its Publications Committee for putting their trust in us; we hope we were able to deliver what the readership will find educationally valuable, but most important, beneficial in improving the care of the vascular patient – a truly must-have resource.

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To Rachel and Mason, who through your character and ethical conduct of your lives, and your unique professional talents and pursuit of your own individual career goals, your amazing accomplishments, and your wisdom beyond your years, make me so proud every day, and to Patti, whose selfless nurturing helps make you who you are.

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We also dedicate this contribution to our vascular surgical colleagues, and all caregivers across the healthcare field, who have given unparalleled and selfless dedicated service during the COVID pandemic – we consider it an honor to have you as our colleagues.

In particular, to our friend, colleague, and contributor to this book, Dr. Robyn Macsata who suddenly left us, we miss your bright outlook and passion for our specialty.

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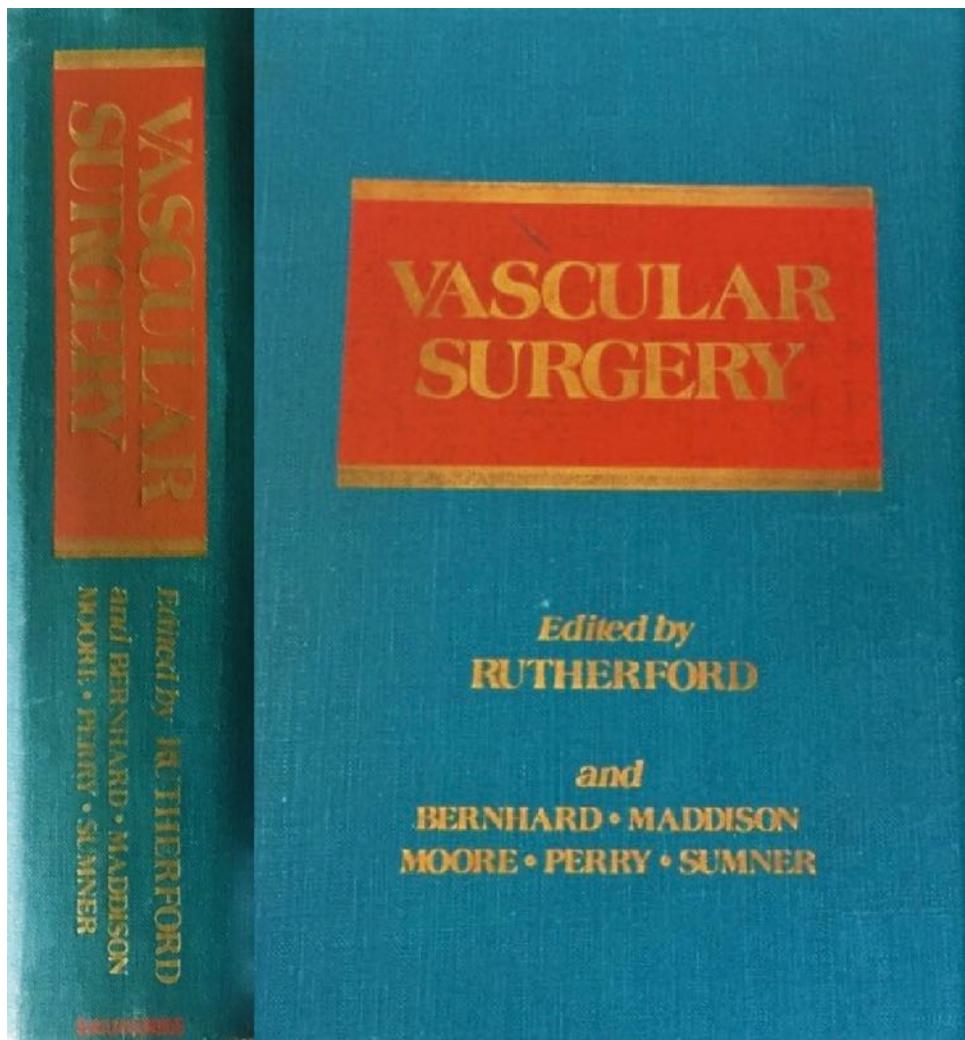
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PREFACE

This year with the publication of the tenth edition of this text, we commemorate the 45th anniversary of the

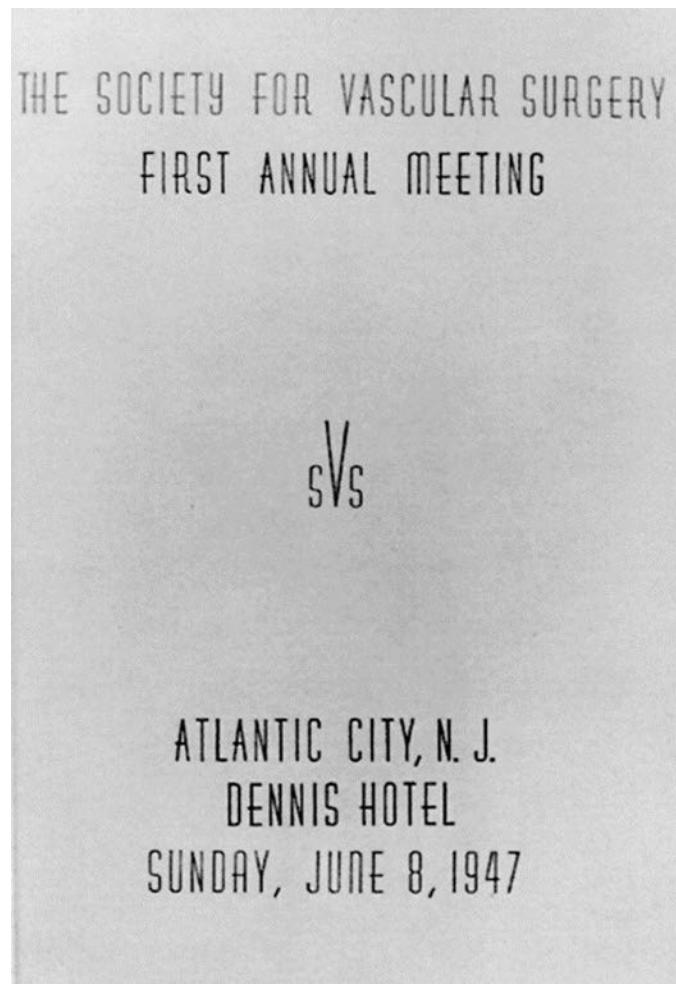
publication of the first edition of *Rutherford's Vascular Surgery* in 1977.



Dr. Robert Rutherford was an internationally recognized iconic leader in vascular surgery, a respected colleague, and a mentor and a dear personal friend to us both, whose impact on the education of students, residents, fellows, and practicing clinicians has been immeasurable. However, we suspect that even Dr. Rutherford could not have predicted the monumental impact that his textbook would have on the field.

This year also commemorates the 75th anniversary of the founding of the Society for Vascular Surgery (SVS) to which Dr. Rutherford entrusted future publication of his textbook. Since its inception in 1946, the SVS and its members have

seen many transformational changes in the diagnosis, management, and prevention of arterial and venous disease as documented in the programs of its annual meetings starting with the first meeting on June 8, 1947 at the Dennis Hotel in Atlantic City, NJ, when a total of nine presentations, including Dr. Alton Ochsner's Presidential Address "Venous Thrombosis," were made, and mainly centered on venous disease. The entire print program fit on one page compared to hundreds of presentations in the SVS Vascular Annual Meeting in 2021 accessed by an interactive format through smart mobile device application.



The transformation of the content of the programs of the annual meetings over the last 75 years chronicles the advancements and evolution in the diagnosis and management of vascular disease; an evolution that we aimed to emphasize in this edition of this text by including the most up-to-date information available.

It was with a sense of deep professional pride and responsibility that we assumed the editorship of the ninth edition of the book, and with that edition we changed the title to *Rutherford's Vascular Surgery and Endovascular Therapy*, to reflect the dramatic evolution in the evaluation and treatment of patients with circulatory disease. It is with an equal level of dedication and enthusiasm that we have devoted the past three years to produce the tenth edition of this work, which we believe is an unparalleled resource to support clinicians in our mission to optimize the care of patients with vascular disease, and to continue as a living testimonial to Dr. Rutherford's memory and legacy.

From its first edition, in the nearly half century of its existence, this textbook has always been, and without question, we believe, remains today, *the definitive* reference text in the field. We are indebted to Dr. Rutherford for his vision and commitment, and to our colleagues, Drs. Jack Cronenwett and Wayne Johnston, who edited the seventh and eighth editions,

for handing over to us a superb text to build on; a book that is without question the *bible* of vascular surgery.

When we assumed the editorship of the ninth edition, it was our core belief that we should commit ourselves to continually improving the textbook. While we were deeply gratified by the overwhelmingly positive book reviews of the ninth edition and the response of our readership, we rededicated ourselves to make the tenth edition even better through significant improvements that will quickly be apparent to the reader.

Technology is advancing and new medical information is developing at a faster rate than at any time in our history with respect to the care of patients with vascular disease. In addition, the practice of medicine in general, and vascular surgery in particular, is becoming increasingly complex and challenging. Indeed, the content of these two volumes reflects the totality of care delivered by vascular surgeons and other specialists devoted to the evaluation and management of the vascular patient in contemporary practice – namely, open surgery, endovascular therapy, and medical management of patients with the entire spectrum of circulatory disease – as well as presenting the most valuable diagnostic modalities; the basic science foundation of vascular disease and its treatment; and, the delivery of vascular care within the context of what is today the business of medicine.

This tenth edition contains 207 chapters organized in 32 focused sections. This is the first edition of this textbook in which we have included in the majority of the chapters concise yet very comprehensive diagnostic and therapeutic algorithms vital to the diagnosis and management of the condition addressed in that particular chapter. These algorithms provide for the reader a practical and succinct summary of the substance of the information in that chapter. The number of chapters has increased not only by adding new chapters in evolving areas of practice, but also by creating more directed presentations of the subject matter in shorter and more focused chapters to allow easier access to the desired information; having that in mind, we also included at the beginning of every chapter a listing of the topics discussed in that chapter.

The authors in this text were carefully selected to represent a roster of multidisciplinary national and international innovative thought leaders in the field, and whose contributions represent collectively the most up-to-date advances in the scientific basis for, and the management of, vascular disease to provide an unparalleled insight into the most appropriate contemporary and future treatment of these conditions. No other text can match the level of expertise assembled in this one book. Optimal patient outcomes increasingly are achieved through multidisciplinary care; therefore, we believe a strength of this text results from the insights of a unique group, these most respected experts from the entire spectrum of medical specialties, as well as vascular surgery and basic science. Likewise, in an increasingly global healthcare system, the international authorship is a strength of this book.

As noted in the ninth edition, we increased the number of chapters in the book not only to present the material in a more concise and focused manner, but also by adding new chapters for this edition. In fact, every chapter in this book, previously published, has been revised for the tenth edition and in some cases the authorship changed. We felt that the expansion in the number of chapters was necessary to incorporate new topics, reflect the rapid generation of new information, and reorganize information on topics that gained more relevance over the years, or add topics that have not been included in past editions. This book covers the totality of commonplace vascular problems as well as esoteric challenges infrequently seen by the vascular specialist, such as vascular oncologic conditions and pediatric vascular aneurysms and tumors. It presents the most up-to-date information on the endovascular management of vascular disease, including complex aneurysms such as aortic arch and thoracoabdominal aortic aneurysms. Recognizing the increasing influence of economic considerations in the delivery of care, we completely revised the section on the business of medicine for the tenth edition, including chapters on the operation of multidisciplinary cardiovascular centers, outpatient vascular centers, and outpatient dialysis care. Further, an entirely new section on the use of technology platforms and social media in vascular surgery includes the most up-to-date information on the marketing of a vascular practice and branding of vascular surgery, including focused chapters on the use of the

internet and social media in vascular practice, and the evolving strategy of telemedicine for the vascular surgeon. Furthermore, we continue in the tenth edition to provide the latest information with respect to managing less common but still occasionally encountered, and especially challenging clinical problems such as medial arcuate ligament syndrome and its contemporary management, vascular reconstructions in oncologic surgery, management of complex regional pain syndrome, and management of chronic compartment syndrome, among others. With the increasing performance of endovascular interventions and consequent reduced exposure to open surgery, we believe a text that devotes significant space to open vascular surgical exposure is more important than ever. This text directly addresses that need with chapters devoted to open surgical exposure in various vascular beds and operative techniques with extensive illustrations and videos.

It was our vision when we began this project to produce a book that would be of enormous value to not only practicing vascular surgeons, as well as interventional radiologists and interventional cardiologists treating patients with circulatory disease, but of equal if not greater value to residents and fellows in these disciplines and medical students and other allied healthcare professionals treating patients with vascular disease, and we believe we have achieved that vision. We also believe this text will be especially valuable to vascular surgical trainees as they prepare for in-service and board certification examinations.

Finally, we have all been functioning in a healthcare system, and in our own communities and personal lives, at a time of unprecedented stress related to the COVID pandemic in this country and globally. Despite all the challenges that this has created, the work of producing this book continued unabated. In that light we are especially indebted to our twelve associate editors, international leaders in the field, who were each responsible for editing specific sections of the book. Words cannot express our deep appreciation to Drs. AbuRahma, Aulivola, Brown, Duncan, Eidt, Forbes, Harris, Henke, Hoballah, Rowe, Upchurch, and Velazquez. Their diligence in working with the contributors to control the size and direct the focus of each chapter was instrumental in allowing us to execute our goal of increasing the number of chapters in the book while meeting our page allotment. We would like to thank our contributors who managed to produce the most up-to-date information available and produce algorithms that summarize such information and put it in a practical format for the readers to benefit from it in the care of their patients; they are the ones who did the majority of the work while following our sometimes burdensome instructions to make the book look and feel as one entity despite the participation of over 350 authors. They did this work mostly in the middle of the COVID-19 pandemic while working hard in untoward and mostly unsafe circumstances risking themselves and their families and loved ones, taking care of infected patients. We also greatly appreciate the hard work and attention to details by the production team at Elsevier, in particular, Joanie Milnes, Senior Content Development

Specialist; Jessica McCool, Content Strategist, Books; and Joanna Souch, Production Manager.

Finally, we would like to again thank the Society for Vascular Surgery and its Publications Committee for putting their trust in us; we hope we were able to deliver what the readership will find educationally valuable, but most important, beneficial in improving the care of the vascular patient – a truly must-have resource.

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Epidemiology and Research Methodology

YIYUAN DAVID HU and PHILIP P. GOODNEY

Based on a previous edition chapter by Louis L. Nguyen and Rebecca E. Scully

EPIDEMIOLOGY 1

The Origins of Epidemiology 1

Modern Developments 1

CLINICAL RESEARCH METHODS 2

Study Design 2

Observational Studies 2

COHORT STUDIES IN VASCULAR CARE 2

COHORT AND CASE–CONTROL STUDIES IN UNCOMMON
VASCULAR CONDITIONS 2

USING COHORTS TO IDENTIFY RISK FACTORS 2

Experimental Studies 3

Special Techniques: Meta-Analysis 5

OUTCOMES ANALYSIS 5

Bias in Study Design 5

Statistical Methods 7

Regression Analysis 7

Survival Analysis 7

Adjusting for Confounding Using Propensity Scoring 8

Type I Error and Type II Error 8

Utility Measures 10

Decision Analysis 10

Cost–Benefit and Cost-Effectiveness Analysis 10

EVIDENCE IN PRACTICE 11

WHAT'S NEXT FOR VASCULAR SURGEONS IN OUTCOMES RESEARCH 11

The goal of this chapter is to introduce to the vascular surgeon principles that underlie the design, conduct, and interpretation of epidemiology and clinical research. Disease-specific outcomes otherwise detailed in subsequent chapters are not covered here. Rather, this chapter discusses the history of epidemiology in medicine, clinical research methods in vascular care, and techniques in outcome analysis. This chapter serves as a foundation for clinicians to better interpret clinical results and as a guide for researchers to further expand clinical analysis.

EPIDEMIOLOGY

The word *epidemiology* is derived from Greek terms meaning “upon” (*epi*), “the people” (*demos*), and “study” (*logos*) or “the study of what is upon the people.” It exists to answer the four major questions of medicine: diagnosis, etiology, treatment, and prognosis.

The Origins of Epidemiology

Hippocrates and his disciples not only marked the beginning of western medicine but were also among the first to

begin to contemplate the role of external factors in disease. As the world learned from the coronavirus epidemic in 2020, epidemiology has long captivated societies as they learn how and why diseases begin, spread, and manifest their effects on populations. Long before we studied the COVID-19 pandemic, John Snow is often cited as the first modern epidemiologist. In the middle of a cholera epidemic in the summer of 1854, Snow, a physician, by mapping the geographic distribution of incident cases, successfully identified the source of the outbreak as contaminated water from the Broad Street pump. He then convinced local officials to remove the pump handle, thus shutting down the pump and stopping the outbreak.¹

Modern Developments

The study of epidemiology continues the work of Hippocrates and Snow, working to investigate the cause and impact of disease. Many of the same basic principles apply: identifying the number of patients who have the disease, as

well as the number of patients who may contract the disease. Further, determining which disease outcomes occur most commonly, in whom, and why, are critical aspects of epidemiologic study. While early epidemiologists achieved these goals with pencil and paper, modern clinical investigators are armed with international registries, terabyte-bearing servers, machine-learning algorithms, and online collaborations. The principles, nonetheless, remain the same: adherence to data quality, sound analytic design, and clear interpretation and presentation of results.

CLINICAL RESEARCH METHODS

Each research project starts with a clinical question. Why do vein grafts fail? When is a patient at risk for aneurysm rupture? What size graft should I use? Which treatment option is best for this patient? Each of these different questions requires a different approach or “study design.” We review several of the basic options herein.

Study Design

Clinical research can be broadly divided into observational studies and experimental studies. Observational studies are characterized by the absence of a study-directed intervention. Experimental studies involve testing a treatment, be it a drug, device, or clinical pathway. Observational studies can follow ongoing treatments but cannot influence choices made in the treatment of a patient. Observational studies can be executed in a prospective or retrospective fashion, whereas experimental studies can be performed only prospectively.

Deciding between these approaches is influenced by a number of factors. A key first step is to determine how common the disease or exposure of interest is. The *prevalence* of disease is the ratio of persons affected for the population at risk and reflects the frequency of the disease at a single time point, regardless of the time of disease development. In contrast, the *incidence* is the ratio of persons in whom the disease develops within a specified period for the population at risk. For diseases with short duration or high mortality, prevalence may not accurately reflect the impact of disease because the single time point of measurement does not capture resolved disease or patients who died of the disease. Prevalence is a more useful parameter in discussing diseases of longer duration, whereas incidence is more useful for diseases of shorter duration.

Observational Studies

There are two main types of observational studies: cohort studies and case-control studies. A cohort is a group that has something in common; in epidemiology this is frequently risk of developing a disease of interest. Cohort studies enroll a population at risk and follow them for a period of time. Individuals who develop the disease in that time are then compared with individuals who remain disease-free.

Cohort studies in vascular care

There have been many cohort studies performed in vascular surgery pertaining especially to the utility of endovascular aneurysm repair (EVAR) versus open surgical repair (OSR) in the treatment of abdominal aortic aneurysms. One prominent trial was conducted by the OVER Veterans Affairs Cooperative Study Group, which recruited 881 patients and randomized them to EVAR or OSR. Notably, this study found that overall survival at 14 years of follow-up was similar between patients randomized to EVAR and those randomized to OSR² (Fig. 1.1A). Other notable cohort studies have been performed in vascular surgery using cohorts of patients described in Medicare claims, as well as cohorts from the Society for Vascular Surgery’s Vascular Quality Initiative (VQI) registry.

For example, Columbo et al. utilized a database that linked the VQI registry to Medicare claims to study 12,911 patients who had undergone EVAR and the long-term effects of the procedure. This group found that a third of EVAR patients were at risk of reintervention and further identified five clinical factors at the time of the initial repair that were associated with a higher risk of reintervention³ (Fig. 1.1B).

Cohort and case-control studies in uncommon vascular conditions

Cohort studies are facilitated by large numbers of patients. However, uncommon vascular conditions require study as well. Two strategies can be employed here. First, a well-designed effort in registry design has tackled the study of uncommon vascular conditions using international cohorts, and case-control studies. One such effort is the UCLA Vascular Low Frequency Disease Consortium (VLFDC), which uses patients from 75 reporting institutions from around the world to generate a cumulative sample size that has enough power to study rare vascular diseases and conditions. To date, the VLFDC has generated data on rare conditions such as renal artery aneurysms, aortic endograft infection, carotid body tumors, etc.⁴ Second, investigators can utilize a longitudinal, single institution approach that retrospectively analyzes all the patients with a condition through an entire time range. This was utilized to study the effect of vascular resection and reconstruction during sarcoma resection. In this study, the investigators studied 50 patients who had undergone this procedure from 2000 to 2014 and studied their outcomes relative to 100 similar patients who had not undergone the same treatment for sarcoma resection.⁵

Using cohorts to identify risk factors

Cohort studies allow us to determine risk factors, or variables, which can be deduced by comparisons between those with the condition and those without the condition. In this retrospective design, an *odds ratio* (OR) is calculated from the ratio of patients exposed to patients not exposed to the risk factors.

Risk factor analysis is a key derivative from large cohort studies in vascular surgery. For example, the Vascular Study Group of Northern New England utilized a prospective cohort of 1387 patients who underwent elective EVAR or OSR between 2003 and 2007. This cohort was representative of a population

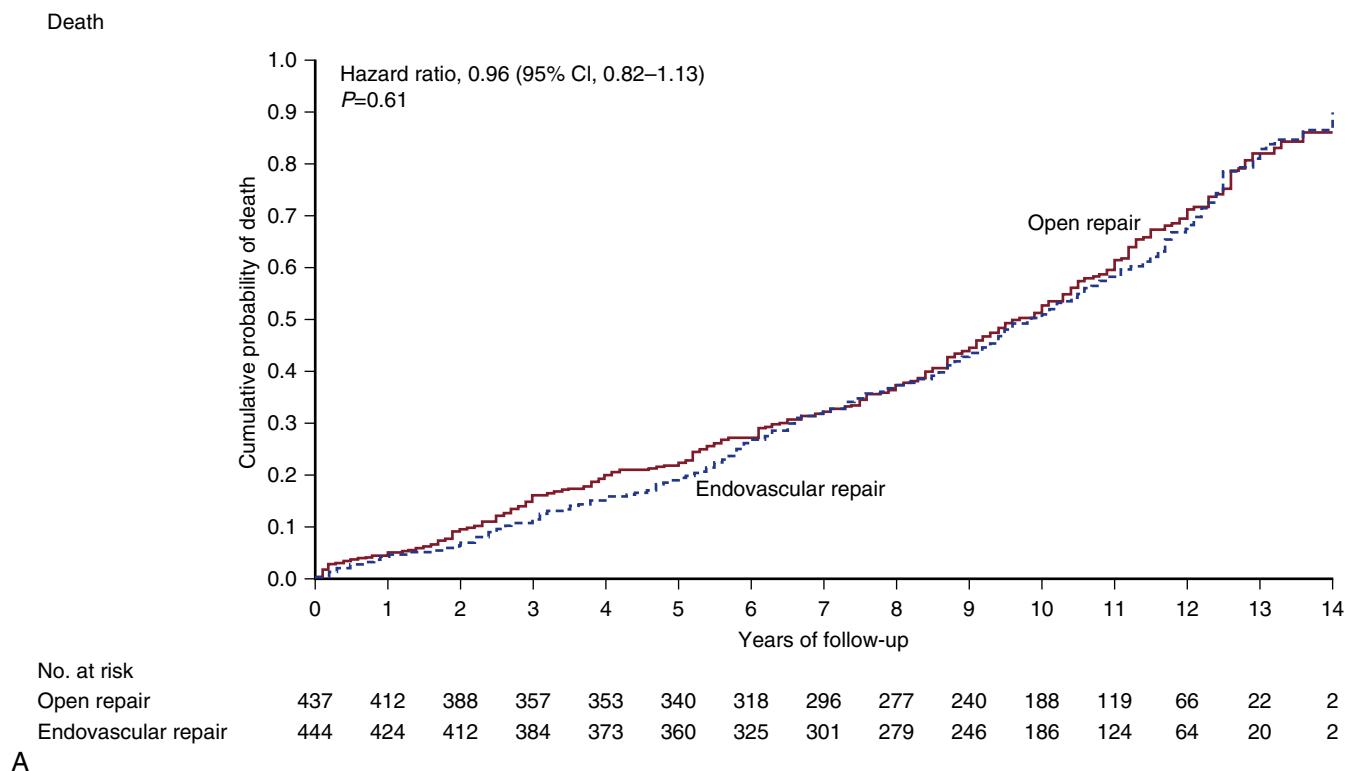


Figure 1.1 (A) Kaplan–Meier plot of cumulative mortality probability over 14 years. (B) This panel shows a Kaplan–Meier plot of cumulative risk of reintervention among the study cohort over 10 years. (C) Risk factors associated with mortality after 1 year. (A, Redrawn from Lederle FA, Kyriakides TC, Stroupe KT, et al., for the OVER Veterans Affairs Cooperative Study Group. Open versus endovascular repair of abdominal aortic aneurysm. *N Engl J Med.* 2019;380:2126–2135; B, Redrawn from Columbo JA, Martinez-Camblor P, O’Malley AJ, et al.; Society for Vascular Surgery’s Vascular Quality Initiative. Long-term reintervention after endovascular abdominal aortic aneurysm repair. *Ann Surg.* 2021;274(1):179–185. C, Redrawn from Beck AW, Goodney PP, Nolan BW, et al.; Vascular Study Group of Northern New England. Predicting 1-year mortality after elective abdominal aortic aneurysm repair. *J Vasc Surg.* 2009;49:838–844.)

Continued

undergoing prophylactic intervention, where it is especially important to have the necessary information to determine in which patients the procedural benefit would outweigh the procedural risk. This study sought to answer this question with a study population consisting of 748 OSR patients and 639 EVAR patients; the investigators identified statistically significant factors associated with 1-year mortality by univariate analysis. Furthermore, using Cox proportional hazard modeling, the group was able to generate a model that predicted patients who were at high risk for 1 year mortality. Their study identified unique factors impacting OSR and EVAR, thereby enabling better risk stratification and decision making when identifying qualified patients (Fig. 1.1C).⁶

Experimental Studies

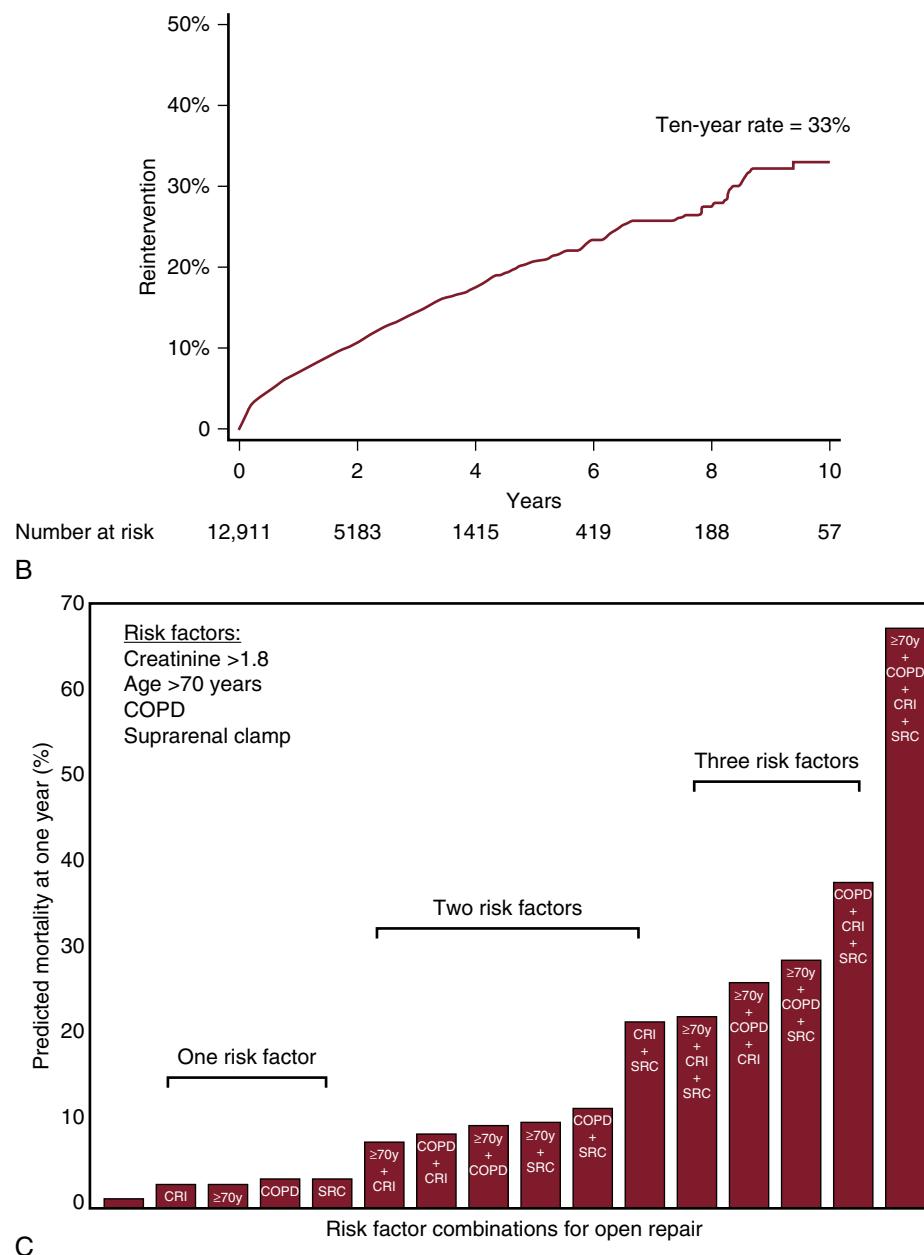
The other large class among study designs is the *experimental study*. Unlike observational studies, experimental trials involve introducing participants to an exposure of interest. One benefit of experimental studies is the ability to randomize participants, commonly via a *randomized controlled trial* (RCT).

The benefit of randomization is the avoidance of bias. Randomization ensures that known factors are evenly distributed between the exposure and control groups. Further, it also

ensures the even distribution of *unknown* factors. Thus, in a well-designed RCT, complex statistical models are not necessary to control for confounding factors, as long as randomization is performed in a well-designed and well-executed fashion.

There are several ways of structuring a randomization to address potential issues including complete randomization of the entire study population, block randomization, and adaptive randomization. For *complete randomization*, each new patient is randomized without prior influence on previously enrolled patients. The expected outcome at the completion of the trial is an equal distribution of patients within each treatment group, although unequal distribution may occur by chance, especially in small trials.

In a *cluster randomization*, groups of individuals (i.e. communities, schools, hospital systems, etc.) are randomized to treatment arms. This methodology is useful when complete randomization is difficult to implement, and other factors can confound randomization at the individual level. One example of such a study is the Preferences for Open Versus Endovascular Repair of Abdominal Aortic Aneurysm (PROVE-AA) trial. This cluster randomized trial aimed to determine the efficacy of a validated decision aid to enable better matching between a patient’s ultimate repair modality and their preoperative preference. This



study's unit of randomization was each participant location. Each location was randomized to receiving a decision aid or not receiving a decision aid. All subsequent patients in each location would then receive or not receive the decision aid on the basis of their treatment location. As treating patients who have used the decision aid may change how a surgeon interacts with those who do not have the decision aid, a cluster randomized trial was used to ensure that a surgeon's actions are not "contaminated" by the decision aid itself. This methodology further ensures better assessment of the decision aid's efficacy.⁷

Experimental studies face stricter ethical and patient safety requirements than their observational counterparts. One basic assumption of experimental trials is *clinical equipoise*, or the existence of more than one generally accepted treatment.⁸ This must exist both to create the situation where the research that is being undertaken will lead to clinical relevant information and

that the treatment options to which a participant is randomized will not be assuming risk of care that is known to be inferior. Whereas you could not randomize people to observation only for a ruptured aortic aneurysm, for certain populations you could make an argument for endovascular versus open repair. This type of situation often arises when clinical experts professionally disagree on the preferred treatment method.⁸ It is worth noting that although the field may have equipoise, individual healthcare providers or patients may have bias for one treatment. In such a case, enrollment in an RCT may be difficult because the patients or their providers are not willing to be subject to randomization. A recent example of a large clinical trial in vascular care where equipoise in treatment options has been compared is the Best Endovascular vs. Best Surgical Therapy in Patients with Critical Limb Ischemia, or BEST-CLI trial (www.bestcli.com). This large multicenter, NHLBI-funded

trial compares open surgery to endovascular treatments using a pragmatic study design. Results from this landmark trial are expected to be reported in the fall of 2020.

Although RCTs represent the pinnacle in clinical design, there are many situations in which RCTs are impractical or impossible. Clinical equipoise may not exist, or common sense could prevent randomization of well-established practices, such as the use of parachutes during free fall.⁹ RCTs can also be costly to conduct and must generate a new control group with each trial. For this reason, some studies are single-arm trials that use historical controls similar to the case-control design. In addition, patient enrollment may also be difficult, particularly if patients or clinicians are uneasy with the randomization of treatment. RCTs can also have methodologic and interpretative limitations. For example, if study patients are analyzed by their assigned randomization grouping (*intent to treat*) studies with asymmetric or high overall dropout and/or crossover rates may not reflect actual treatment effects. Given the cost and time required, RCTs are often conducted in high-volume specialty centers; as a result, enrollment and treatment of study patients may not reflect the general population with the disease or providers in the community. Finally, as with any analysis, inaccurate assumptions made in the initial power calculations may lead to failure to capture a true effect.

Special Techniques: Meta-Analysis

Meta-analysis is a statistical technique that combines the results of several related studies to address a common hypothesis. The first use of meta-analysis in medicine is attributed to Smith and Glass in their review of the efficacy of psychotherapy in 1977.¹⁰ By combining results from several smaller studies, researchers may decrease sampling error and increase statistical power, thus helping to clarify disparate results among different studies.

The related studies must share a common dependent variable. *Effect size* specific to each study is then weighted to account for the variance in each study. Because studies may differ in patient selection and their associated independent variables, a test for heterogeneity should also be performed. Where no heterogeneity exists ($P > 0.5$), a fixed-effects meta-analysis model is used to incorporate the within-study variance for the studies included. A random-effects model is used when concern for between-study variance exists ($0.5 > P > 0.05$). When heterogeneity among studies is found, the OR should not be pooled and further investigation for the source of heterogeneity may then exclude outlying studies.

The weighted composite dependent variable is visually displayed in a forest plot along with the results from each study included. Each result is displayed as a point estimate, with a horizontal bar representing the 95% confidence interval for the effect. The symbol used to mark the point estimate is usually sized proportional to other studies to reflect the relative weight of the estimate as it contributes to the composite result. For example, Columbo and colleagues examined bleeding risk associated with continuing aspirin during non-cardiac surgery, with an effect size shown in the forest plot shown in **Figure 1.2**. Classically, meta-analyses have included only RCTs, but observational studies can also be used.^{11,12} Inclusion of observational

studies can result in greater heterogeneity through uncontrolled studies or controlled studies with selection bias.

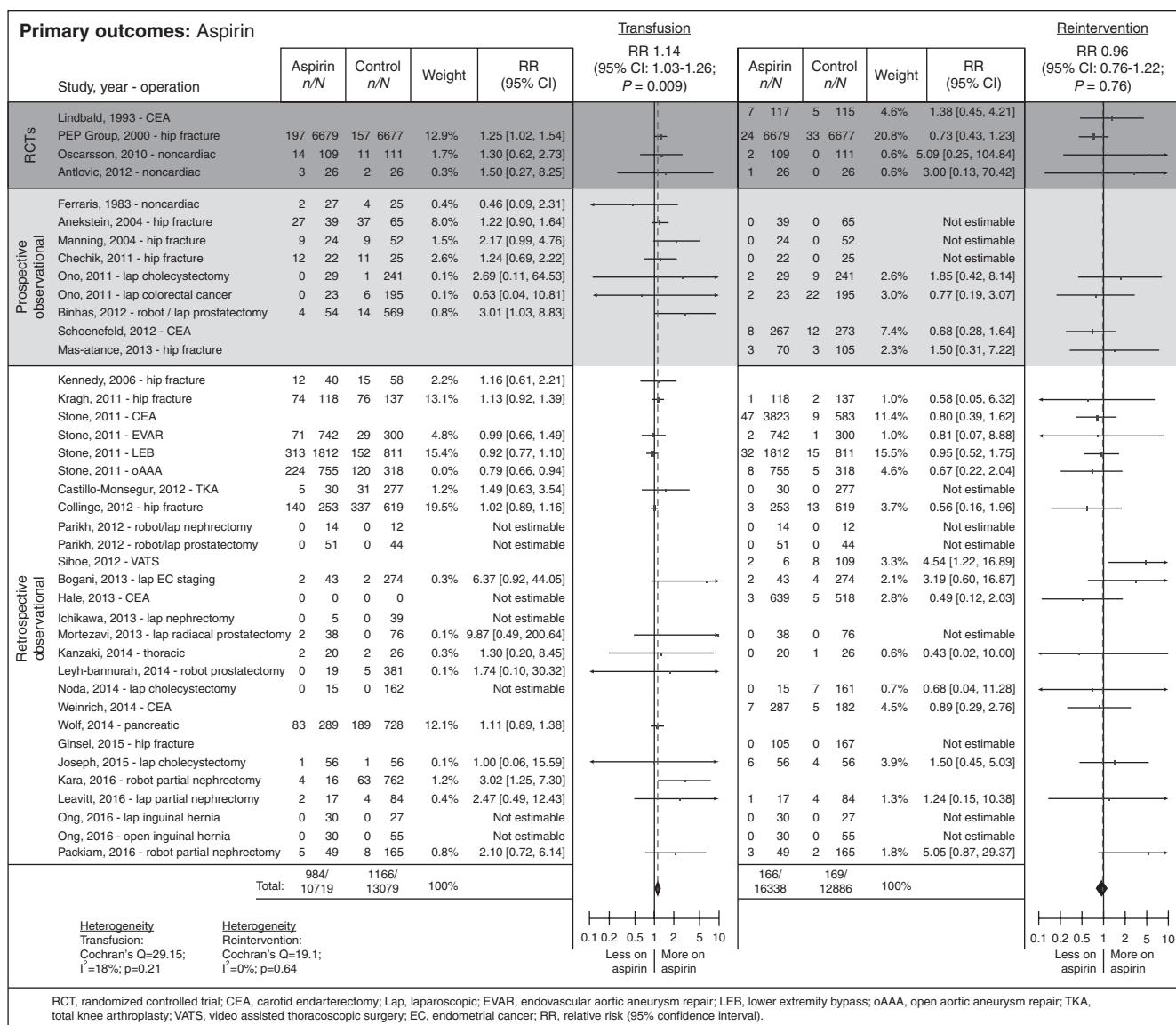
The strength of a meta-analysis comes from the strength of the studies that make up the composite variable. Furthermore, if available, the results of unpublished studies can also potentially influence the composite variable, because presumably many studies with nonsignificant results are not published. Therefore, an assessment of *publication bias* should be included with every meta-analysis. Publication bias can be assessed graphically by creating a *funnel plot* in which the effect size is compared with the sample size or another measure of variance. If no bias is present, the effect sizes should be balanced around the population mean effect size and decrease in variance with increasing sample size. If publication bias exists, part of the funnel plot will be sparse or empty of studies. *Begg's test* for publication bias is a statistical test that represents the funnel plot's graphic test.¹³ The variance of the effect estimate is divided by its standard error to give adjusted effect estimates with similar variance. Correlation is then tested between the adjusted effect size and the meta-analysis weight. An alternative method is *Egger's test*, in which the study's effect size divided by its standard error is regressed on 1/standard error.¹⁴ The intercept of this regression should equal zero, and testing for the statistical significance of nonzero intercepts should indicate publication bias.

OUTCOMES ANALYSIS

As physicians, we can usually see the natural progression of disease or the clinical outcome of treatment. Although these observations can be made for individual patients, general inferences about causation and broad application to all patients cannot be made without further analysis. Clinical analysis attempts to answer these questions by either observing or testing patients and their treatments. Because clinical analysis can be performed only on a subset or sample of the relevant entire population, a level of uncertainty will always exist in clinical analysis. Statistical methods are an integral aspect of clinical analysis because they help the researcher understand and accommodate the inherent uncertainty in a sample in comparison to the ideal population. In the following sections, common clinical analytic methods are reviewed so that the reader can better interpret clinical analysis and also have foundations to initiate an analysis. Reference to biostatistical and econometric texts is recommended for detailed derivation of the methods discussed.

Bias in Study Design

In discussing statistical methods, it is important to remember that clinical analysis can estimate only the "true" effects of a disease or its potential treatments. Because the true effects cannot be known with certainty, analytic results carry potential for error. All studies can be affected by two broadly defined types of error: random error and systematic error. *Random error* in clinical analysis comes from natural variation and can be handled with the statistical techniques covered later in this chapter.



RCT, randomized controlled trial; CEA, carotid endarterectomy; Lap, laparoscopic; EVAR, endovascular aortic aneurysm repair; LEB, lower extremity bypass; oAAA, open aortic aneurysm repair; TKA, total knee arthroplasty; VATS, video assisted thoracoscopic surgery; EC, endometrial cancer; RR, relative risk (95% confidence interval).

Figure 1.2 Example of a forest plot from a meta-analysis of carotid artery stenting (CAS) versus carotid endarterectomy (CEA) to determine 30-day risk for stroke and death. CI, confidence interval. (Redrawn from Brahmanandam S, Ding EL, Conte MS, et al. Clinical results of carotid artery stenting compared with endarterectomy. *J Vasc Surg*. 2008;47:343–349.)

Systematic error, also known as *bias*, affects the results in one unintended direction and can threaten the validity of the study. Bias can be further categorized into three main groupings: selection bias, information bias, and confounding.

Selection bias occurs when the effect being tested differs among patients who participate in the study as opposed to those who do not. Because actual study participation involves a researcher's determination of which patients are eligible for a study and then the patient's agreement to participate in the study, the decision points can be affected by bias. One common form of selection bias is self-selection, in which patients who are healthier or sicker are more likely to participate in the study because of perceived self-benefit. Selection bias can also occur at the level of the researchers when they perceive potential study patients as being too sick and preferentially recruit healthy patients.

Confounding is a significant factor in epidemiology and clinical analysis. Confounding exists when a second spurious variable (e.g., race/ethnicity) correlates with a primary independent variable (e.g., type 2 diabetes) and its associated dependent variable (e.g., critical limb ischemia). Researchers can conclude that patients in certain race/ethnicity groups are at greater risk for critical limb ischemia when diabetes is the stronger predictor. **Confounding by indication** is especially relevant in observational studies. This can occur when, without randomization, patients being treated with a drug can show worse clinical results than untreated counterparts because treated patients were presumably sicker at baseline and required the drug *a priori*. Confounding can be addressed by several methods: assigning confounders equally to the treatment and control groups (for case-control studies), matching confounders equally (for cohort studies), stratifying the results according to confounding groups, and multivariate analysis.

BOX 1.1**Choosing Statistical Tests Based on Research Question and Data Characteristics****Is There a Difference Between Means, Medians, and Proportions?****One Group**

- Parametric data: one sample *t*-test
- Nonparametric data: sign test, Wilcoxon signed rank test, transform data for *t*-test
- Proportions: exact binomial test, *z* approximation to exact test

Two Independent Groups

- Parametric data: *t*-test
- Nonparametric data: Wilcoxon rank-sum test
- Proportions: chi-squared or Fisher's exact test

Two Related Groups

- Parametric data: paired *t*-test
- Nonparametric data: sign test, Wilcoxon signed-rank test
- Proportions: McNemar's test or kappa statistic

Three or More Independent Groups

- Parametric data: ANOVA

- Nonparametric data: Kruskal-Wallis test
- Proportions: chi-squared or Fisher's exact test

Three or More Related Groups

- Parametric data: repeated-measures ANOVA
- Nonparametric: ANOVA by ranks

Is There an Association?**Two Comparable Variables**

- Nominal data: relative risk
- Ordinal data: Spearman's rank correlation test
- Continuous data: linear regression

One Dependent Variable and Two or More Independent Variables

- Binary dependent variable: logistic regression
- Categorical dependent variable: ANCOVA
- Continuous dependent variable: multiple linear regression
- Censored observations: CPH model
- Clustered or hierarchic parametric data: linear mixed models
- Clustered or hierarchic semiparametric data: GEE

ANCOVA, analysis of covariance; ANOVA, analysis of variance; CPH, Cox proportional hazards; GEE, generalized estimating equations.

Statistical Methods

At the beginning of most clinical analyses, *descriptive statistics* are used to quantify the study sample and its relevant clinical variables. *Continuous variables*, or variables that can take on any value in a range between a minimum and a maximum, such as weight or age, are expressed as means or medians; *categorical variables*, or variables that have only a discrete value, such as institution of treatment or TASC classification, are expressed as numbers or percentages of the total. A subset of categorical variables are *ordinal variables*, in which categories have some structure or relative value, such as good, better, best. Study sample characteristics and their relative distribution of comorbid conditions help determine whether the sample is consistent with known population characteristics and hence addresses the issue of generalizability of the clinical results to the overall population.

The next step in clinical analysis is *hypothesis testing*, in which the factor or treatment of interest is tested against a control group. The statistical methods used in hypothesis testing depend on the research question and characteristics of the data under comparison (Box 1.1). At its core, hypothesis testing asks whether the observable differences between groups represent true differences or if they just appear different because of random chance. A wide variety of tests exist and each attempts to answer this question in a way that is appropriate to the data in question.

Regression Analysis

Among the statistical tests available, a few deserve special mention because of their common application to the clinical analysis of studies of vascular patients. *Regression analysis* is a mathematical technique in which the relationship between a dependent (or response) variable is modeled as a function of

one or more independent variables, an intercept, and an error term. Models often describe a linear relationship between dependent and independent variables; however, they can also take on polynomial relationships, including quadratic and cubic functions. Regression analysis produces regression coefficients for each variable of interest. Regression coefficients, or *betas* (β), describe the magnitude of the effect that each independent variable (x) has on the dependent variable (y). For binary dependent variables, a *logistic (logit)* regression is used, whereas for continuous dependent variables, a *linear regression* is used (see Box 1.1). The *goodness of fit* for the model is tested by using the R^2 value (R squared) and the analysis of residuals. R^2 is the proportion of variability that is accounted for by the model and has a range of 0 to 1. Although larger R^2 values imply better fit, there is no defined threshold for goodness of fit and R^2 can be artificially inflated by adding more variables to the model. Thus, an adjusted R^2 , which also accounts for the number of variables in the model, should be used.

Survival Analysis

Survival analysis was developed to assess patient survival, and while death is often the primary event of interest, survival analysis can also be used to assess treatment failure, such as time to loss of graft patency or amputation. Rather than simply addressing frequency, survival analysis also captures an element of time to an event. It also incorporates *censorship*, in which data about the event of interest are unknown because of withdrawal of the patient from the study. Traditionally in clinical analysis, *death* is the event variable, and *loss to follow-up* is the censorship variable. In vascular surgery, where graft patency is more often the endpoint of interest, *graft patency* is treated as the event variable and *death* and/or *study withdrawal* is treated as

the combined censoring variable. This assumes that censorship (death) is not due to the event (loss of graft patency); however, this assumption cannot be held true in other fields, such as oncology (death attributable to failure of cancer treatment) or cardiac surgery (death caused by loss of coronary artery bypass graft patency).

In essence, survival analysis accounts for event status between fixed periods of measurement. For example, in traditional methods, if graft patency is measured only after 1 year, a graft that fails at 30 days is statistically treated the same as a graft that fails on day 364. Similarly, a graft that was patent at 360 days but was lost to follow-up is treated the same as a graft that was patent but lost to follow-up at 60 days. In contrast, *life tables* measure events at fixed intervals (e.g., every 30 days), so occurrences before 365 days are accounted for (Fig. 1.3).¹⁵ Such analysis allows greater precision of events, but resolution is still limited to fixed time points. These limitations are addressed by using the *Kaplan–Meier (KM) method*. KM captures each event at the time of occurrence without the need for fixed time frames (Fig. 1.4).¹⁶ Although the KM method allows more precise analysis of events and censorship, life tables are still appropriate when only predetermined periodic measurement of events is available or when arbitrary important milestones are of interest, such as 1-year graft patency or patient survival. The strength of survival analysis lies in the ability to statistically account for censored data. Censoring means that the subject leaves the analysis before the failure endpoint has occurred.

Several tests are commonly used to test for differences between survival functions. The *log-rank test* adds observed and expected events within each group and sums them across all time points containing events. The log-rank statistic serves as the basis for the proportional hazards model (further on). In contrast, the *Wilcoxon test* is the log-rank test weighted by the number of patients at risk for each time point. *Cox proportional hazards models* assume that the underlying hazard (risk) function is proportional over time, so that parameters can be estimated without complete knowledge of the hazard function. The Cox proportional hazards assumption allows the application of survival analysis techniques to multivariable models

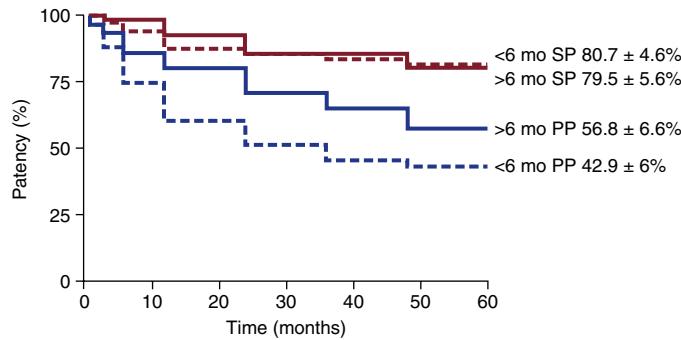


Figure 1.3 Example of life-table analysis of primary patency (PP) and secondary patency (SP) of bypass grafts after being revised before or after 6 months from the index operation. (Redrawn from Nguyen LL, Conte MS, Menard MT, et al. Infrainguinal vein bypass graft revision: factors affecting long-term outcome. *J Vasc Surg*. 2004;40:916–923.)

because individual hazards do not have to be specified for each independent variable in the model.

Adjusting for Confounding Using Propensity Scoring

In comparing two groups that were not randomized, *propensity scoring* is a technique that can be employed to control for confounding and is often used when treatment groups are related to indication; for example, in comparing endarterectomy with stenting for carotid artery stenosis, as patients who underwent stenting may have more comorbidities or a different disease pattern than patients selected for carotid endarterectomy. Each individual included in the trial is assigned a propensity score that reflects the conditional probability of being in a treatment group based on selected variables. This balances the treatment groups for the variables on which the score is based.

As with other methods, propensity scoring can control only for known confounders, again falling short of the randomized controlled trial in its ability to balance unknown factors. Further, to appropriately balance but not confound the outcome, the variables used to create the propensity score should be related to treatment assignment but not to the outcome of interest.

Despite these drawbacks, when an RCT is not possible or practical, propensity scoring methods are useful for providing some insight in comparing treatment groups. Propensity score methods have also been used for other purposes, including nesting covariates for multivariable regression models and generating reweighted estimating equations to rebalance weights from missing data. RCTs further have the advantage of limiting the number of variables in a model and thus preserving power.

Another technique of estimating causal relationships when RCTs are not practical is to utilize the instrumental variables method. This regression analysis technique adjusts for confounding variables through the use of a variable, termed the “instrument,” that has a causal effect on the treatment but not on the outcome. If the instrument is then found to be correlated with the outcome, then this provides evidence for the causal relationship between the treatment and the outcome while controlling for confounding variables that were unmeasured. Recently, advances in the instrumental variable techniques have enabled the use of risk adjustment in the Cox regression model, which has allowed for a more accurate study of the impact of carotid endarterectomy on survival when compared to traditional means.¹⁷ This technique, termed the instrumental-variable Cox model, also found that patients with peripheral artery disease who were treated with atherectomy were four times more likely than patients treated with stenting to have an amputation.¹⁸

Type I Error and Type II Error

Two types of errors can be made when statistical comparisons are employed. A *type I error* is rejection of the null hypothesis when the null hypothesis is in fact true. Alpha (α) is the probability of making a type I error. The P value is calculated from statistical testing and represents the probability of obtaining a result as extreme or more extreme than the results observed. Commonly, α is set at 0.05, and a P value less than α would

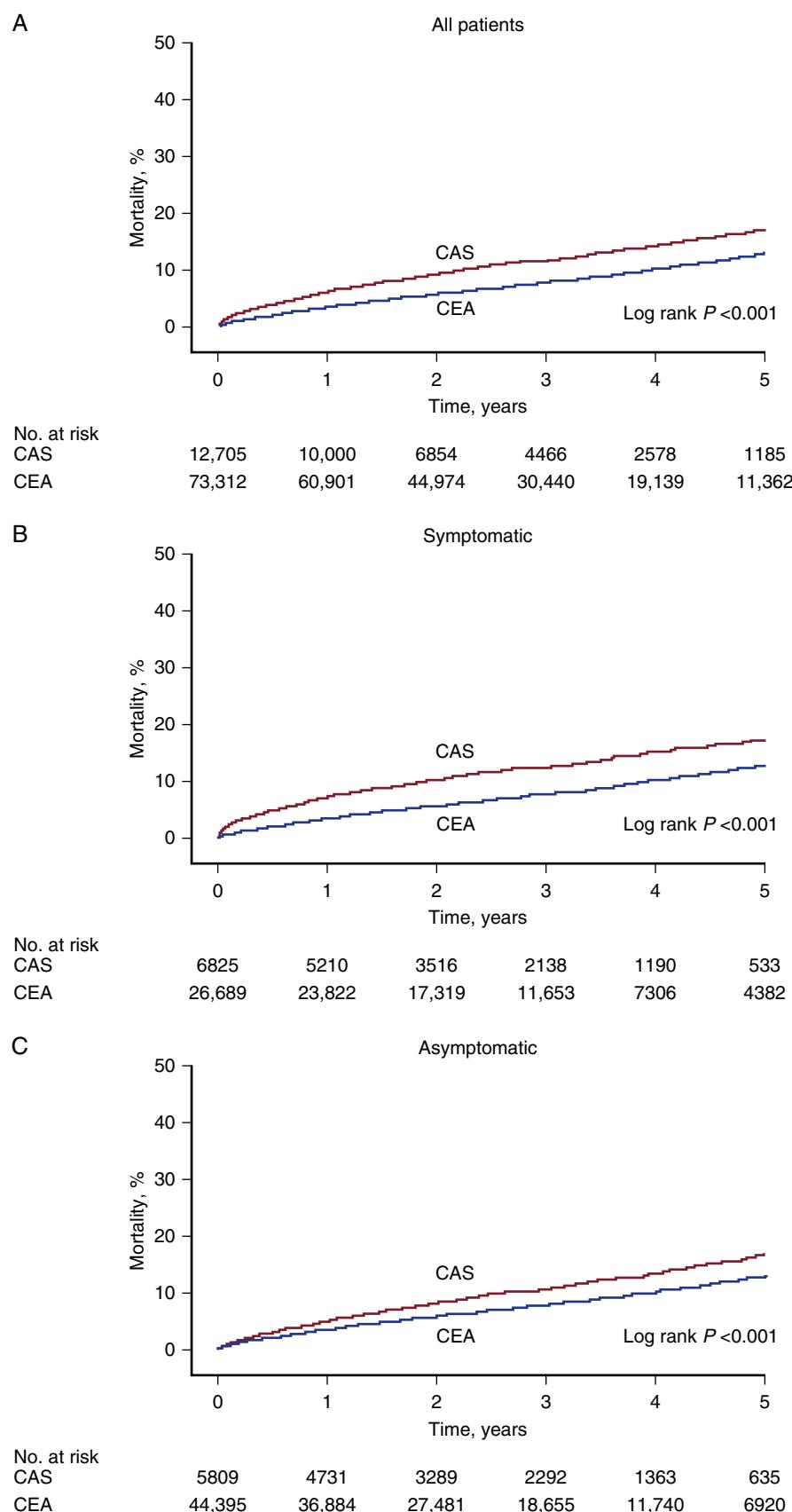


Figure 1.4 Example of Kaplan–Meier analysis of survival after carotid endarterectomy (CEA) and carotid artery stenting (CAS). (From Columbo JA, Martinez-Camblor P, MacKenzie TA, et al. Comparing long-term mortality after carotid endarterectomy vs carotid stenting using a novel instrumental variable method for risk adjustment in observational time-to-event data. *JAMA Netw Open*. 2018;1:e181676.)

reject the null hypothesis. Because 0.05 is a somewhat arbitrary setting, many will report actual P values to more precisely communicate statistical significance. Stricter α can be used when concern for a type I error is heightened, as in the testing of a large number of independent variables.

A *type II error* is failure to reject the null hypothesis when the null hypothesis is false. Beta (β) is the probability of making a type II error. *Power* is defined as the probability of rejecting the null hypothesis when it is false (or concluding that the alternative hypothesis is true when it is true). In other words, power is the ability of a study to detect a true difference. Power is calculated as $1 - \beta$ and is closely related to sample size. Power analysis can be performed before or after data collection. *A priori power analysis* is used to determine the sample size needed to achieve adequate power for a study. *Post hoc power analysis* is used to determine the actual power of the study. Power analysis requires specification of several parameters, including α (usually 0.05), the level of power desired (usually 80%), expected effect size, and variance. Variance is simply estimated from previous measurements of related outcomes. However, the expected effect size is a parameter most susceptible to unintended influence. Setting the expected effect size too small decreases type I error but also decreases power and results in the necessity of enrolling larger numbers of patients. Setting the expected effect size too large allows lower enrollment numbers, but at the cost of type I error.

Utility Measures

In economic analysis and decision analysis, patients are considered to be in distinct "states" governed by specific diagnoses or symptoms; for example, asymptomatic versus symptomatic carotid artery stenosis or claudication versus critical limb ischemia. *Utility measures* capture the value a person places on a state of health. Such measures then can be used in decision trees and cost-effectiveness analysis. The Health Utilities Index and the EQ-5D are two widely used utility measures. The simplest method of determining utility is to ask patients to value their own health or a hypothetical state of health by using a rating scale. This information is then transformed into a utility measure by using data from a reference population. Transformations of health states from descriptive instruments (e.g., SF-36) have also been created, although low correlations between descriptive and preference measures have been demonstrated. For transformations to be meaningful, the reference population itself has to be subject to utility assessment.

Direct assessment of utility can be performed by using the *standard gamble*, in which, for a given health state, patients are asked whether they would choose to remain in that state or take a gamble between death and perfect health. The question is then repeated with varying gamble probabilities. The utility of the health state is then derived from the probability of achieving perfect health when the patient is at equilibrium (or indifferent between the choices) between taking the gamble or remaining in the known state of intermediate health. Another common direct utility assessment method is the *time tradeoff*, in which the patient is asked to choose between a length of life in a given compromised state and a shorter length of life in a

perfect state. The utility of the health state is then derived from the ratio of the shorter to the longer life expectancy at the point of equilibrium.

Decision Analysis

Decision analysis is the formal methodology of addressing decisions by defining a problem, considering alternative choices, and then modeling the consequences of these alternatives based on an estimated risk of each alternative. This process has the potential to capture varying outcomes for individual types of patients and can also demonstrate critical factors that may alter their decisions.

One of the primary tools used in decision analysis is the decision tree, where the relevant factors are represented in chronologic relationship flowing left to right. The alternative choices (diagnostic tests, natural history states, treatments, etc.) are visually represented as branches of the tree, and each branch point is a decision node (usually represented as a square) in which a choice is possible, or a chance node (usually represented as a circle) in which the probability of a consequence is conditional on the events that preceded it. The end of each branch has an outcome that is based on the one that preceded it and their probabilities. Each outcome (life expectancy, quality-adjusted life years [QALYs], etc.) is given a value and the expected value for each alternative is then calculated on the basis of cumulative probabilities and outcome values. Decisions can then be made to optimize an outcome value, such as the lowest cost or highest QALYs.

The results from decision analysis are strongly influenced by the event probabilities and outcome values used. Ideally, these figures are derived from strong clinical studies in the field, although a consensus on precise figures and values can be difficult. *Sensitivity and threshold analysis* can then be performed to test the results of decision analysis under different probability and outcome assumptions. Sensitivity analysis is performed mathematically by setting the key probability as an unknown variable to be solved algebraically. This results in a probability value threshold around which the analysis can change to favor different decisions. If the threshold value (or probability) is within accepted estimated clinical probabilities for that event, researchers can have greater confidence in the applicability of the decision analysis results.

Cost–Benefit and Cost-Effectiveness Analysis

At the heart of cost analysis is the assumption that resources are constrained. If unlimited resources are available, all testing and treatment would be offered as long as they were not harmful. However, in an environment of limited resources, cost analysis helps policymakers and clinicians decide on the greatest utility of the resources available. Cost analysis is certainly not the only or necessarily the best criterion for making health policy decisions. It is, however, an objective, quantitative tool that yields important information about the efficacy of clinical practice and does help to better clarify healthcare decisions.

From an economic standpoint, tests or treatments can be measured on two parameters: health improvement and cost savings. Treatments that both improve health and save costs

are the goal and should be readily adopted. At the opposite end of the spectrum, treatments that worsen health and increase costs should be abandoned. Less clear cut are those interventions that fall within the spectrum – treatments that improve outcomes but also increase costs or those that save costs but fail to show improved outcomes. *Cost-effectiveness analysis* compares treatments based on a common measure of costs and effectiveness. The measure of effectiveness can be represented by the number of lives saved, cases cured, cases prevented, and preference-based utility measures such as QALYs. *Cost-benefit analysis* seeks to quantify costs and effectiveness in monetary terms. Cost-benefit analysis is useful for comparing very different choices of treatments or interventions. Because many involved in healthcare are uncomfortable with the monetary valuation of life and life-years, cost-effectiveness is more commonly used in health-related analysis, whereas cost-benefit is more prevalent in economically oriented healthcare analysis.

The cost-effectiveness measure is the ratio of cost to effectiveness, typically dollars per QALY gained. Comparative choices (treatments, programs, tests) are subsequently ranked in order of lowest cost-effectiveness ratio to highest. These ratios can then be used to drive funding decisions. If funding is distributed to programs starting with the lowest cost per unit of efficacy, the cost-effectiveness ratio of the last funded program in this algorithm is defined as the *permissible cost-effectiveness threshold* for other programs to meet. In the United Kingdom, the National Institute for Health and Care Excellence (NICE) has adopted a cost-effectiveness threshold range of £20,000 to £30,000 (\$28,400 to \$42,600 in US dollars) per QALY gained. In the United States, no official threshold has been adopted, although many in practice have used the threshold of \$60,000 per QALY. This figure is based on the calculated average cost of hemodialysis per person per year and Medicare's special coverage of renal failure patients regardless of age.

EVIDENCE IN PRACTICE

Evidence-based medicine is a relatively modern approach to the practice of medicine that aims to qualify and encourage the use of currently available clinical evidence to support a particular treatment paradigm. This practice encourages the integration of an individual practitioner's clinical expertise with the best currently available recommendations from clinical research studies.² Reliance on personal experience alone can lead to biased decisions, whereas reliance solely on results from clinical research studies can lead to inflexible policies. Evidence-based medicine stratifies the strength of the evidence from clinical research studies based on study design and statistical findings (Table 1.1). The criteria differ when the evidence is sufficient to support a specific therapeutic approach, prognosis, diagnosis, or other health services research. Criteria also differ among research institutions, including the US Preventive Services Task Force and the UK National Health Service. However, common themes can be seen among the different fields. Systematic reviews with homogeneity are preferred

over single reports, whereas RCTs are preferred over cohort and case-control studies. Even within similar study design groupings, the statistical strength of each study is evaluated, with preference for studies with large numbers, complete and thorough follow-up, and results with small confidence intervals. Clinical recommendations are then based on the available evidence and are further graded according to their strengths (Table 1.2).

WHAT'S NEXT FOR VASCULAR SURGEONS IN OUTCOMES RESEARCH

Although the clinical care of patients will continue to challenge us, the way we conduct and finance healthcare will also have a profound impact. Healthcare is a continuum from advancements in basic sciences, patient applications, clinical outcomes,

TABLE 1.1 Levels of Evidence for Therapeutics

Level	Evidence
1a	Systematic reviews of RCT studies with homogeneity
1b	Individual RCT with narrow confidence intervals
1c	"All or none" trials ^a
2a	Systematic reviews of cohort studies with homogeneity
2b	Individual cohort studies
2c	Clinical outcomes studies
3a	Systematic reviews of case-control studies with homogeneity
3b	Individual case-control studies
4	Case-series studies
5	Expert opinion without critical appraisal or based on bench research

^aIn which all patients died before the therapeutic became available, but some now survive with it, or in which some patients survived before the therapeutic became available, but now die with it.

RCT, randomized controlled trial.

Modified from Oxford Centre for Evidence-Based Medicine (2001).

TABLE 1.2 Grades of Recommendation

Grade	Recommendation	Basis
A	Strong evidence to support practice	Consistent level 1 studies
B	Fair evidence to support practice	Consistent level 2 or 3 studies or extrapolations from level 1 studies
C	Evidence too close to make a general recommendation	Level 4 studies or extrapolation from level 2 or 3 studies
D	Evidence insufficient or conflicting to make a general recommendation	Level 5 evidence or inconsistent studies of any level

Modified from U.S. Preventive Services Task Force Ratings (2003) and Oxford Centre for Evidence-based Medicine (2001).

efficacy analysis, and policy. The care of patients with vascular disease is no different, and will require us to rise to these challenges.

A greater understanding of technical success, vessel patency, and treatment durability will allow further improvements in the treatments themselves. Therefore, measurement of clinical outcomes is a multimodal technique involving the use of integrated components that measure several aspects of success and failure. Meeting these challenges in order to further knowledge and improve care require collaboration across industries, academia, and government regulators. One such organization has been the Vascular Implant Surveillance and Interventional Outcomes Network (VISION). This Food and Drug Administration (FDA) funded venture brings together experts from the private sector, the government (FDA), academic institutions, and existing initiatives (VQI) to try to improve the data sources used in observational analyses of vascular outcomes by measuring outcomes not just one year after intervention, but many years.¹⁹ In a field as fast moving, innovative, and evidence driven as vascular surgery, a coordinated, public–private, multi-institution approach is best suited to identify the priorities of the field, generate data and databases, and organize ever more complex clinical trials aiming to improve care for the vascular patient.

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A complete reference list can be found online at www.expertconsult.com.

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Embryology and Developmental Anatomy

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Based on a previous edition chapter by Eric D. Endean

FORMATION OF EMBRYONIC BLOOD VESSELS	13
Molecular Signaling of Vasculogenesis and Angiogenesis	13
Early Angiogenesis	14
Aortic Arch	14
Normal Arch Development	14
Aortic Arch Anomalies	15
PATENT DUCTUS ARTERIOSUS	15
COARCTATION OF THE AORTA	15
DOUBLE AORTIC ARCH	15
RIGHT AORTIC ARCH	15
ABERRANT RIGHT SUBCLAVIAN ARTERY	17
Carotid Artery Anomalies	17
DEVELOPMENT OF THE DESCENDING (THORACIC AND ABDOMINAL) AORTA	17
DEVELOPMENT OF THE LIMBS	18
Upper Limb	19

Normal Development	19
Upper Limb Vascular Anomalies	19
Lower Limb	21
Normal Development	21
Lower Limb Vascular Anomalies	21
PERSISTENT SCIATIC ARTERY	21
POPLITEAL ARTERY ENTRAPMENT SYNDROME	22
POPLITEAL AND TIBIAL ARTERY VARIATIONS	22
DEVELOPMENT OF THE VENOUS SYSTEM	23
Superior Vena Cava	23
Inferior Vena Cava and Associated Vessels	23
Anomalies	26
Superior Vena Cava	26
Inferior Vena Cava Duplication and Left-Sided Inferior Vena Cava	26
Renal Vein Anomalies	26
DEVELOPMENT OF THE LYMPHATIC SYSTEM	26

The fundamental purpose of the vascular system is to supply the organism with oxygen and nutrients, and to remove metabolic waste products. During the first three weeks of gestation, simple diffusion is sufficient to support the embryo; however, by the fourth week, a functional cardiovascular system must be in place to support the rapidly developing embryo. The cardiovascular system is one of the earliest systems in the embryo to appear and function. Isolated blood vessels and blood components form in the yolk sac by day 17, while just a day later, blood vessels begin to appear in the embryo proper. The heart begins to beat by day 22 and pumps blood by day 24. Failure of the vascular system to properly develop may result in a number of anatomic variations and abnormalities that can have significant clinical ramifications. By understanding the embryology of the vascular system, the vascular surgeon will be better able to recognize vascular anomalies and understand how they developed.

FORMATION OF EMBRYONIC BLOOD VESSELS

Molecular Signaling of Vasculogenesis and Angiogenesis

Development of the vascular system is a complex orchestration of signaling molecules, receptor molecules, and transcription factors. This process starts with *vasculogenesis*, which begins with the modification of splanchnic mesodermal cells into angioblasts that form vesicular aggregates in the splanchnic mesoderm of the embryo and extraembryonic regions (Fig. 2.1).^{1,2} These angioblasts develop into flattened endothelial cells that form small vessel cords, which coalesce to form a primitive capillary plexus. This process is driven in large part by the signaling

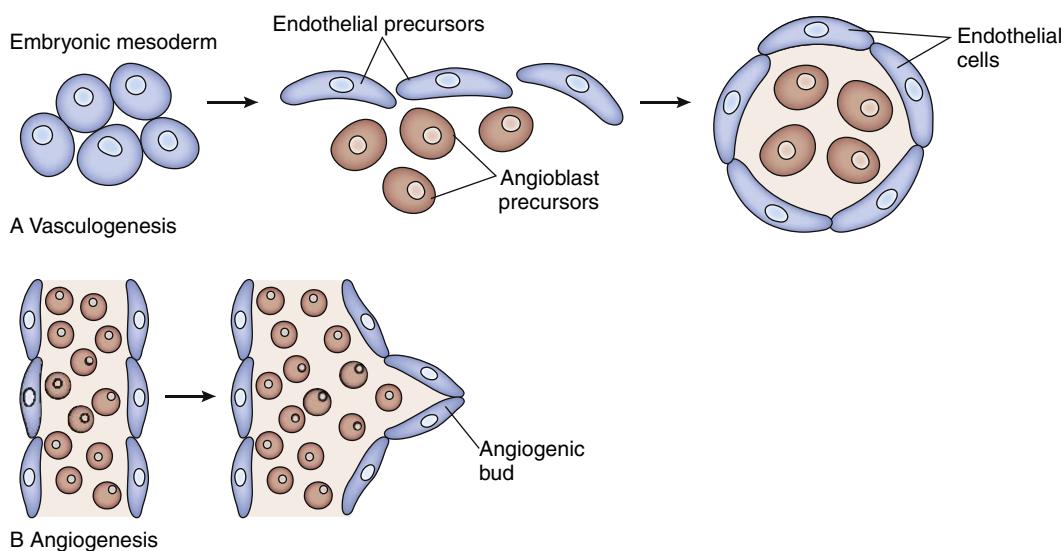


Figure 2.1 (A) The embryonic mesoderm differentiates into endothelial cells and angioblast precursors, which then develop into a primitive capillary plexus. (B) The growth of new blood vessels from pre-existing blood vessels in response to angiogenic signals including hypoxia-induced factor 1 α (HIF-1 α) and vascular endothelial growth factor (VEGF).

protein vascular endothelial growth factor (VEGF). The process of vasculogenesis is restricted to embryogenesis.

Once endothelial cells are established as vascular elements, they begin to sprout and bud, forming simple capillary networks in response to the growing embryo in a process termed *angiogenesis*. These new vessels form in response to hypoxic tissues that release hypoxia-inducible factor 1 α (HIF-1 α) as well as VEGF, which ultimately lead to a stable vascular network.² Capillary networks then remodel into arterial, capillary, and venous systems as determined by the surrounding environment. In contrast with vasculogenesis, angiogenesis occurs during embryogenesis as well as during adult life when it provides sprouting to bring blood to ischemic tissue.

Veins and arteries are vessels whose direction of blood flow is either toward (veins) or away from (arteries) the heart, but vessel identity is established prior to blood flow. Arterial and venous differentiation is determined early in the developmental process through cell surface markers including Eph-B2 and Eph-B4.² Briefly, arterial differentiation occurs as a result of VEGF binding to the VEGF receptor 2 (VEGF-R2). This leads to Notch activation and suppression of chicken ovalbumin upstream promoter transcription factor II (COUP-TFII), which result in Eph-B2 expression. Venous differentiation is driven by COUP-TFII, which suppresses Notch and results in Eph-B4 expression on endothelial cell surfaces. Lymphatic development occurs as a result of Sox18 and prospero homeobox transcription factor 1 (PROX1). PROX1 forms a heterodimer with COUP-TFII, which leads to expression of VEGF receptor 3 (VEGF-R3) and subsequent lymphatic development.

Early Angiogenesis

As major vascular structures including the heart, dorsal aortas, umbilical vessels, vitelline vessels, and cardinal veins are being formed by vasculogenesis, the embryo is already beginning to remodel the vascular system by the processes of angiogenesis

and vascular intussusception. Factors related to the heart and dorsal aortas have a profound effect on the configuration of the vascular system. By day 20, the growth of the neural tube forces the cardiogenic area ventrally and caudally to its final position in the thoracic region, whereas lateral folding of the embryo causes the two endocardial tubes to fuse along the midline, resulting in a single endocardial tube with a more cranially placed outflow and a more caudally located inflow region. As the developing heart lengthens, a series of swellings become visible. Beginning at the inflow end, these include the sinus venosus with its right and left horns, primitive atrium, primitive left ventricle, bulbus cordis (future right ventricle), and truncus arteriosus. The aortic sac, the rostral dilation of the truncus arteriosus, connects the developing heart to the dorsal aortas. Between days 22 and 24, the developing heart moves into its ventral position, and the attached dorsal aortas are forced into a dorsoventral bend that forms the first aortic arch. The first aortic arch is contained in the thickened mesoderm of the developing first pharyngeal arch surrounding the pharynx. Aortic arches two through six develop from mesenchyme within their own pharyngeal arches in a rostral to caudal sequence between days 26 and 30. Lateral folding of the embryo also forces the paired dorsal aortas, beginning at the fourth thoracic somite, to fuse with one another as a common aorta; however, rostral to this level, the dorsal aortas remain as separate vessels. By the beginning of the fourth week, intersegmental vessels between each somite arise from the dorsal aortas. Each intersegmental vessel has a dorsal branch, a lateral branch, and a ventral branch that supplies the individual somite regions.

Aortic Arch

Normal Arch Development

The aortic arch is formed by six pairs of arteries that branch from the ventrally positioned aortic sac to the two dorsal aortas. Diagrammatic representation of these changes would

suggest that the aortic arches are all present at the same time, but in reality, the first arches are already regressing while others are still developing. The right horn of the aortic sac forms the brachiocephalic (innominate) artery, right common carotid, and origin of the right subclavian arteries. The left horn of the aortic sac forms the initial portion of the aortic arch.

The first two aortic arches appear and regress quickly and contribute very little to adult structures, whereas the fifth aortic arch never develops in humans (Fig. 2.2A). The third aortic arches become the common and proximal segments of the internal carotid arteries. The distal segments of the internal carotid arteries are derived from the dorsal aorta between the first and third arches. The external carotid arteries sprout from the common carotid arteries. The dorsal aorta on each side of the embryo between the third and fourth arches disappears, thus directing blood through the third aortic arch system to the head and neck regions (Fig. 2.2B). The fourth aortic arches are asymmetrical with regard to their fate. The left aortic arch forms the part of the adult aortic arch between the left common carotid and left subclavian arteries, whereas the right aortic arch becomes the proximal segment of the right subclavian artery. The remainder of the right subclavian artery is derived from the right dorsal aorta and its right seventh intersegmental artery. The right dorsal aorta distal to the seventh intersegmental artery and proximal to the fused common aorta involutes (Fig. 2.2C). The proximal portions of both sixth arches become the right and left pulmonary arteries. The distal segment of the right sixth arch disappears, but the distal segment of the left sixth arch becomes the ductus arteriosus during fetal life and atrophies after birth to become the ligamentum arteriosum (Fig. 2.2D).

Aortic Arch Anomalies

In light of the complexity of events that must occur for normal development of the aortic arch and its branches, anomalies do occur.^{3–9} Anomalies result when segments of the primitive aortic arch that should disappear persist, or vice versa. Variations in the development of vessels as they arise from the aortic arch are relatively common, with a “normal” developmental pattern occurring in about 65% of the population, as illustrated in Figure 2.2D. In 15%–30% of the population, the left common carotid artery originates from the brachiocephalic trunk rather than from the aortic arch in what is termed a “bovine arch”.^{10,11} In this case, the brachiocephalic trunk gives rise to the right subclavian, right common carotid, and left common carotid arteries, whereas the left subclavian artery originates from the aortic arch as normally expected, and accounts for 73% of all arch anomalies. Many other variations, each occurring in less than 3% of the population, have been described. Some of these anomalies include a shortened brachiocephalic trunk that bifurcates immediately into the right subclavian and right common carotid arteries, with the left common carotid arising from the aortic arch at the base of the brachiocephalic trunk and a normal origin for the left subclavian artery from the aortic arch. The left vertebral artery can originate directly from the aortic arch between the left common carotid and left subclavian arteries. A left brachiocephalic trunk may be

present, which bifurcates into left subclavian and left common carotid arteries.³

Patent ductus arteriosus

The ductus arteriosus develops from the distal portion of the left sixth aortic arch and is responsible for shunting blood from the immature, developing fetal lungs to the systemic circulation. A patent ductus arteriosus is the most common vascular anomaly, with an increased incidence in children who are born prematurely. At birth, the ductus normally closes in response to increased oxygen tension and a decrease in prostaglandin production with lung expansion and function. By the age of 1 month, the ductus normally obliterates to become the ligamentum arteriosum. If the ductus arteriosus does not constrict and remains patent, blood is shunted from the high-pressure thoracic aorta to the low-pressure pulmonary system, eventually resulting in significant pulmonary hypertension.

Coarctation of the aorta

Coarctation is a congenital narrowing that can occur at any level of the aorta. The most common location is just distal to the ligamentum arteriosum, and is termed postductal; the preductal type occurs immediately proximal to the ligamentum arteriosum (Fig. 2.3). The etiologic mechanisms related to coarctation remain undefined, however, they are thought to resemble those processes that result in normal, physiologic obliteration of the ductus arteriosus. Oxygen-sensitive muscle tissue from the ductus arteriosus is incorporated into the wall of the aorta. This smooth muscle, which constricts when exposed to high oxygen tension, results in narrowing of the aorta. Eventually chronic changes develop, and the constriction becomes permanent. In the preductal type, the ductus arteriosus remains patent to maintain distal perfusion. In the postductal type, collateral vessels including the internal thoracic, anterior spinal, and intercostal arteries provide perfusion to the lower body. Radiographically, notching on the inferior aspect of the third to eighth ribs may be seen as a consequence of the increased collateral blood flow in the intercostal arteries.^{9,12,13}

Double aortic arch

The right dorsal aorta distal to the right seventh intersegmental artery may fail to involute, resulting in a double aortic arch. This segment passes posterior to the esophagus and joins the left aortic arch, which passes anterior to the trachea. As a result, a vascular ring forms around the esophagus and trachea. Symptoms may develop secondary to compression of the esophagus and/or trachea (Fig. 2.4).

Right aortic arch

Involution of the left dorsal aorta distal to the left seventh segmental artery with persistence of the right dorsal aorta (opposite the normal sequence) creates a right aortic arch (Fig. 2.5). The ligamentum arteriosum arises from the distal right sixth arch instead of the distal left sixth arch, but still connects to the aorta. If the arch passes to the left side and posterior to the esophagus, a vascular ring is formed with the ligamentum arteriosum and is known as a right aortic arch with an aberrant

left subclavian artery or a retroesophageal component. If the right aortic arch passes anterior to the esophagus and trachea, a vascular ring is not formed, and this is considered a right aortic arch with mirror image branching. The former anomaly may

initially be a double aortic arch in which the left dorsal aorta later regresses. The latter is a mirror image of normal anatomy and is associated with a higher incidence of congenital heart malformations, including tetralogy of Fallot.⁹

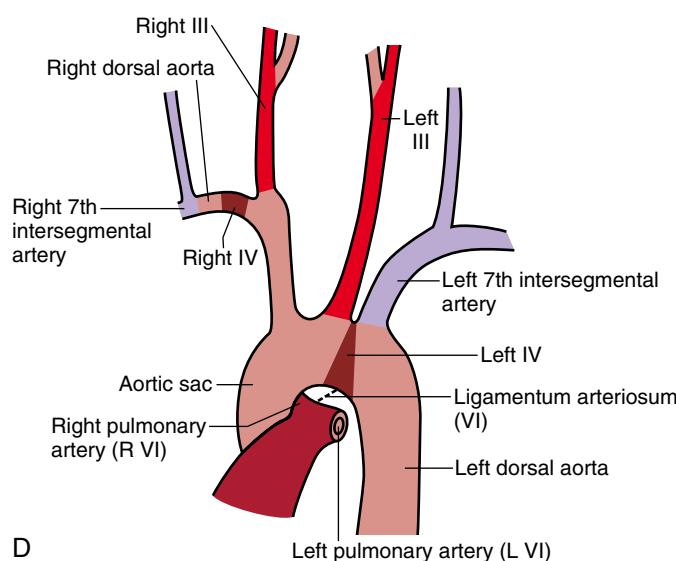
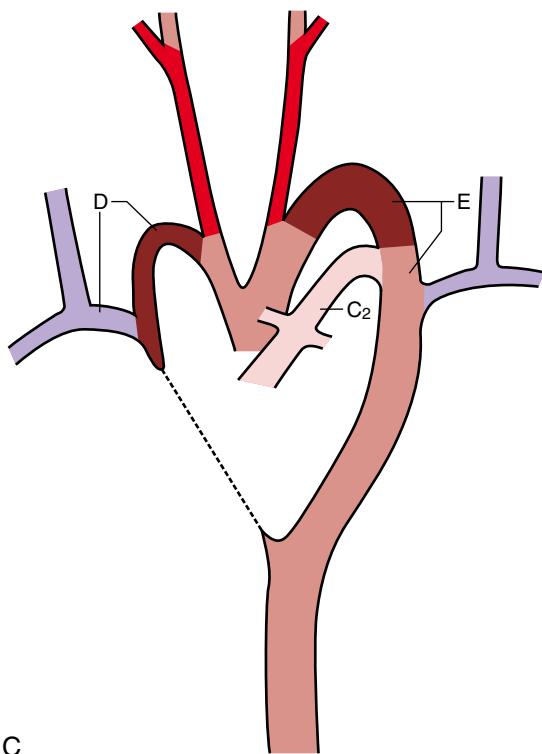
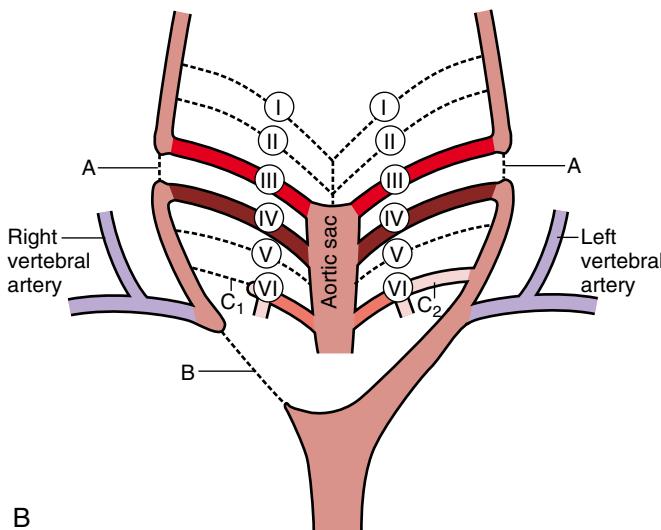
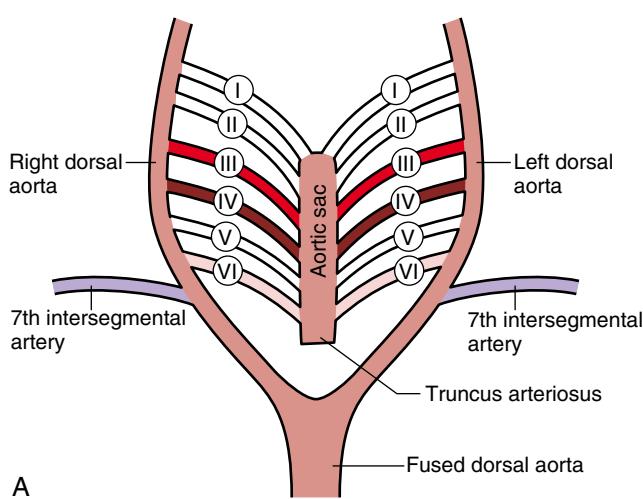


Figure 2.2 (A) The six primitive aortic arches. The first, second, and fifth arches disappear. The paired dorsal aortas fuse at the level of the seventh cervical vertebra to become the thoracic and abdominal aorta. (B) Differentiation of the aortic arches. The dorsal aorta between the third and fourth arches (A) involutes so that blood entering the third arch perfuses the head only. The third arch and its branches become the carotid system. The right dorsal aorta distal to the right seventh intersegmental artery (B) involutes so that blood entering the right fourth arch perfuses the right upper limb. This system becomes the right subclavian artery. The distal part of the right sixth arch (C_1) involutes, but the distal part of the left sixth arch (C_2) persists and becomes the ductus arteriosus. (C) Development of the aortic arches. The right fourth arch, the dorsal aorta distal to the right fourth arch, and the right seventh intersegmental artery become the right subclavian artery (D). The left fourth arch and the left dorsal aorta distal to it become part of the aortic arch (E). The left seventh intersegmental artery becomes the left subclavian artery. Pulmonary arteries form from the sixth arch with the ductus arteriosus (C_2) present. (D) Segments of the aortic arches that produce the adult aortic arch. The patent ductus arteriosus becomes the ligamentum arteriosum.

Aberrant right subclavian artery

The right subclavian artery normally forms from the right fourth aortic arch and the seventh intersegmental artery, connected by the intervening segment of the right dorsal aorta. An aberrant right subclavian artery forms as a result of abnormal involution of the right fourth aortic arch and the connected right dorsal aorta (Fig. 2.6). The right dorsal aorta distal to the right intersegmental artery persists instead of involuting and

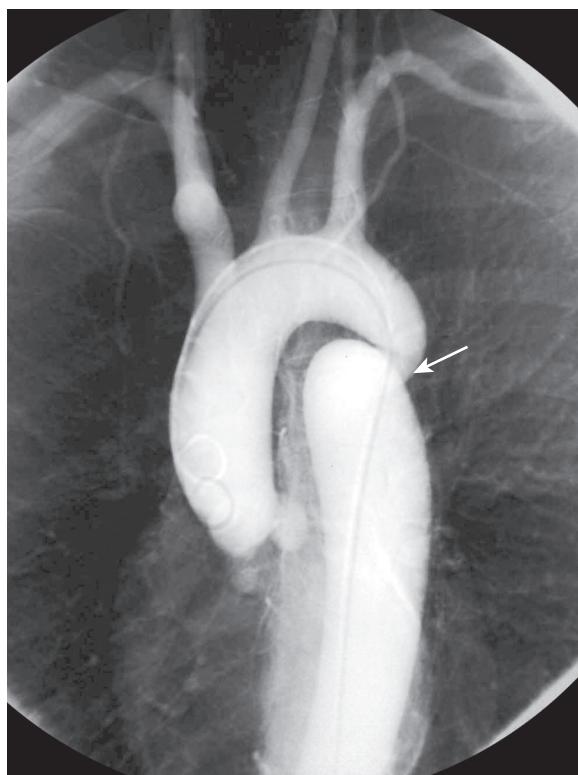


Figure 2.3 Coarctation of the aorta (arrow), with poststenotic dilation of the aorta.

joins the right seventh intersegmental artery to form the right subclavian artery. As the proximal portion of the aortic arch develops from the aortic sac, the right subclavian artery is forced to the left side distal to the left subclavian artery and takes a retroesophageal path behind the esophagus to supply the right upper limb. Most patients with this entity are asymptomatic, but about 5% have compression of the esophagus resulting in difficulty with swallowing, termed *dysphagia lusoria*.⁵ In addition, this anomaly is prone to aneurysmal degeneration, a condition termed *Kommerell diverticulum*. These aneurysms can lead to compressive symptoms (dysphagia, cough, dyspnea), thromboembolism, rupture, or dissection.

Carotid Artery Anomalies

The internal and common carotid arteries arise from the third aortic arch and dorsal aorta. Although rare, several variations exist including carotid artery agenesis, aplasia and a retropharyngeal course of the internal carotid artery. Agenesis and aplasia may occur across the entire length of the carotid arteries, corresponding with the associated third aortic arch or segments of the dorsal aorta.^{14,15} An internal carotid artery with a retropharyngeal course has been acknowledged since the 1900s as an important anomaly to recognize during tonsillectomy. It is thought to arise from incomplete straightening of the carotid arteries with persistence of the embryonic angulation.¹⁶ In addition to otolaryngologic procedures, recognition of this anomaly is relevant for carotid interventions and intubation.

DEVELOPMENT OF THE DESCENDING (THORACIC AND ABDOMINAL) AORTA

During the fourth week, the paired dorsal aortas fuse to become the descending (thoracic and abdominal) aorta. At each somite

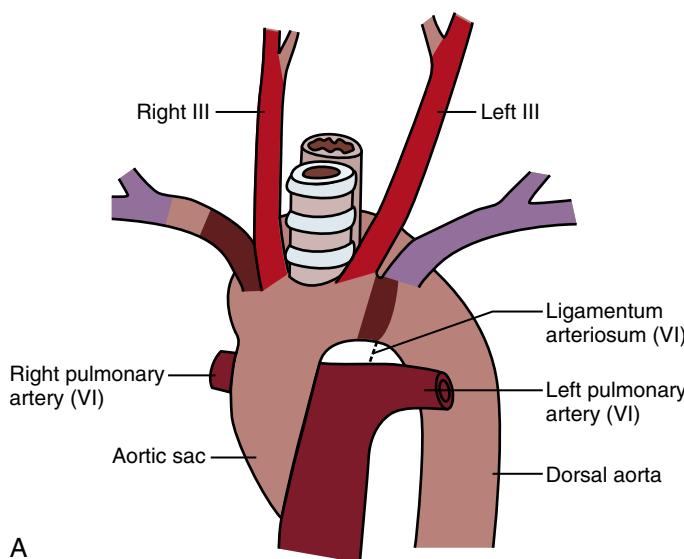
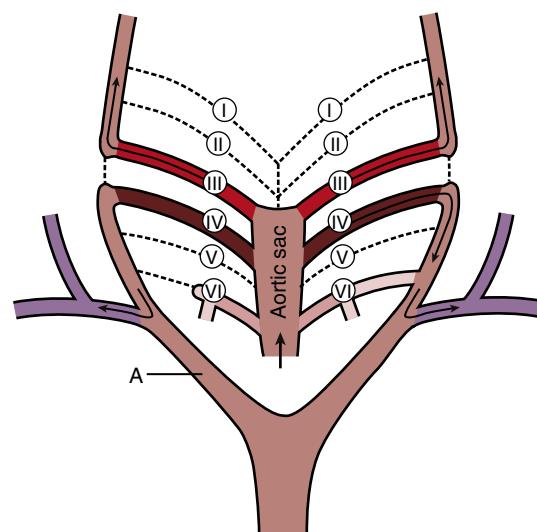


Figure 2.4 Double Aortic Arch. (A) The aortic arch passes anterior and posterior to the trachea and esophagus to form a vascular ring. (B) The right dorsal aorta distal to the right seventh intersegmental artery (A), which normally involutes, persists to become part of the double aortic arch.



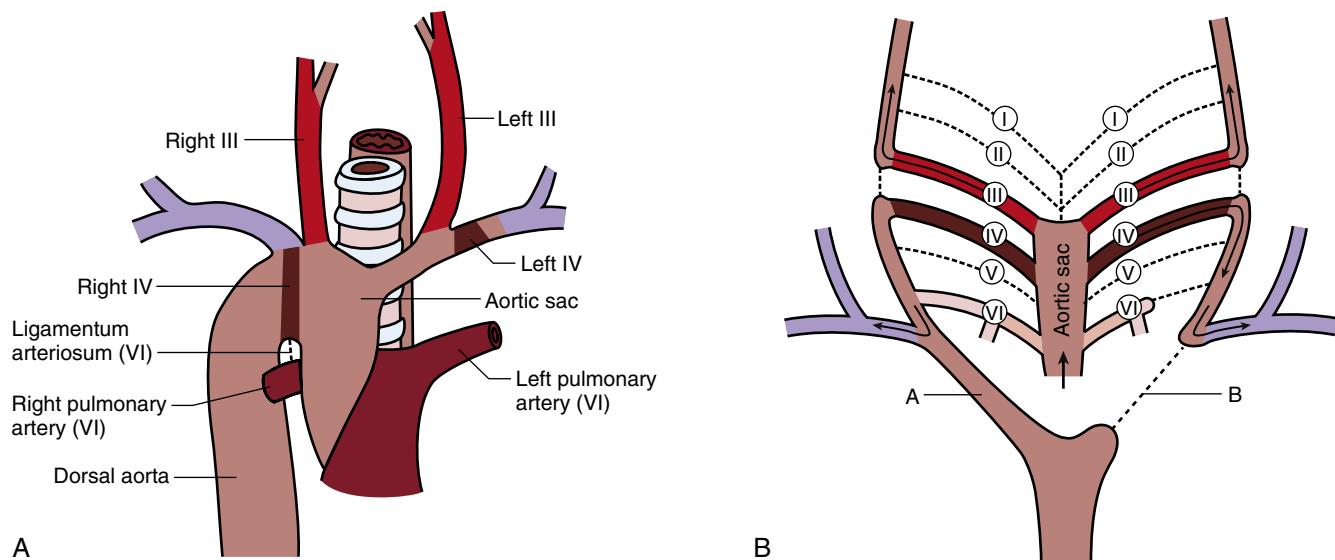


Figure 2.5 Right Aortic Arch. (A) One of two types in which the right arch passes anterior to the trachea and esophagus so that no vascular ring is formed. If the right arch passes posterior to the trachea and esophagus, the ligamentum arteriosum and the right arch form a vascular ring. (B) The right dorsal aorta distal to the right seventh intersegmental artery persists (A), but the left dorsal aorta distal to the left seventh intersegmental artery involutes (B), the opposite of the normal occurrence.

level, segmental arteries continue to form dorsal, ventral, and lateral branches. The dorsal branches supply the developing neural tube and epimere that become the epaxial muscle of the back, whereas the ventral branches supply the hypomere that become the intercostal muscles, external and internal obliques, and transversus abdominus and overlying skin. In cervical regions, the dorsal intersegmental branches anastomose with one another to form the vertebral arteries as well as the deep cervical and ascending cervical arteries. In thoracic regions, the dorsal branches form the intercostal arteries. Ventral branches anastomose with one another and contribute to the formation of the superior thoracic, internal thoracic, and the superior epigastric arteries. At the lumbar level, the dorsal intersegmental branches become lumbar arteries, with the fifth lumbar pair remaining as the common iliac arteries. The lumbar ventral branches form allantoic and vitelline vessels. The segmental arteries forming the allantoic vessels become the umbilical arteries, whereas the other paired ventral segmental vessels connect to the yolk sac and develop into the vitelline arteries. As the gastrointestinal tract develops, the vitelline arteries become its blood supply and fuse to become the three unpaired gut vessels: the celiac artery supplying the foregut, the superior mesenteric artery supplying the midgut, and the inferior mesenteric artery supplying the hindgut. The embryonic-derived components of the umbilical arteries are originally the ventral branches of the dorsal aorta. The bases of these segmental vessels later connect with the fifth intersegmental branches. The proximal portions involute, ultimately leading to the formation of the internal iliac arteries in the adult.

The lateral segmental arteries supply the primitive urogenital ridge, which is the source of the gonads and kidney. The most rostral part of the urogenital ridge develops two sets of primitive kidneys, the pronephros and mesonephros, which over time disappear. The most caudal part of the urogenital ridge becomes the metanephros, which develops into the adult kidney. At the

seventh week of gestation, the “rostrally migrating” metanephros establishes and loses connection with a series of arteries, each of which will eventually regress. The metanephric kidneys establish connections with several rostral lateral segmental arteries that persist and fuse with one another to form definitive renal arteries. Not surprisingly, considerable variations exist in the arterial supply to the kidney.^{17,18} Most individuals (71%) have a single artery to each kidney, whereas the remaining have a variety of combinations of hilar and accessory branches to the poles of the kidneys. Horseshoe kidneys develop with fusion of the caudal kidney poles. Such fusion arrests rostral migration of the kidney, and multiple segmental arteries persist to provide blood supply to the fused kidney. Similarly, ectopic kidneys frequently have several segmental arteries instead of a single renal artery. Thus, when a patient is found to have either a horseshoe or an ectopic kidney, the clinician should suspect an anomalous arterial blood supply.¹⁹ Other lateral segmental branches become the adrenal and gonadal arteries.

DEVELOPMENT OF THE LIMBS

The limbs begin as an outgrowth of tissue originating from the embryonic trunk, called a *limb bud*. Within each limb bud, an apical ectodermal ridge is formed along the anteroposterior plane that differentiates ventral structures (palm of the hand) from dorsal structures (back of the hand). The first structure to differentiate in the limb bud is the skeletal tissue from a cluster of mesenchymal cells. Shortly thereafter, muscle tissue develops from myogenic cells located in the ventral portion of the dermomyotome of the somite. Motor neurons appear in the limb muscles around the fifth week, followed by sensory neurons. Blood vessels that arise from endothelial cells in the segmental branches of the aorta, cardinal veins, and angioblasts within the limb bud mesoderm are the last tissues to be differentiated.

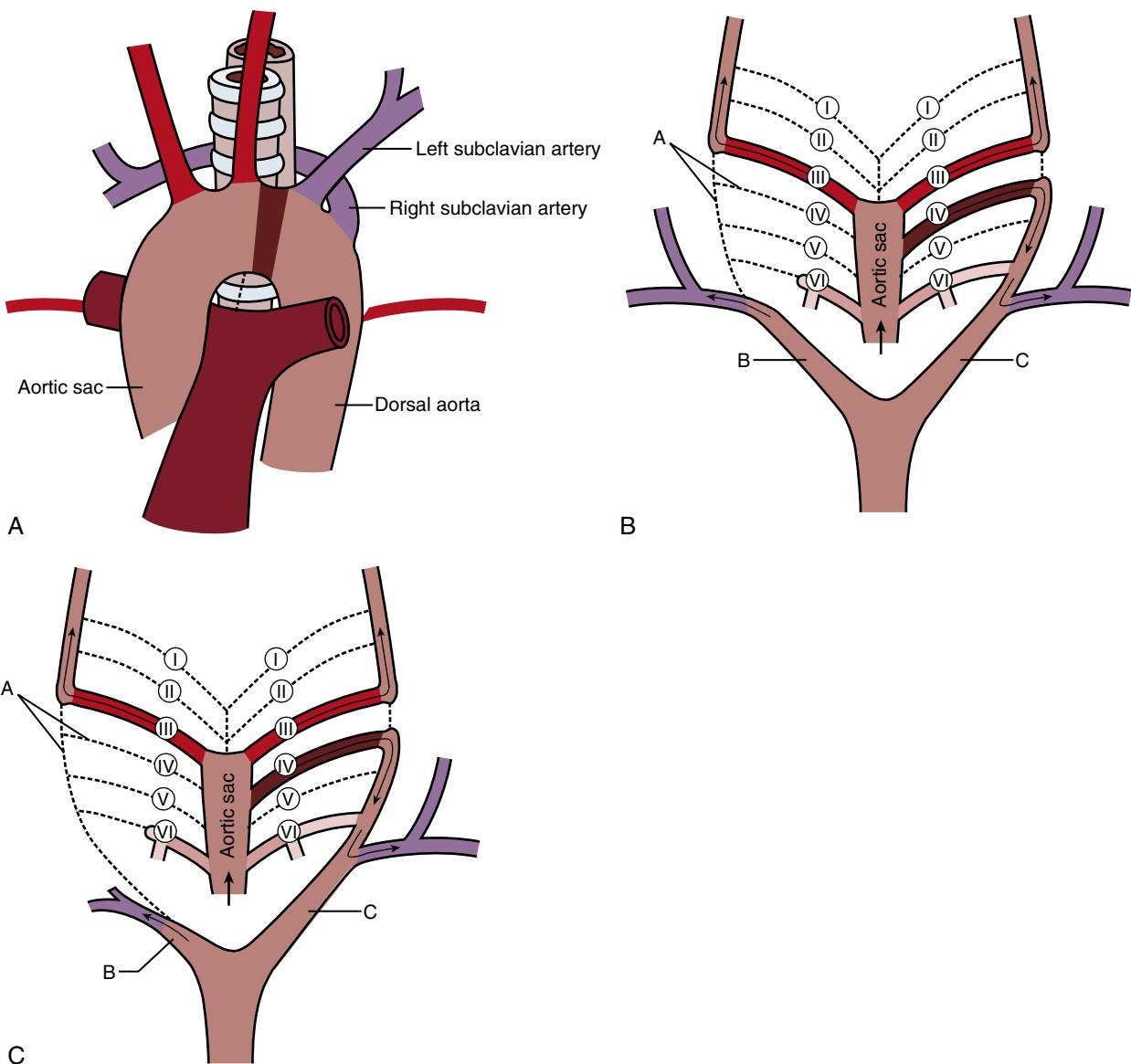


Figure 2.6 Retroesophageal Right Subclavian Artery. (A) The right subclavian artery originates distal to the left subclavian artery and reaches the right upper limb by passing posterior to the trachea and esophagus. (B) The right fourth arch and the dorsal aorta distal to the right fourth arch (A) abnormally involute, and therefore cannot contribute to formation of the right subclavian artery. The right subclavian artery is then formed by the right dorsal aorta distal to the right seventh intersegmental artery (B), which normally involutes (C). This becomes part of the thoracic aorta. (C) The left dorsal aorta (C) elongates and enlarges to become the thoracic aorta. The right dorsal aorta (B) shortens considerably to become a branch of the thoracic aorta leading to the subclavian artery (A). This section abnormally involutes (see B).

Initially, a fine capillary network provides blood supply to the limb, but preferential enlargement of vessels gives rise to a single, large central artery. Blood from this central artery feeds into a peripheral capillary bed and then collects in a marginal sinus located beneath the apical ectodermal ridge.¹

Upper Limb

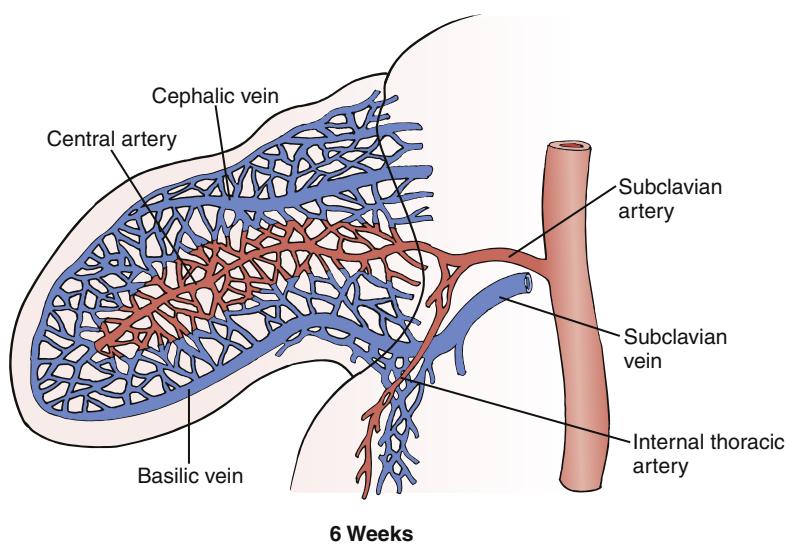
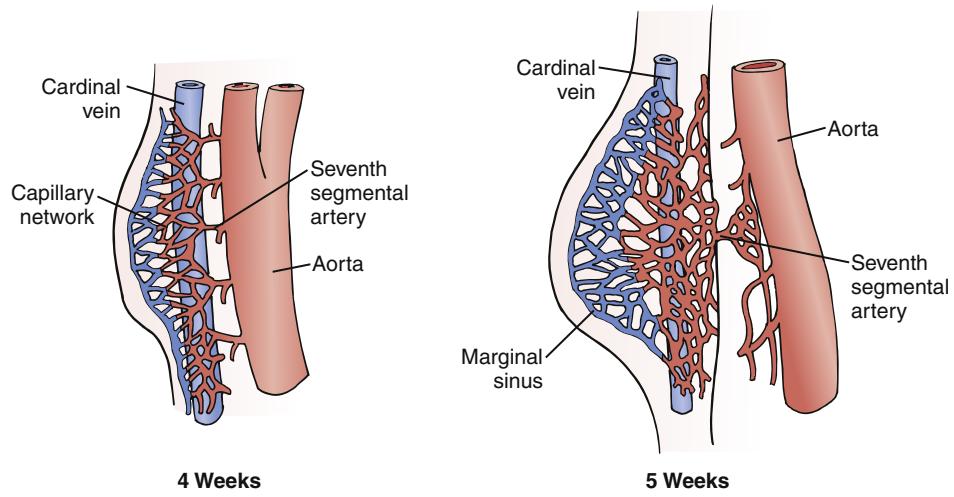
Normal Development

The arterial supply to the upper limb is derived from the seventh cervical intersegmental artery, which supplies the upper limb by joining with the axial artery that develops within the limb bud (Fig. 2.7). The developing axial artery becomes the brachial

artery, whereas the portion of the axial artery in the forearm becomes the anterior interosseous artery. In the hand, a portion of the axial artery persists as the deep palmar arch. The ulnar, radial, and median arteries sprout from the axial artery to take over the supply of the forearm as the interosseous artery regresses. Over time, the radial and ulnar arteries replace the anterior interosseous artery as the dominant arteries in the hand, and the median artery regresses after eight weeks of intrauterine life.

Upper Limb Vascular Anomalies

Although there are few anomalies of the upper arm, several variations exist. The main variations are a high origin of the radial artery proximal to the level of the elbow at the cubital



A

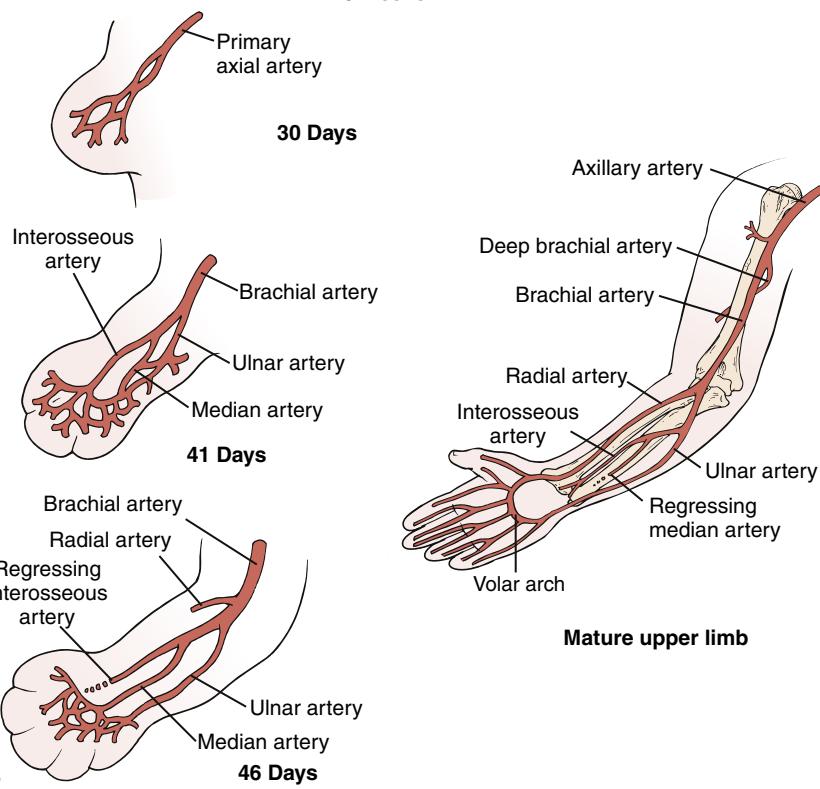


Figure 2.7 Arterial Supply to the Right Upper Limb. (A) Formation of the vascular system within the limb bud. Note how the capillary bed separates into an arterial and a venous network. At about 6 weeks, the marginal sinus becomes the basilic and cephalic veins, and a central artery forms from the arterial network. (B) The primary axial artery becomes the brachial artery, which gives rise to a number of branches in the newly developing forearm.

fossa, which occurs in 14% of the population, and persistence of the median artery into the palm, which is present in up to 12% of the population.²⁰

Lower Limb

Normal Development

Development of the arterial supply to the lower limbs is more complex than that of the upper limb (Fig. 2.8).²¹ The umbilical arteries join with the proximal portion of the fifth lumbar dorsal intersegmental arteries, giving rise to the common iliac arteries. The external iliac artery forms as a second branch of the fifth lumbar intersegmental artery, developing into the iliofemoral artery. The sciatic (axial) artery, an extension of the internal iliac artery, follows the course of the sciatic nerve, and provides perfusion to the lower limb during early development. In the lower part of the thigh, the sciatic artery joins the iliofemoral system at the level of the popliteal fossa. The sciatic artery regresses by the beginning of the eighth week, although parts of it persist as the popliteal artery and a portion of the peroneal artery. The remainder of the lower limb arteries develop later as an extension of the external iliac artery.

The popliteal artery develops from the union of two arteries: (1) the deep popliteal artery, which is part of the sciatic system and lies anterior to the popliteus muscle, and (2) the later-developing superficial popliteal artery from the iliofemoral system (Fig. 2.9). The distal section of the deep popliteal artery regresses, whereas the superficial popliteal artery unites with the proximal portion of the deep popliteal artery to form the definitive popliteal artery and lies posterior to the popliteus muscle.

Lower Limb Vascular Anomalies

Persistent sciatic artery

If the iliofemoral artery fails to develop, the sciatic artery may persist as the dominant vessel supplying blood to the thigh. This is a rare anomaly, with an incidence of 0.05% with variable morphology, ranging from a complete persistent sciatic artery from its origin at the internal iliac artery to its union with the popliteal artery, to an incomplete sciatic artery that connects with the internal iliac or popliteal artery through small collaterals.^{22,23} A persistent sciatic artery is anatomically located next to the sciatic nerve and thus passes into the thigh through the sciatic notch and posterior to the adductor magnus

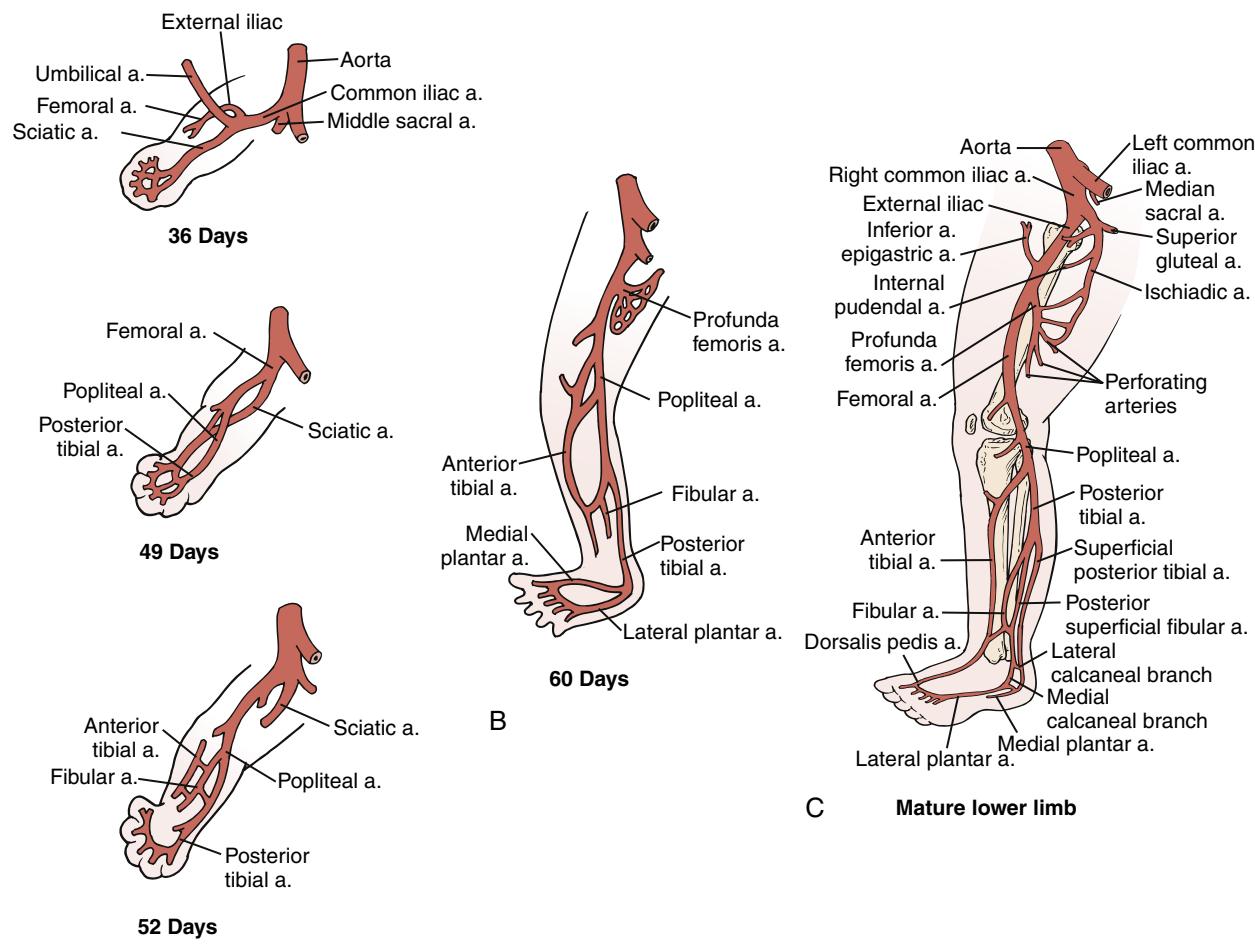


Figure 2.8 Arterial Supply to the Left Lower Limb. (A) The sciatic artery forms as a branch of the umbilical artery and initially supplies the entire leg. (B) The sciatic artery regresses, and the external iliac artery develops into the common femoral artery to supply the thigh. Note that the sciatic artery communicates with the popliteal artery just above the knee. (C) The sciatic artery disappears, although small portions remain to form the popliteal and peroneal arteries.

until it enters the popliteal fossa to join the popliteal artery. On physical examination, patients with a complete persistent sciatic artery may have an absent femoral pulse but a normal popliteal pulse. A persistent sciatic artery is superficial in the

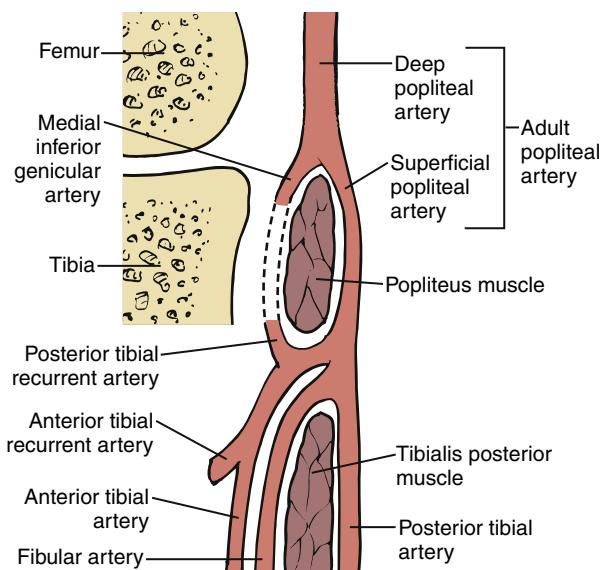


Figure 2.9 Embryologic Development of the Popliteal Artery. The deep popliteal artery anterior to the popliteus muscle regresses, and the superficial popliteal artery posterior to the popliteus muscle becomes the mature popliteal artery. (From Gibson MHL, Mills JG, Johnson GE, et al. Popliteal entrapment syndrome. *Ann Surg*. 1977;185:341; modified from Senior HD. The development of the arteries of the human lower extremity. *Am J Anat*. 1919;25:55.)

buttocks and can be traumatized by normal activity such as sitting. This causes early atherosclerotic changes, and aneurysmal degeneration has been noted in up to 48% of patients.^{23–25} An aneurysm in a sciatic artery can compress the sciatic nerve and result in neurologic symptoms (Fig. 2.10).

Popliteal artery entrapment syndrome

Popliteal artery entrapment syndrome is caused by anatomic variations between the popliteal artery and surrounding musculoskeletal structures leading to compression of the popliteal artery.^{21,26} Normally, at the time of attachment of the medial head of the gastrocnemius, the deep popliteal artery has involuted, and the mature popliteal artery has formed in its normal location. However, if the mature popliteal artery develops prior to the migration of the medial head of the gastrocnemius, it is pushed medially, resulting in the most common form of popliteal entrapment syndrome (type I).²⁷ Popliteal entrapment syndrome tends to be diagnosed in patients at an early age. Clinically, compression of the artery against the femur by the medial head of the gastrocnemius can cause claudication and formation of a popliteal artery aneurysm.

Popliteal and tibial artery variations

Variations in the branching pattern of the popliteal and tibial arteries are common, and found in approximately 10% of the population.²⁸ Although many variations exist, the most common include a hypoplastic or aplastic posterior tibial artery, high origin of the anterior tibial artery, and trifurcation of all

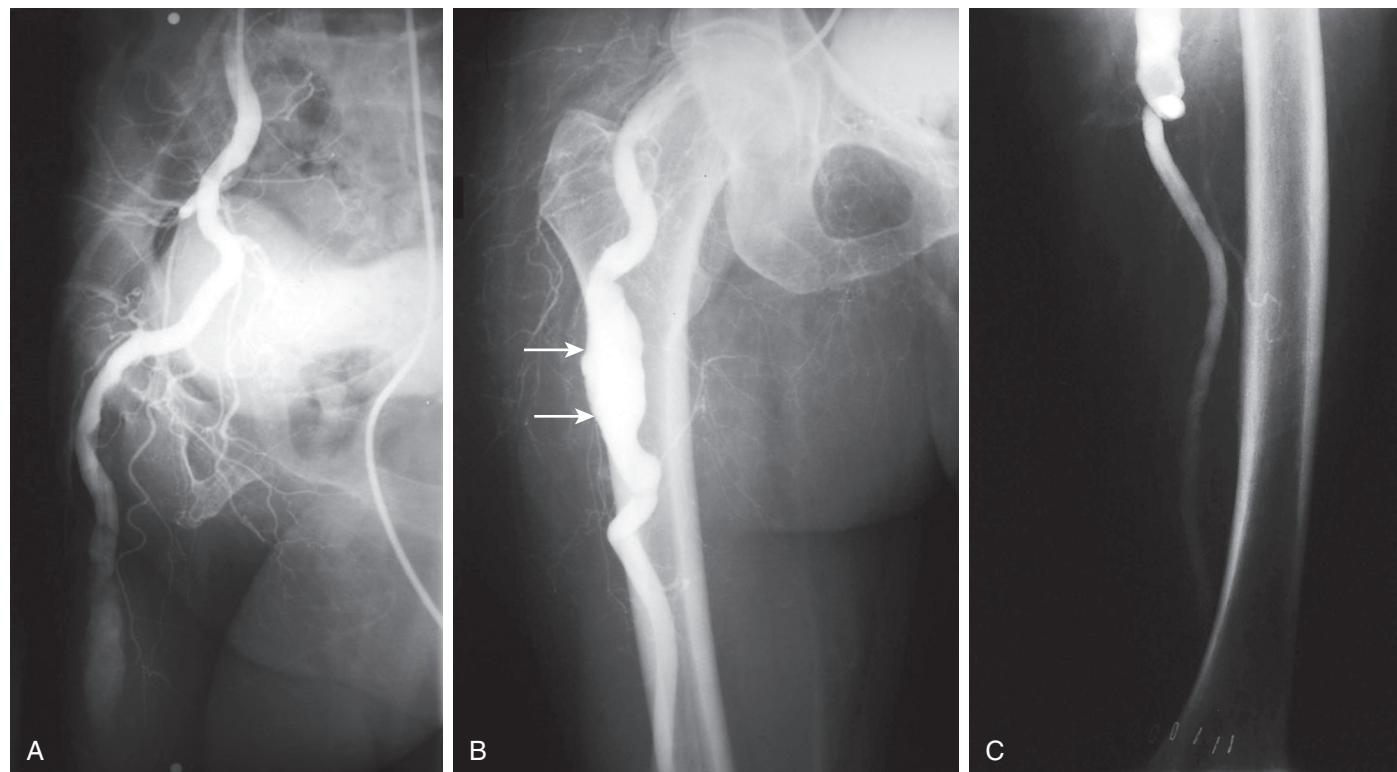


Figure 2.10 Persistent Sciatic Artery. (A) The sciatic artery in the pelvis is coursing posteriorly. (B) In the thigh, the sciatic artery has developed aneurysmal changes (arrows) and courses lateral to the normal location of the femoral artery. (C) More distally in the thigh, the sciatic artery is located laterally and ultimately joins the popliteal artery in the popliteal fossa (not seen on the radiograph).

three tibial vessels at the same level.^{28,29} In patients with anatomic variation in one limb, contralateral variation is common and may be present in up to 50% of patients.^{29–31}

As the sciatic artery regresses during development, the distal sciatic artery remains and forms the popliteal and peroneal arteries.²¹ The anterior tibial artery arises as a branch of the popliteal artery, which is initially positioned anterior to the popliteus muscle in the embryo. As development progresses, the deep popliteal artery regresses and is replaced by the superficial popliteal artery, which runs posterior to the popliteus and gives rise to the anterior tibial artery. If this fails to happen, high takeoff of the anterior tibial artery can be observed, and it is positioned anterior to the popliteus. Failed development or persistence of the distal femoral artery likely leads to hypoplasia or aplasia of the posterior tibial artery.³⁰

DEVELOPMENT OF THE VENOUS SYSTEM

Within four weeks of gestation, a cluster of capillary networks begins to enlarge and develop into the definitive veins of the embryo.¹ Greater anatomic variation is possible within the venous system because it begins with multiple venous channels that can develop into numerous configurations. At four weeks, three paired venous systems form the vitelline, umbilical, and cardinal veins. The vitelline veins drain the yolk sac, the umbilical veins return oxygenated blood from the placenta, and the cardinal veins return blood to the embryonic heart (Fig. 2.11).

The right and left vitelline veins drain blood from the yolk sac and the developing gastrointestinal tract. Before the vitelline vein enters the sinus venosus (the venous end of the heart), it forms a venous plexus in the developing liver, resulting in the formation of the liver's hepatic sinusoids (Fig. 2.12).¹ The left vitelline vein regresses, resulting in blood being shunted to the enlarged right vitelline vein as it passes through the developing

liver. The enlarged right vitelline vein in the liver becomes the ductus venosus, and the cranial portion develops into the inferior vena cava between the liver and heart. The portion of the right vitelline vein caudad to the liver becomes the portal vein and superior mesenteric vein. Left-to-right vitelline anastomoses remodel to form the splenic and inferior mesenteric veins, bringing blood from the foregut and hindgut, respectively, to the distal end of the portal vein.

The umbilical veins bring oxygenated blood from the placenta to the heart. Initially, the umbilical veins are paired, but as the fetus develops, the right umbilical vein regresses, while the left persists. The left umbilical vein forms a direct anastomosis with the ductus venosus, which bypasses oxygenated blood from the placenta around the liver's sinusoids to directly enter the inferior vena cava. After birth, the ductus venosus atrophies and becomes the ligamentum venosum, whereas the left umbilical vein also atrophies to become the ligamentum teres (hepatis) contained within the falciform ligament.

The paired cardinal veins provide the blueprint for development of the intraembryonic venous system. The anterior cardinal veins, located cranial to the heart, join the posterior cardinal veins, located caudal to the heart, to form the paired short common cardinal veins, which drain blood into the right and left horns of the heart's sinus venosus. The paired anterior cardinal veins eventually become the superior vena cava and its major tributaries; the posterior cardinal veins, subcardinal veins, and supracardinal veins contribute to formation of the inferior vena cava, its tributaries, and the azygos system.¹

Superior Vena Cava

The cranial portions of the paired anterior cardinal veins become the internal jugular veins and connect with the external jugular veins, which develop from venous plexuses of the face.¹ The subclavian veins are formed from venous plexuses of the upper limb bud and empty into the proximal anterior cardinal veins. At the seventh week of gestation, an anastomosis develops between the left and right anterior cardinal veins, forming the left brachiocephalic vein. The portion of the left anterior cardinal vein caudal to the developing left brachiocephalic and the left posterior cardinal veins regresses, directing blood from the left side of the head and neck to enter the heart through the left brachiocephalic vein to the superior vena cava. The superior vena cava, which develops from the junction of the left and right brachiocephalic veins and then enters the right atrium, is an enlargement of the right anterior cardinal and right common cardinal veins. The left common cardinal vein becomes the coronary sinus (Fig. 2.13).

Inferior Vena Cava and Associated Vessels

The inferior vena cava, common iliac veins, renal veins, and gonadal veins develop from a series of vitelline and cardinal veins that have multiple anastomoses with one another and eventually coalesce to shunt blood from the left to the right side of the embryo.¹ The suprahepatic portion of the inferior vena cava is formed from the enlargement of the right vitelline vein in

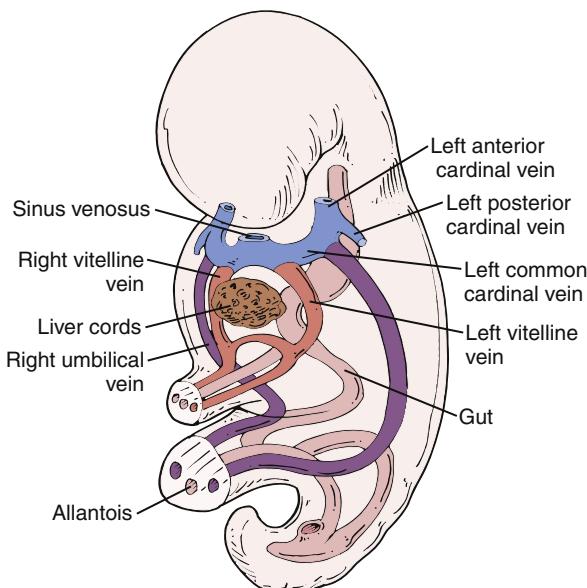


Figure 2.11 Venous System of a 4-Week-Old Embryo.

the liver. The remainder of the inferior vena cava arises from parts of three parallel but different sets of veins that appear sequentially between the sixth and tenth weeks of gestation (Fig. 2.14A,B). These veins go through various changes but eventually coalesce to form the inferior vena cava and other veins of this region. The paired posterior cardinal veins appear first and are located on the posterior aspect of the fetus but eventually regress, with the exception of: (1) the most distal portions,

which fuse to become the caudal portion (fourth part) of the inferior vena cava and the common iliac veins; and (2) the most proximal part persists as the root of the azygos vein. The next pair of veins, the subcardinal veins, arise from the posterior cardinal veins before the latter regresses. They are located anterior and medial to the posterior cardinal veins and drain the mesonephric kidneys. The subcardinal veins anastomose with one another at the level of the metanephric kidneys. The right

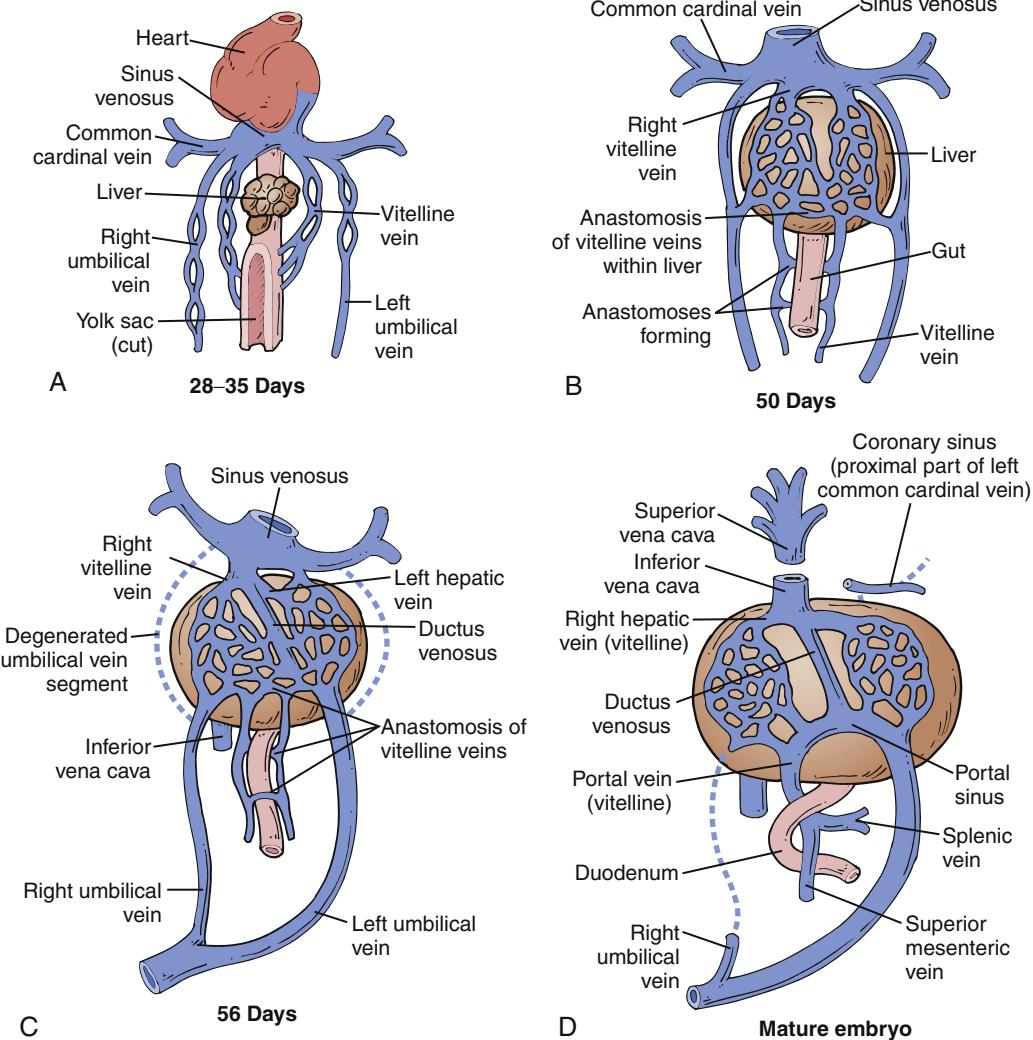


Figure 2.12 (A–D) Development of the umbilical and hepatic portal veins and the intrahepatic circulation.

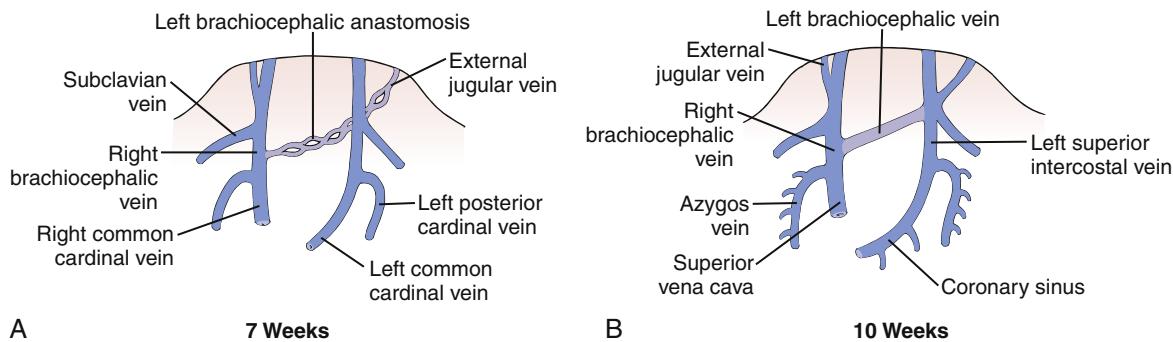


Figure 2.13 (A) Development of the superior vena cava and its major branches. **(B)** Completed development of the superior vena cava and its major branches.

subcardinal vein enlarges and becomes the renal portion of the inferior vena cava (second part), whereas the left subcardinal vein forms an anastomosis with the right subcardinal vein at the level of the kidneys to form the left renal vein. This anastomosis has both anterior and posterior components that surround the aorta. The posterior vein regresses, whereas the anterior vein persists to become the left renal vein (Fig. 2.14C,D).

The left subcardinal vein rostral to the subcardinal anastomosis regresses, whereas the caudal portion remains as the left gonadal vein. The last pair of veins to appear, the supracardinal veins, also arise from the posterior cardinal veins, and drain the body wall. The thoracic and abdominal portions of the supracardinal veins have different fates. In the abdomen, the right supracardinal vein anastomoses with the right subcardinal portion of the

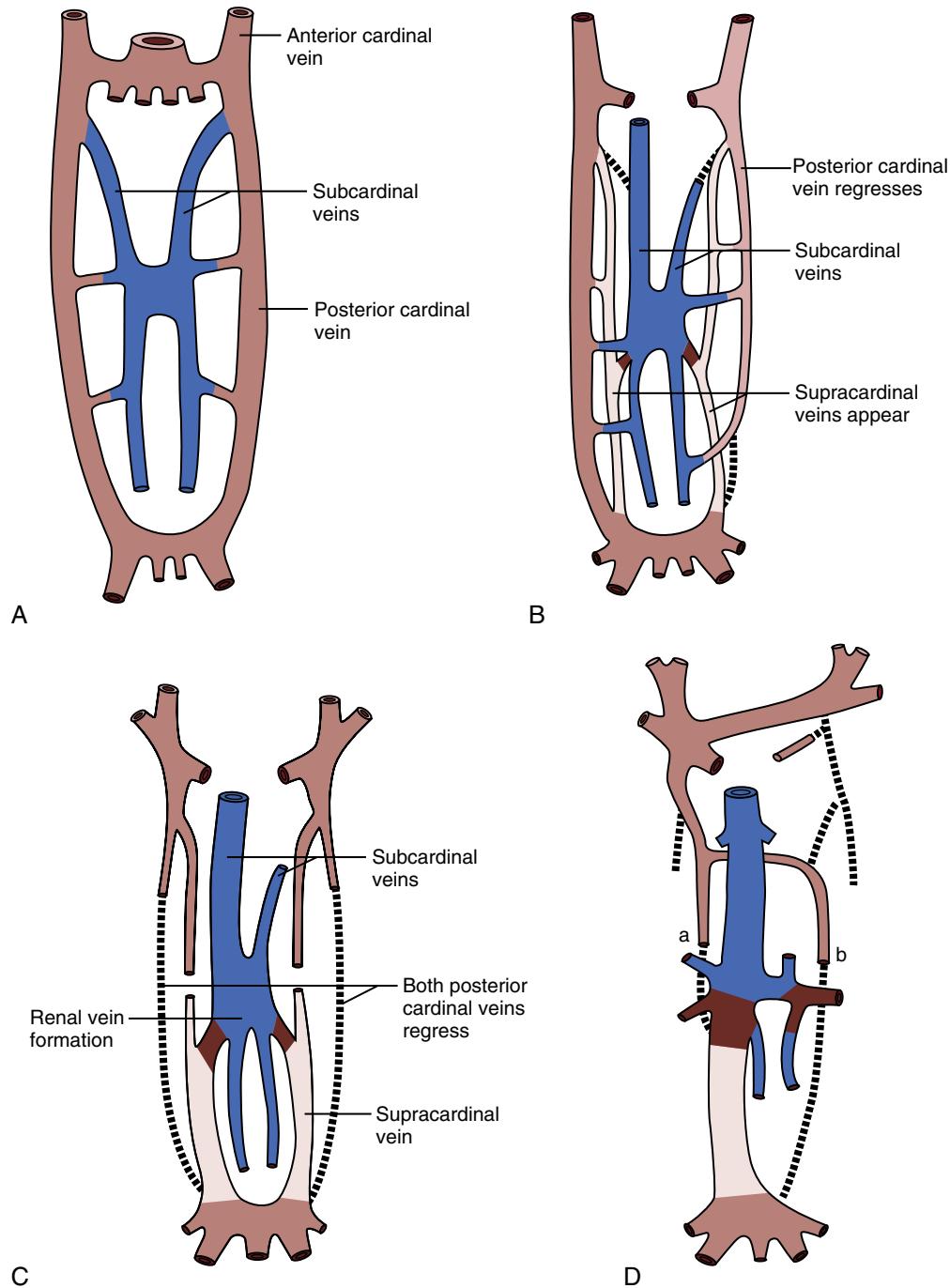


Figure 2.14 Development of the Inferior Vena Cava. (A) At 6 weeks of gestation, the posterior cardinal veins are dominant and the subcardinal system is beginning to appear. (B) At 7 weeks of gestation, the subcardinal veins are dominant and the supracardinal system begins to appear. The posterior cardinal veins are beginning to regress. (C) At 8 weeks of gestation, the subcardinal veins form the suprarenal inferior vena cava. The supracardinal veins form the infrarenal inferior vena cava. The posterior cardinal veins regress. (D) The adult inferior vena cava. Portions of the posterior cardinal veins persist to form the azygos system (*a*) and the hemiazygos system (*b*). (From Giordano JM, Trout HH, III. Anomalies of the inferior vena cava. *J Vasc Surg*. 1986;3:924–928.)

inferior vena cava, thus becoming the infrarenal portion of the inferior vena cava (third part). At its caudal end, it will anastomose with the fused left and right posterior cardinal veins (fourth part of the inferior vena cava). The thoracic portion of the supracardinal veins drains the posterior wall by a series of intercostal veins. They drain into the left and right horns of the sinus venosus by way of the rostral remnants of the posterior cardinal veins. Eventually the left supracardinal vein loses its communication with the left horn of the sinus venosus and instead anastomoses with the right supracardinal vein before its entrance into the right horn of the sinus venosus. The right supracardinal vein becomes the azygos vein, whereas the left supracardinal vein becomes the hemiazygos vein.

Anomalies

Superior Vena Cava

Two anomalies that can occur from development of the superior vena cava are described: double superior vena cava and left-sided superior vena cava. Neither anomaly is clinically important, except that they produce unusual shadows on chest radiographs. A double superior vena cava develops when the caudal section of the left anterior cardinal vein fails to regress.³² This anomaly may occur with or without development of the brachiocephalic vein. A left-sided superior vena cava occurs if the caudal section of the right anterior cardinal vein regresses, but the caudal section of the left anterior cardinal vein remains open – the opposite of normal development.³³ A brachiocephalic vein forms, but in this anomaly, it connects the right anterior cardinal vein to the left anterior cardinal vein, becoming a left-sided superior vena cava.

Inferior Vena Cava Duplication and Left-Sided Inferior Vena Cava

Duplication occurs if the left supracardinal vein fails to regress. If both supracardinal veins persist and join at the level of the renal arteries, a double inferior vena cava is observed. If the left supracardinal vein persists and the right supracardinal vein regresses (the opposite of normal), a left-sided inferior vena cava is seen. In this anomaly, the vein crosses to the right side at the level of the renal arteries and is a mirror image of normal anatomy. In the case of a left-sided inferior vena cava, the right adrenal and gonadal veins, instead of emptying into the inferior vena cava as normal, empty into the right renal vein; likewise, the left adrenal and gonadal veins empty into the inferior vena cava instead of the left renal vein. Recognition of a duplicated inferior vena cava or a left-sided vena cava is important when placing an inferior vena cava filter.

Renal Vein Anomalies

The most common venous anomaly is persistence of the posterior component of the left renal vein. This results in either a retro-aortic left renal vein if the anterior component regresses or a left circum-aortic renal vein if both anterior and posterior renal veins persist. In cases of circum-aortic renal veins, both veins join before entering the inferior vena cava, resulting in a venous collar around the aorta. Awareness of a retro-aortic renal

vein is important during dissection of the aorta at the level of the renal arteries to avoid injuring the vein (Fig. 2.15).^{34–40}

DEVELOPMENT OF THE LYMPHATIC SYSTEM

The lymphatic system plays key roles in the regulation of fluid transport, absorption of lipids from the intestines, and inflammation. It is involved in the removal of fluids from the interstitial space and provides a pathway for lymphatic cells involved in the immune response. During the early twentieth century, two distinct hypotheses were proposed to explain its development: the “centrifugal” or “venous origin” theory which proposed that the lymphatic system budded from the developing venous system to form discrete lymph sacs in specific regions; and the “centripetal”, “local” or “non-venous” origin theory which proposed that the lymphatic system developed de novo from embryonic mesoderm adjacent to developing veins.^{41,42} Recent evidence supports a dual origin of lymphatic cells that can arise from venous endothelial cells or non-venous lineages in specific tissues.⁴³ Our understanding of the molecular mechanisms involved in lymphatic development has also advanced. Lymphatic specification occurs through several transcription factors including PROX1, SOX18, and the vascular endothelial growth factor C (VEGF-C)/VEGF receptor 3 (VEGF-R3) axis.⁴³

Development of the lymphatic system (Fig. 2.16) begins as lymph sacs, two paired and two unpaired, that begin to develop in a cranial to caudal sequence. These lymph sacs begin to appear in the fifth week and are complete by the end of the sixth week in the human embryo.⁴² First to appear are a pair of jugular lymph sacs that bud off from the anterior cardinal veins (at the junction of the presumptive subclavian veins and internal jugular veins), whereas a second pair, the posterior lymph sacs, bud from the caudal segment of the posterior cardinal veins (at the junction of the internal and external iliac veins). The posterior lymph sacs appear late in the sixth week of development. The remaining lymph sacs, the retroperitoneal lymph sac and cisterna chyli, are unpaired and appear between the end

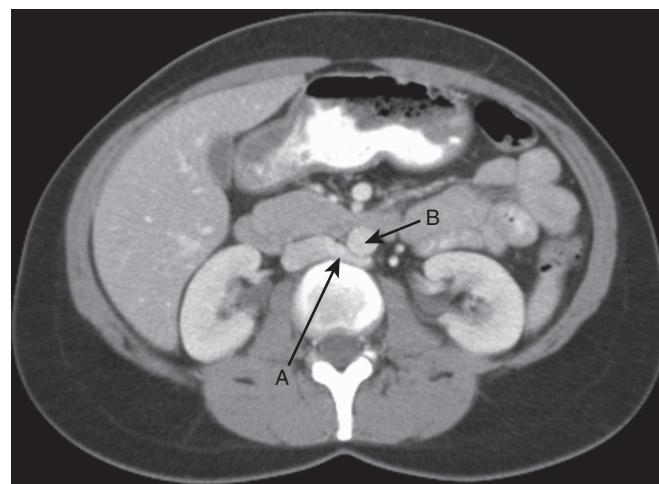


Figure 2.15 Retro-Aortic Left Renal Vein. The left renal vein (A) can be seen posterior to the aorta (B).

of the fifth week and beginning of the sixth week. The retroperitoneal lymph sac develops from the part of the mesonephric venous system near the developing suprarenal gland, whereas the cisterna chyli buds from veins at lumbar levels three to four.

Additional lymphatic vessels sprout from these six initial lymph sacs and follow the course of the developing veins. Extensions of the two jugular sacs extend caudally to meet with extensions of the cisterna chyli, forming a pair of thoracic lymph vessels that empty into the venous system at the internal

jugular and subclavian junction on both the left and right sides. These two thoracic vessels fuse at midthoracic levels, creating the definitive thoracic duct. The right jugular sac remains connected to the venous system and will eventually form the right lymphatic duct. Additional lymphatic vessels bud from existing lymphatic vessels to form deep lymphatic vessels.

Lymphatic vessels and nodes do not invade the central nervous system, meninges, eye, internal ear, cartilage, epidermis, or spleen. All the lymph sacs except the rostral part of the

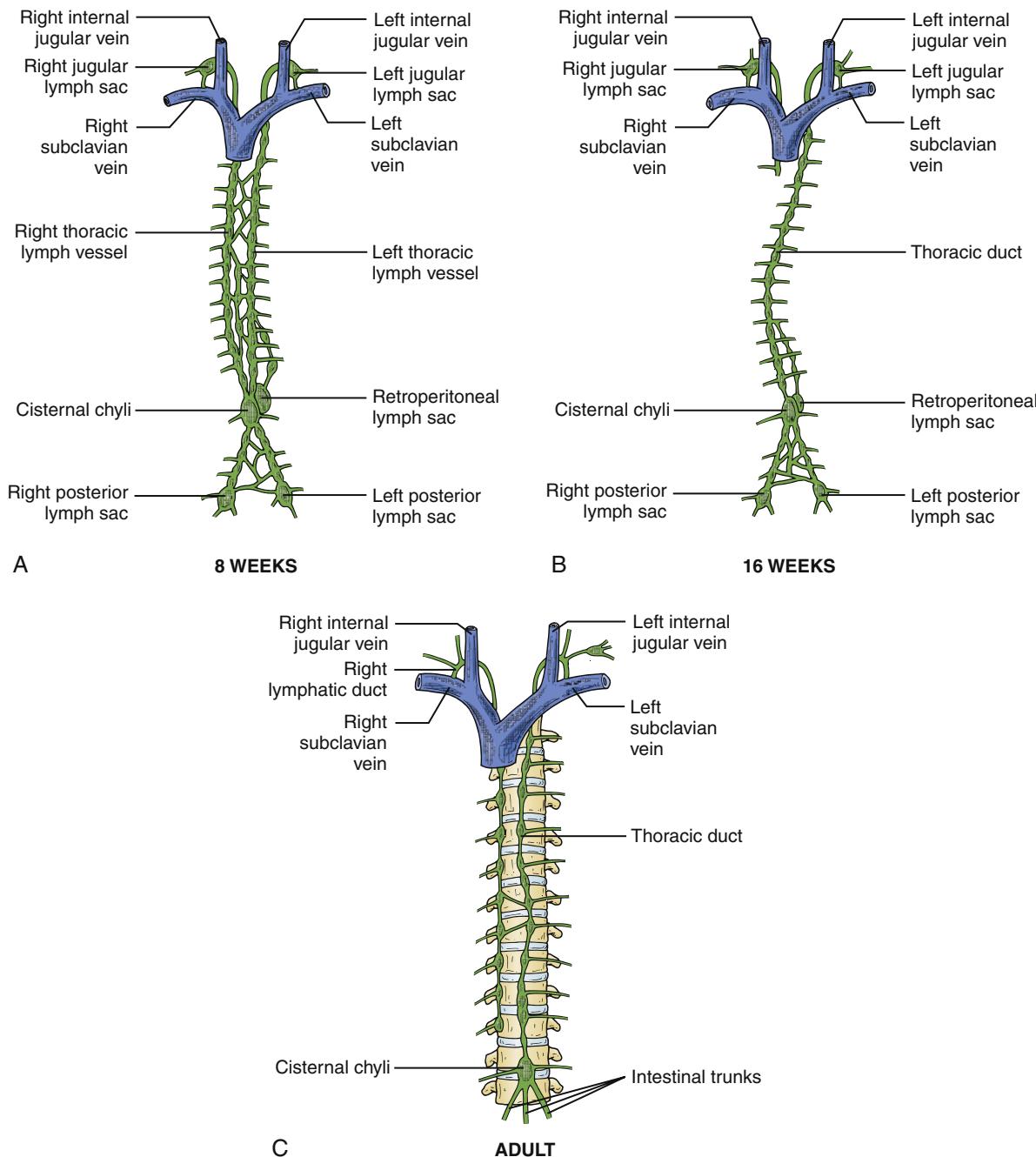


Figure 2.16 Development of the Lymphatic System. (A) By 8 weeks, lymphatic vessels are established as outgrowths of the six lymph sacs that budded off of the vascular system. At this point in time the embryo has a pair of thoracic lymph vessels that connect the cisterna chyli to the venous system superiorly. (B) By 16 weeks, portions of the right and left thoracic lymph vessels have been remodeled to form a single thoracic duct. (C) By the adult stage, all the embryonic lymphatic sacs have regressed with the exception of the cisterna chyli. The definitive thoracic duct and right lymphatic duct are present.

cisterna chyli develop connective tissue bridges and are seeded with lymphatic cells, which, in turn, form lymph nodes. The lymphatic connections to the venous system are lost, with the exceptions of the final connections of the right lymphatic duct and thoracic duct.

More recent work has shown that alternative, non-venous lineages can also contribute to lymphatic development in specific tissues.⁴³ For example, dermal lymphatic vessels are organized into a superficial and deep network, the latter having valves that can direct flow. Multiple reports have supported a dual origin of related lymphatic endothelial cells from venous and non-venous precursor cells. Failure of normal dermal lymphatic development can lead to hypoplasia that manifests as lymphedema. In this condition, improper lymphatic drainage results in accumulation of interstitial fluid. The role of dermal lymphatic development in pathological conditions such as primary lymphedema has not been elucidated. Several genetic mutations have been associated with primary lymphedema including Sox18 and various treatments have demonstrated efficacy in restoring the lymphatic network in pre-clinical modes. Data from clinical trials, however, are lacking.

The previous sections have addressed the development of the arterial, venous, and lymphatic systems in isolation. However, it should be apparent that these systems develop simultaneously and likely interact with each other. As the initial fine capillary network forms (the retiform plexus stage), errors in morphogenesis can result in various vascular malformations. Mutations in genes that regulate vessel specification may lead to various arteriovenous malformations and venous malformations such as Klippel–Trénaunay syndrome. Likewise, abnormal lymphatic

system development can be manifested concurrently or separately as the venous and arterial systems develop. Failure of normal lymphatic development can lead to hypoplasia that manifests as lymphedema, and has been linked to mutations responsible for early lymphatic development including Sox18. Future efforts to better understand the molecular basis of vascular development and vascular anomalies may lead to novel therapies that improve the care of patients with vascular disease.

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Vessel Wall Biology

JOHN T. LANGFORD and ALAN DARDIK

Based on a previous edition chapter by Bauer Sumpio and Jason Chin

WALL SYSTEM FUNCTION 29

Large Arteries 29

Small Arteries 30

Arterioles 30

Capillaries 30

Venules 31

Veins 32

Venous Valves 32

Venae Cavae 32

Lymphatics 32

Initial Lymphatics 32

Precollecting Lymphatics 32

Collecting Lymphatics 33

STRUCTURE OF THE VESSEL WALL 33

Artery 33

Intima 33

Endothelium 33

Basal Lamina 34

Internal Elastic Lamina 35

Media 35

Adventitia 35

Vein 36

Intima 36

Media 36

Adventitia 36

Lymphatics 36

MOLECULAR IDENTITY OF ARTERIES, VEINS, LYMPHATICS 36

Ephrins and Eph Receptors 37

Arteries 38

Veins 38

Lymphatics 38

HEMODYNAMICS AND VASCULAR WALL BIOLOGY 39

Shear Stress 39

Endothelium 39

Circumferential Stretch 40

Hemodynamic Effects on Vessel Identity 40

Complex organisms rely on the fluid, nutrient, and waste transport capabilities of the cardiovascular system, whose subdivisions each have unique structure and biology to perform crucial metabolic and mechanical functions. The arterial network reacts to hemodynamic forces through constriction or dilation and also via activation of signaling pathways. Its venous counterpart is an adaptable system, with unique mechanisms for accommodating the hemodynamic stresses of the body. The lymphatic system is a distinct but closely related network that augments fluid transport and immunologic functions (Table 3.1).

WALL SYSTEM FUNCTION

Vessel wall composition changes at different locations to allow optimal function. For arteries they are mainly structured to be “resistance vessels” while properties of veins allow them to be

“capacitance vessels.” Below we discuss the differences in the vessels from large arteries leaving the heart, down to capillaries and then back to large veins which return to the heart. We also discuss the lymphatic system which is a separate system that begins in interstitium and eventually returns lymph into the venous circulation.

Large Arteries

The arterial system commences with the aorta and its branches, whose primary function is to provide a conduit for blood flow to the peripheral tissues and to smooth out the pulsations of intermittent ventricular ejection. In general, larger arteries are elastic arteries while smaller arteries are muscular arteries. The larger arteries are predominantly under control of downstream resistance vessels, and there is little evidence that vasoconstrictor changes in large arteries occur independently of those in small

TABLE 3.1**Vessel Wall Characteristics of Circulatory Systems**

	Arterial Wall	Venous Wall	Lymphatic Wall
Collagen content	Moderate	High	Mixed
Elastic fiber content	High	Moderate	Mixed
Central pressure	High	Very low	Low
Shear stress	High	Low	Low
Stretch force	High	Low	Low
Pulsatility	High	Low	Low
Compliance	Moderate	High	High
Oxygen tension	High	Low	Low
Intrinsic propulsion	None	None	Predominant
Valves	None	Some	Many

resistive arteries.¹ Changes in caliber of large arteries occur passively through changes in transmural pressure and actively through changes in vascular smooth muscle cell (SMC) contraction. Arteriolar vasomotion may induce systemic or local blood pressure changes that alter the diameter of the artery and its viscoelastic properties. Arteriolar constriction or dilation may also modify arterial SMC tone through the endothelium-dependent mechanism of high-flow dilation.

The large arteries cushion the pulsations that are characterized by the relationships between oscillatory pressure, flow, and frequency, which in turn depend on arterial diameter and elasticity.² Because of their significant size, the aorta and the large arteries offer little resistance to blood flow. Large arteries store a large portion of the blood volume during systole and drain it during diastole. They are able to accommodate the stroke volume ejected with each contraction of the heart. This ability allows for propagation of the pulse and also offers dynamic resistance to the oscillatory components of pulsatile flow.

Blood pressure is highest at the origin of systemic circulation, although decreases in blood pressure are not linear with vessel diameter or distance down the vascular tree.² Blood pressure decreases to 30% to 40% of aortic pressure in vessels 250 to 50 µm in diameter. Significant pressure drop occurs in the terminal arterioles, which branch into small capillaries with diameters less than 100 µm (Fig. 3.1). In vessels smaller than 60 µm, no correlation has been found between central arterial pressure and microvascular pressure, suggesting that perfusion pressure is controlled directly in these blood vessels and those with smaller diameters.³

Small Arteries

Small arteries are considered muscular arteries and are involved in vascular resistance. Initially, it was believed that resistance arteries consisted solely of arterioles with only one layer of SMC and diameters of less than 30 to 50 µm. However, approximately 50% of precapillary resistance lies proximal to the arterioles and to vessels with diameters of <100 µm.⁴ The

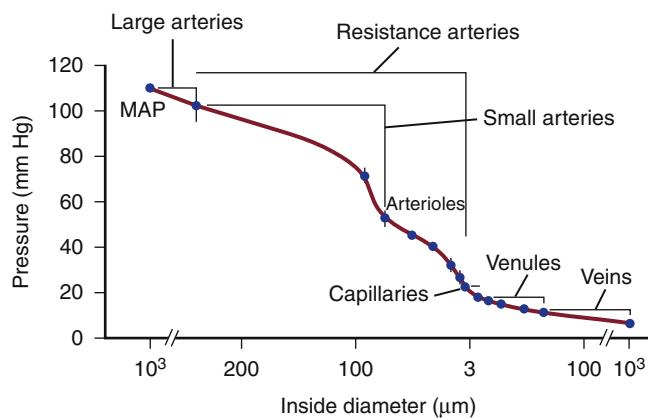


Figure 3.1 Pressure Drop Through Hamster Cheek Pouch Circulation. MAP, mean arterial pressure, measured in the femoral artery. Bars indicate standard error. (Redrawn from Mulvany M, et al. Structure and function of small arteries. *Physiol Rev*. 1990;70:922–949.)

resistance provided by a small artery is a function of the diameter-pressure relation. This compliance depends on the amount, arrangement, and characteristics of the connective tissues and SMC and on the activation level of SMC. There are approximately six layers of SMC in 300-µm vessels that decrease to a monolayer in 30- to 50-µm arterioles. The volume fraction of SMC in the media of small arteries as seen by electron micrographs is from 70% to 85%, a fraction that is greater than in larger vessels.⁴

Arterioles

Arterioles are the chief source of vascular resistance and control the distribution of flow. Smooth muscle contraction and dilation is the primary source of vascular resistance. Abnormal constriction and dilation cause systemic hypertension and hypotension, respectively. The regulation of constriction and dilation is controlled by both sympathetic and parasympathetic innervation. Vasomotor responses depend on arteriolar territory, as in the case of emotional stress; emotional stress dilates arterioles of skeletal muscles through activation of cholinergic vasodilator fibers and release of epinephrine, while it constricts the splanchnic and renal arterioles.²

Capillaries

Capillaries are responsible for the transfer of nutrient materials and waste products between blood and tissues. The blood volume of all capillaries is significantly larger than that of the aorta, causing a decrease in blood pressure and flow rate. The low rate of blood flow and large surface area facilitate the provision of nutrients and oxygen to surrounding tissue; the absorption of nutrients, waste products, and carbon dioxide; and the excretion of waste products from the body. Capillaries have a wide variety of configurations dependent on the structure of the endothelium, which is in turn related to the function of their specific vascular bed. They may be nonfenestrated continuous, fenestrated continuous, or discontinuous (Fig. 3.2).

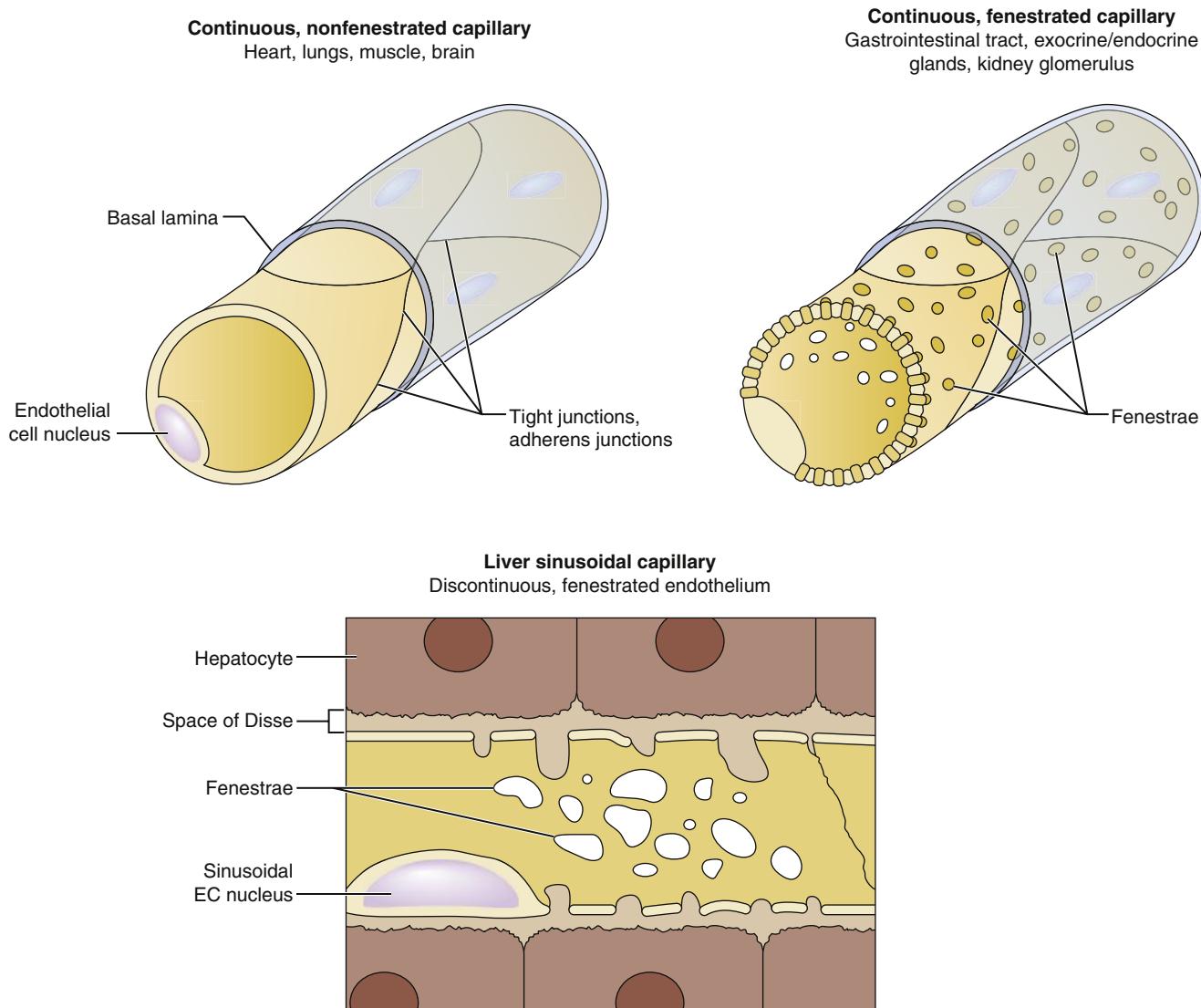


Figure 3.2 Nonfenestrated Continuous, Fenestrated Continuous, and Discontinuous Capillary Endothelium. These configurations affect the vascular permeability of the capillary bed with highly selective circulations such as the blood–brain barrier having continuous nonfenestrated capillary endothelium, and high fluid and macromolecule exchange circulations such as the liver having discontinuous capillary endothelium. (Redrawn from Kanasty RL, Whitehead KA, Vegas AJ, Anderson DG. Action and reaction: the biological response to siRNA and its delivery vehicles. *Mol Ther*. 2012;20(3):513–524.)

Nonfenestrated continuous endothelium functions as a selective filter and exists in capillaries all throughout the body. Fenestrated endothelium occurs in locations of increased filtration or transendothelial transport, including the capillaries of exocrine and endocrine glands, gastric and intestinal mucosa, choroid plexus, glomeruli, and renal tubules. The majority of fenestrae have a 5- to 6-nm nonmembranous diaphragm across their openings, although fenestral density varies in differing vascular beds and increases with greater need to absorb or secrete.^{5–7} Discontinuous endothelium possesses larger fenestrations, ranging from 100 to 200 nm in diameter. These fenestrations lack a diaphragm and contain gaps or pores within individual cells.⁸ Such capillaries form large irregularly shaped vessels, such as in hepatic sinusoids.

Venules

Postcapillary venules are the smallest of venous vessels and form from the confluence of several capillaries. Their dimensions have ranges of 50 to 650 µm in length and 10 to 50 µm in diameter. Similar in structure to large capillaries, they have an endothelium and pericytes but no SMC layer. The attenuated endothelium is 0.2 to 0.4 µm wide. The endothelium is loosely organized and leaky with only a few intercellular junctions linking cells. A thin basal lamina is present. Given these characteristics and their slow flow, they are the main sites for white blood cell diapedesis and tissue exudate from circulation to interstitial tissue during inflammation.^{9,10} Postcapillary venules drain into collecting venules, which are larger but structurally similar with more pericytes. These continue

to drain into muscular venules, which have one or two layers of SMC.¹⁰

Veins

Veins form from the confluence of multiple venules. Typical small and medium-sized veins are 1 to 9 mm in diameter and contain valves. Compliance is extremely high for veins in comparison with arteries. A relaxed vein can increase in volume by up to 200% for a small increase in transmural pressure from 0 to 10 mm Hg. This increase reflects a change in geometry, with a low-pressure vein having an ellipsoidal cross-section that becomes circular with a small pressure increase, greatly enlarging the cross-sectional area.¹¹ The adventitia, usually the thickest layer, consists primarily of longitudinally oriented collagen fibers. It may be continuous with the surrounding collagen of the supporting tissue.

Venous Valves

Venous valves are an important structural distinction found in veins that are not present in arteries; venous valves are characteristic of small and medium-sized veins more than 2 mm in diameter.¹⁰ Composed of local semilunar infoldings of wall intima, the valves project into the lumen in the direction of blood flow and prevent backflow as the blood returns to the heart against gravity. A reverse velocity of 30 cm/s appears required for valve closure.¹² Structurally, valves are composed of a mixed collagen–elastic fiber core of connective tissue covered by thin endothelium, making them stronger and more elastic than the vein wall.⁹ They are typically found distal to the confluence of venous branches forming larger veins. The valve sinus is wider than the lumen above and below the cusps. Generally, valves assist in the caudal-to-cephalad flow of blood, but also ensure the flow from the superficial veins to the deep system. A notable exception to this is in the feet, where flow is from the deep muscles in the sole to the superficial veins in the dorsum of the foot.¹³ Valves disappear more proximally in the venous system and are absent in the common iliac veins and inferior vena cava, as well as the veins of the head and neck.

The exact starting point for the development of venous diseases such as chronic venous insufficiency and varicose veins is not completely clear; however, there is little doubt that venous valve incompetence plays a role. A reduction in the number of valves per unit length has been seen in chronic venous insufficiency.¹⁴ The normal architecture of valve leaflets is also changed in chronic venous insufficiency, with significant infiltration by monocytes and macrophages.¹⁵ Valves exposed to abnormally high pressures experience increased MMP-2 and MMP-9 levels, favoring the accumulation of ECM; remodeling with reductions in leaflet height, width, and total disappearance also occurs.¹⁶

Venae Cavae

The superior and inferior venae cavae are the largest veins and deliver deoxygenated blood into the right atrium of the heart. At the entrance to the heart, venae cavae and pulmonary veins

have extensive vasa vasorum and can have a small amount of cardiac tissue in the adventitia.⁹ Other large systemic veins, such as the portal, pulmonary, azygos, splenic, and mesenteric veins, have similar features. The innermost layers have a distinct layer of fibroelastic tissue and a thin layer of circumferentially oriented SMC. A thick adventitia is most notable for the numerous bundles of longitudinally oriented collagen and smooth muscle fibers. Elastic fibers may be scattered throughout all the layers.

Lymphatics

The lymphatic system is a unidirectional flow network originating in the interstitial space throughout the body, draining fluid from the ECM into initial lymphatics, which in turn contribute to gradually larger precollecting lymphatics, collecting lymphatics, trunks, and ducts.¹⁷

Initial Lymphatics

The lymphatic system starts in connective tissue spaces at blind-ended vessels called initial lymphatics. These are most abundant in the connective tissue of the skin; beneath the mucous membranes of the respiratory, gastrointestinal, and genitourinary tracts; and in the connective tissue of the liver.⁹ Skin on the lower extremities has a denser network of lymphatics than other areas. The capacity for transport is also higher in the lower extremities to compensate for increased interstitial fluid filtration due to gravity.¹⁸

The influx of lymph is enabled by endothelial microvalves. When the vessels are collapsed, the endothelial cells (EC) have overlapping extensions of cell membrane interconnected by discontinuous buttonlike junctions.¹⁹ Gaps are made possible by anchoring filaments, 6 to 10 nm in diameter, that anchor the EC externally to the surrounding connective tissue.^{20,21} When the interstitial fluid pressure increases and the surrounding matrix expands, the EC anchored to this matrix are prevented from collapsing and allow fluid to flow through the resultant gaps between them.¹⁷ As the interstitial fluid decreases and internal pressure rises, the EC come together, overlap, and the gaps close (Fig. 3.3). In this way, the unidirectional flow of lymph into the lymphatics is ensured.²²

Precollecting Lymphatics

Precollecting lymphatics are segments of vessels connecting the initial lymphatics to collecting vessels. Like vein walls, lymphatic walls project bicuspid, one-way valves (termed secondary valves) into the vessel lumen. Unlike in larger collecting lymphatics, these valves are irregularly spaced and sometimes constitute only a single leaflet. At this level, SMC also start to appear around the vessels in one or more layers. These smooth muscle layers may contract and begin the forward propulsion of lymph through the system. However, many areas still are without any muscular layer or basal lamina and can act as a primary valve. Therefore, precollecting lymphatics fulfill a dual function in absorbing more lymph and also propelling it forward.^{17,23}

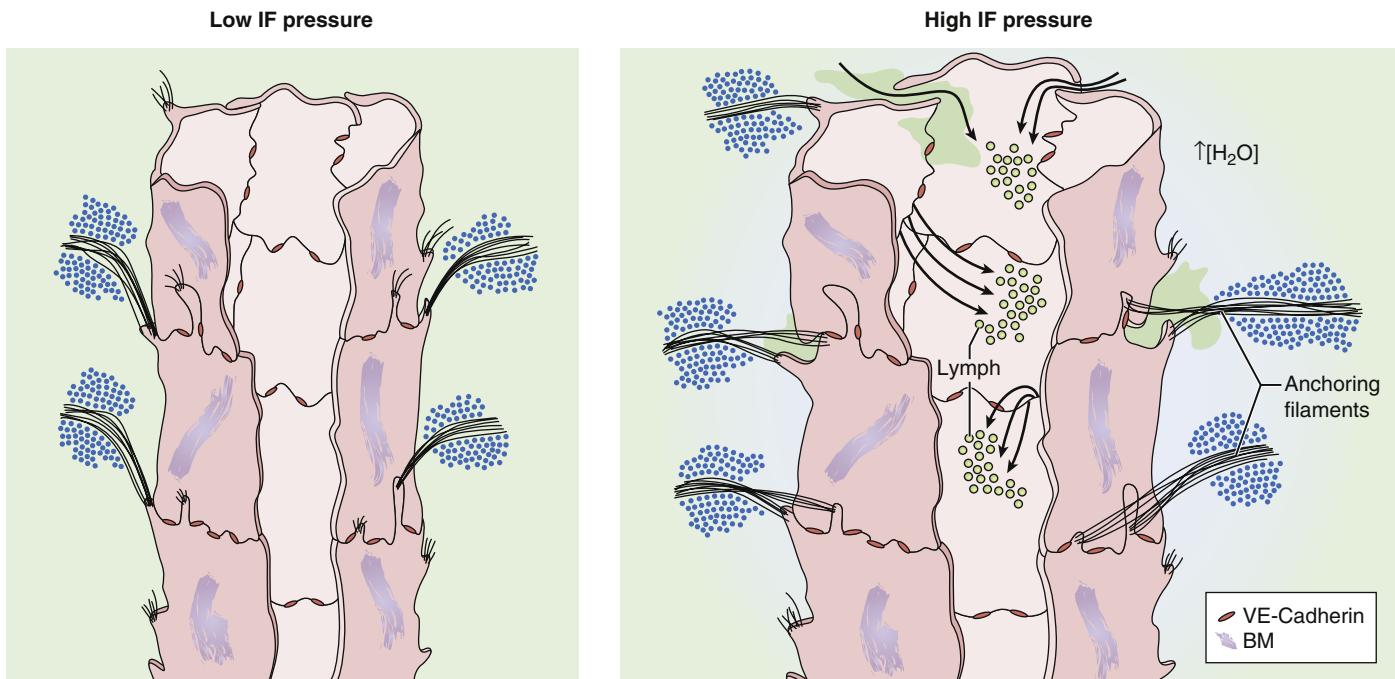


Figure 3.3 Endothelial Microvalve Drainage Response to Fluid Pressure Changes. When interstitial fluid (IF) pressure is low, junctions between endothelial cells are closed, not allowing flow into the lumen. When IF pressure is high, anchoring filaments pull the overlapping junctions apart and allow fluid entry into the lumen. (Redrawn from Bazigou E, Wilson JT, Moore JE Jr. Primary and secondary lymphatic valve development: molecular, functional and mechanical insights. *Microvasc Res.* 2014;96:38–45.)

Collecting Lymphatics

At the level of the collecting lymphatics, the basal lamina surrounding the endothelium of the lymph vessel becomes complete. The luminal side is bordered by an intimal monolayer of EC. The vessel wall contains one to three layers of SMC intermixed with collagen and elastic fibers. The muscle bundles are arranged in a helicoidal manner. This is all surrounded by an adventitia composed of fibroblasts, connective tissue, and nerve terminals.²⁴ Secondary valves also become more regularly spaced.

Lymphangions each represent the vessel space between two of these secondary valves. In humans, lymphangions of collecting lymphatics in the head and neck average 0.2 mm in diameter with a length of 2 mm. Those of the lower extremities may have a diameter of 1 to 2 mm with a similar 2-mm length. Lymphangions are innervated by sympathetic and parasympathetic nerves and rhythmically contract to propel lymph forward.¹⁷

STRUCTURE OF THE VESSEL WALL

Arteries and veins have three main layers of the vessel wall. Lymphatics also have these layers but they are less well defined. The three layers are known as the intima, media and adventitia. In the arteries there are well developed layers that separate these three compartments; these boundary layers are the internal elastic lamina (IEL) and the external elastic lamina (EEL). In veins these layers are disorganized, less prominent and are difficult to define by histology (Fig. 3.4). Depending on location and size of vessels the vessel wall layers will change according to the function of the vessel, as previously discussed.

Artery

Arteries, in general, are thicker vessels with more SMC in the media than veins. The composition of the media will dictate whether an artery is categorized as either elastic or muscular. These changes allow the arteries to withstand the pulsations from the heart and respond to different stimuli to contract or dilate to augment resistance and blood pressure.

Intima

The intima, the innermost luminal layer, extends from the lumen to the IEL. The intimal luminal surface is lined by the endothelium. The intimal layer is very thin, consisting primarily of a few scattered leukocytes, SMC, and connective tissue fibers. The matrix fibers consist mainly of elastic and collagen fibers and proteoglycans.

Endothelium

The endothelium provides a continuous lining throughout the luminal surface of arteries which is smooth and regular and made up of flat polygonal EC.²⁵ The cell thickness varies from less than 0.1 µm in the capillaries to 1 µm in the aorta.²⁶ By interacting with its cellular and acellular environments, the endothelium is able to play an important role as a regulator of vaso-motor tone, hemostatic balance, permeability, cell proliferation, survival, and immunity.²⁶ Both physiologic and pathologic processes are regulated by the endothelium as it reacts to physical forces, chemical signaling, and immunologic mediators.²⁷

EC, which are typically aligned in the direction of blood flow, tend to overlap immediately adjacent cells. The nature

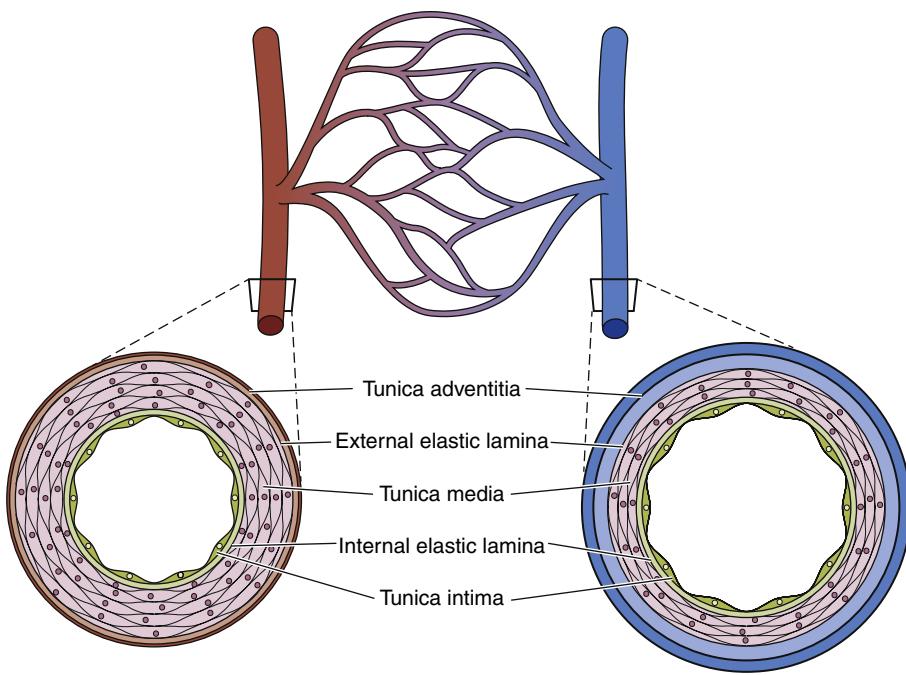


Figure 3.4 Anatomy of the Vessel Wall. Arteries and veins maintain the same basic structure, which consists of the tunica adventitia, tunica media, and tunica intima. However, the composition and prominence varies between the two.

of the cell bonding is relevant to both the mechanical strength and the permeability of the endothelial layer. Membranes of adjacent cells are mainly parallel and separated by an intercellular space of 15 to 20 nm in size.² Sites of firmer attachment include tight junctions (*zona occludens*) and adherens junctions (*zona adherens*). Tight junctions form a barrier to transport between EC and help maintain polarity between the luminal and abluminal sides of the cells. This tight seal prevents the passage of molecules between the lumen and cell base. Large artery EC have a well-developed system of tight junctions due to high rates of pulsatile blood flow, whereas junctions become progressively looser as the system transitions to arterioles and capillaries.²⁶ Adherens junctions exist primarily in large arteries and permit inter-EC communication via movement of ions, metabolites, and regulatory factors.²

While the EC function mainly as a barrier they also function to transport material into the vessel wall. Pinocytic vesicles in EC regulate the permeability of the endothelial layer. The pinocytic vesicles are responsible for the movement of material from the lumen of the vessel into its wall. The vesicles exist primarily in muscular small blood vessels and are found in large numbers in the heart and lung and to a lesser extent in the retina and the brain.²⁸ Caveolae are cup-shaped, cholesterol-enriched sections of cell membrane that function in vesicular transport and have critical functions during EC signal transduction.²⁹ Caveolin-1 is a major structural protein in caveolae that is necessary for caveolae formation and is linked to several other proteins including eNOS; caveolin-1 inhibits eNOS and plays a role in regulating vascular tone. Interestingly caveolin-1 is also associated with Eph receptors, a class of proteins involved in vascular identity that will be discussed later.^{30,31}

The EC are covered by a glycocalyx responsible for the antithrombogenic properties of the endothelial surface.² The glycocalyx reduces friction from blood flow and serves as a barrier for fluid loss through the vessel wall. Its thickness varies across the vascular tree.³² During inflammation, the glycocalyx is sheared off, permitting the attachment of leukocytes and the transport of water from microvessels, and possibly initiating the development of atherosclerotic lesions.³³

Basal Lamina

The basal lamina, which borders the EC on their abluminal surfaces, forms a generally continuous boundary between the endothelium and the immediately underlying intimal structure. The basal lamina is involved in the regeneration and attachment of the endothelial layer, blood vessel permeability, initiation of blood clotting, and provides a barrier to cellular migration.³⁴ Most importantly it strengthens the vessel wall and connects the EC compartment to subjacent cell layers and SMC.³⁵

The basal lamina consists of two zones: a clear inner zone (*lamina rara*) and a dense fibrillar zone (*lamina densa*) adjacent to the interstitial connective tissue.²⁷ The clearer inner layer contains the glycoprotein laminin, whereas the dense layer is composed of type IV collagen. Mechanically, the basal lamina strengthens the vascular wall. Collagen type IV chains form a covalently stabilized framework and provides mechanical resistance. Laminin forms a second polymer network and connects EC as well as heparan sulfate to the collagen.^{35,36} Entactin binds laminin and collagen. Fibronectin transmits mechanical forces between integrin receptors on SMC and the ECM to redistribute the mechanical load within the arterial wall.³⁷

The basal lamina provides a pliable extensive bond, which permits EC to comply with changes in configuration related to cardiac pulsation and to vessel torsion and flexion.^{2,38} Furthermore, discrete adhesion points of increased electron density have been observed between basal peripheral cytoplasmic dense bodies of EC and the IEL, which prevent slippage of the lining cells when shear stress increases.³⁸

Internal Elastic Lamina

The IEL separates the subendothelial intimal layer from the medial layer and is composed of elastic fibers 70 to 100 nm in thickness. Experimental evidence suggests that both abluminal SMC and luminal EC are collaboratively responsible for elastin formation and IEL synthesis.^{39,40} Elastin is organized into fenestrated cylindrical lamellae that are separated from neighboring lamellae by single layers of SMC.⁴¹ Lamellar units are determined prenatally, and postnatal changes in thickness or circumference of the media accompany parallel changes of the thickness and circumference of the elastic lamellae.^{42,43}

Collagen- and proteoglycan-based fenestrations permit flow, driven by a pressure gradient, across the arterial wall tissues into the intimal layer.^{2,44,45} The IEL and elastic lamellae in large arteries may function as barriers to macromolecular accumulation in the vascular wall. Structural defects within the IEL are directly implicated in the onset of intimal thickening in human arteries.⁴⁴ Fragmentation of the IEL and disruption of medial layers is common in advanced plaques in apolipoprotein E knockout mice.⁴⁶ Defective IEL may result in abnormal attachment with EC, causing gaps in the endothelial surface facilitating the entry of macromolecules, lipids, and leukocytes into the intima.^{44,47,48}

Although macromolecules are believed to cross the normal arterial EC layer through vesicular transport, macromolecules are believed to cross the IEL via diffusion through the fenestrations. The diffusion rate is inversely dependent on its size.⁴⁹ Owing to this difference in transfer rate, the IEL most likely plays a significant role in regulating macromolecular accumulation in the intima, restricting plasma-borne macromolecules from entering the media, and regulating discrete microenvironments between the intima and the media.⁴⁹

Media

The media extends from the IEL to the adventitia. At low and physiologic pressures, the media is the chief determinant of arterial wall properties. As mentioned before, arteries are classified as either elastic or muscular according to the proportion of cellular and fibrous components.⁵⁰ In general, the central arteries have the most elastin and more distally, collagen becomes the predominant component.⁵¹

In elastic arteries, well-defined elastic lamellae and collagen bundles are prominent in the media, whereas connective tissue fibers are less frequent. Arterial media elasticity dictates arterial distensibility and the capacitive effects of larger arteries.⁵² The prominent elastic arteries are those with large diameters and in close proximity to the heart – the aorta, the brachiocephalic trunk, and the iliac arteries. Elastic arteries consist of lamellar units, which are close associations of elastin, collagen,

and SMC. Between the lamellae, SMC, collagen fibers, elastin microfibrils, and glycosaminoglycans are organized into functional units. Although the number of lamellar units depends on the arterial diameter, humans have approximately 40 to 60 units in the aorta, which decreases to less than 10 in the peripheral arteries.² The genetic program that determines the amount of elastin in arteries is completed early in life and then is silenced, establishing a fixed amount of elastin for the life of an individual.⁵³ With aging, elastin is slowly degraded and replaced by collagen, a much stiffer protein; this change in vessel composition leads to stiffer vessels and may contribute to the pathogenesis of hypertension.⁵⁴

Muscular arteries predominate in the second- or third-order branch arteries. The media consists primarily of SMC with fewer connective tissue fibers. Nonparallel branching elastin strands increase the capacity to change diameter under neurohumoral stimulation. The SMC layers consist of groups of similarly oriented cells, each surrounded by a common basal lamina in a closely associated interlacing basketwork of type III collagen fibrils. The fibrils are arranged to tighten around the cell groups as the media is brought under tension. This configuration holds the groups of cells together and prevents excessive stretching or slippage. At low and physiologic pressures, collagen fibers are slack and not substantially load-bearing.⁵⁵ The thickness and abundance of collagen bundles depends largely on vessel diameter with the average tangential tension per medial layer approximately 2000 dynes/cm, regardless of species.⁵⁰

Normally SMC are in a contractile state in arteries. The SMC are surrounded by a basement membrane composed mainly of laminin, collagen IV, heparan sulfate proteoglycan, entactin/nidogen, and fibronectins, which prevent the proliferation and migration of the SMC. This arrangement maintains the SMC in a contractile state as opposed to the synthetic state.⁵⁶ In the contractile state in normal arterial vessels, SMC are differentiated and contain abundant contractile and intermediate filament proteins. In certain physiologic, pathologic, and *in vitro* tissue culture conditions, the SMC abandon the contractile phenotype for an immature synthetic phenotype. This phenotype change permits cell migration into the intima, proliferation, and secretion of ECM components.⁵⁷

Adventitia

The adventitia extends from the EEL to an ill-defined boundary usually contiguous with the perivascular connective tissue. The adventitia varies in thickness and organization. Whereas the aorta has minimal adventitial fibrous connective tissue, large muscular arteries contain prominent adventitial elastic and collagen fibers. Here, adventitia may be more prominent than media. Generally, adventitial cells are sparse and consist mainly of fibroblasts.

The adventitia contains vasa vasorum and nerves, which provide nutrition to the adventitia and media and contribute to the regulation of the medial smooth muscle function. The vasoconstrictor nerve fibers induce vasoconstriction or vasodilation via adrenergic receptors. Nervous stimuli are transmitted to the outer SMC through neuromuscular junctions. The signal

is transmitted to the inner cells by electrical coupling between adjacent SMC.

Vasa vasorum are present in arteries with diameters larger than 200 µm. Also, when the medial layer has more than 29 lamellar units, the outer part of the media is irrigated by the vasa vasorum. Nutrients may also be provided from the luminal blood flow for the inner part of the media. The vasa vasorum are composed of small arteries, arterioles, capillaries, and venous channels, as well as nerves that mediate smooth muscle tone. Vasa vasorum arise from the parent artery at branch junctions and arborize in the adventitia. In thick-walled arteries, mural stresses and deformations may affect the vasa vasorum. Hypertension may impair vasa flow. A lymphatic network in the adventitia collects the proteins, ions, and water from the blood and transports these substances through the vessel wall.

An important function of the adventitia is to act as a reservoir of progenitor cells. Pericytes and perivascular adipose tissue (PVAT) have been identified as sources of cells that do just that. Pericytes are specialized cells found around microvessels and vasa vasorum of larger arteries;^{58–60} these cells have multiple morphologies and functions but are important sources of progenitor cells, which play a role in vasculogenesis and angiogenesis.⁶¹ PVAT acts to protect vessels and also functions as an endocrine organ; PVAT releases multiple biologically active molecules that influence the vasculature.⁶² Inside the PVAT are mesenchymal stem cells that participate in vascular regeneration.⁶³

Vein

In general, veins have thinner walls than arteries with a more limited quantity of cellular and fibrous components. The composition of vein walls is also different, with a relative abundance of collagen fibers, particularly in large veins, and a paucity of elastic fibers, as might be assumed from the diminished elastic laminae (Fig. 3.5). The abundance of adventitial collagen and lack of medial elastin allow the vein wall to accommodate 60% to 80% of the body's blood volume.¹²

Intima

The intima consists of one layer of EC sitting on an incomplete elastic basement membrane.⁹ Under normal healthy conditions, the EC have essential roles in venous wall integrity and function. These cells produce vasorelaxants and platelet inhibitors, such as PGI₂ and NO. NO also negatively affects the expression of chemical mediator secretion and inflammatory cell adhesion molecules including ICAM-1 and VCAM-1.^{64–66} These functions of the venous intima and EC in their normal state play important roles in pathologic processes such as neointimal hyperplasia when under arterial pressure.

Media

The media is composed of circularly arranged SMC, and varies greatly according to location; however, in general it is less developed than that of the arterial system and may contribute to the development of varicosities in lower extremity veins. The medial SMC are held in a quiescent state in the normal venous

environment by various factors.⁶⁷ Transforming growth factor-β downregulates their mitogenesis and stabilizes the ECM against SMC migration. In addition, heparin and heparin-like molecules neutralize fibroblast growth factor (FGF) to downregulate cell proliferation. This process is important because these factors keep the normal vessel wall in a state of low cell turnover with low rates of proliferation and apoptosis. Injury or changes to the environment, as when veins are exposed to arterial flow, can increase rates of proliferation or apoptosis.⁶⁷

Adventitia

The adventitia is generally the thickest layer in large veins, sometimes blending with the media. Bundles of longitudinally oriented SMC are interspersed with collagen and elastic fibers. Collagen fibers may be particularly abundant in large veins as well and can have either a longitudinal or helical orientation. Compared with the vasa vasorum of arteries, vasa vasorum are much more extensive in venous adventitia and penetrate deeper regions as well.⁹ Lower oxygen tension in venous blood is a possible explanation for this phenomenon.

Lymphatic

Although lymphatic vessels have been long known, the absence of large, consistent vessels has delayed more active research in the function of lymphatics; however, advances in imaging and tissue engineering have showed distinct structural and functional differences of lymphatics.

The lymphatic wall morphology has distinct anatomy at different levels. These differences play important roles in the drainage and propulsion of lymph through vessels. Briefly, at the level of the capillaries and initial lymphatics, a single layer of EC is present, with large gaps between cells and an incomplete basal lamina.⁹ These smaller lymph vessels coalesce into large vessels with thin walls that resemble veins. Like veins and arteries, large lymph vessels have three concentric layers. These layers, however, are not as readily delineated as they are in the cardiovascular system.⁹ As in veins, valves are present within the lymph vessel lumen but are also much greater in number.

MOLECULAR IDENTITY OF ARTERIES, VEINS, LYMPHATICS

The vascular system is one continuous structure, but arteries, veins and lymphatics are all unique in function. EC, the innermost layer of the cell wall, are molecularly distinct between arteries, veins, and lymphatics.⁶⁸ The molecular determinants of vessel identity are present prior to the first heartbeat and influence vascular identity along with hemodynamic factors. In addition to being embryonic determinants of cell fate they are also present as identity markers in adult arteries, veins, and lymphatics. Ephrin-B2 and Eph-B4 are found on arteries and veins, respectively, while VEGF-R3 is found in lymphatics. A more complete list can be found in Table 3.2.

SMC in the vascular wall, like EC, are also molecularly unique depending on their location; the origin of SMC cell

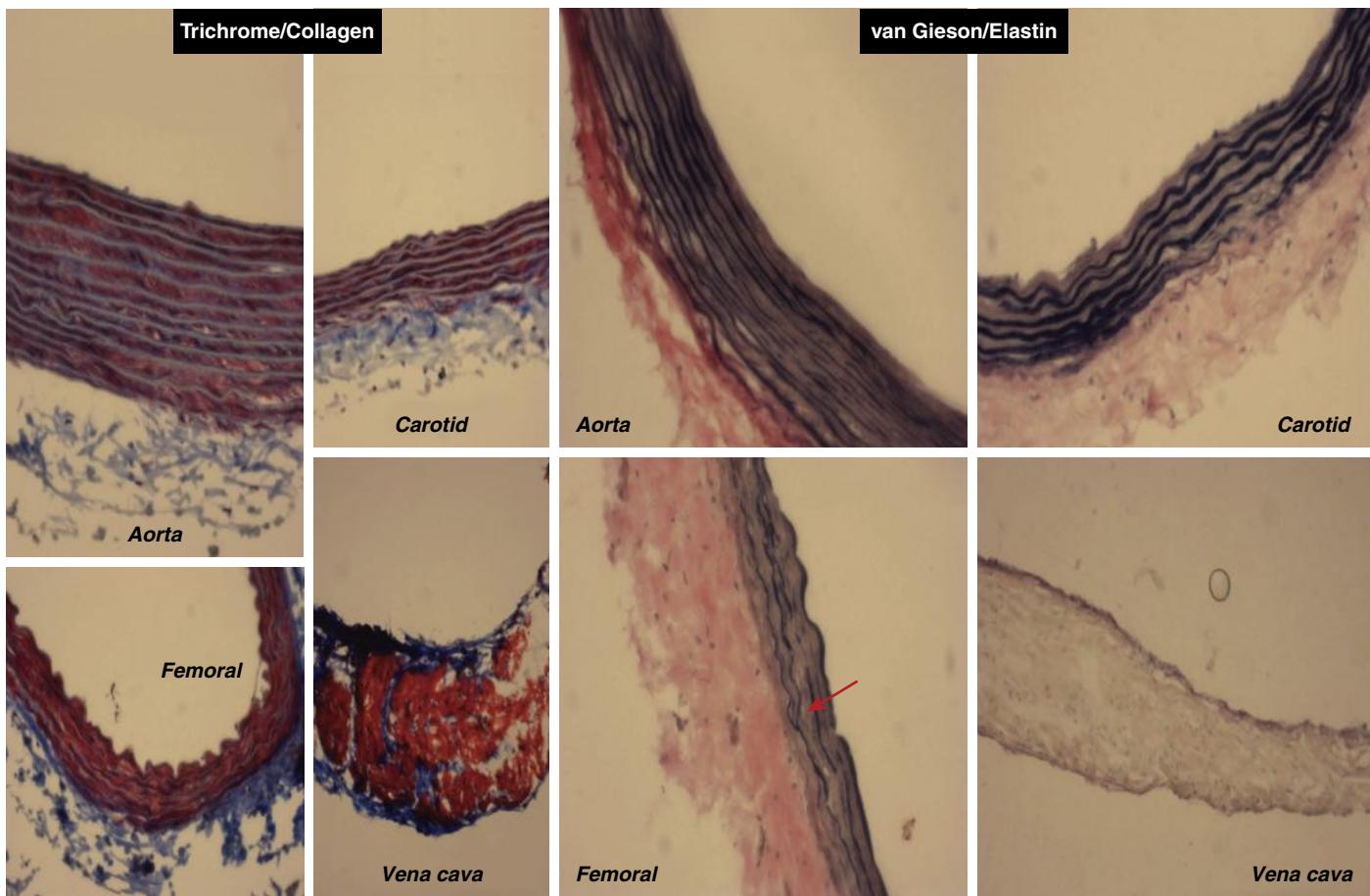


Figure 3.5 Masson Trichrome Blue Staining of Collagen, and Van Gieson Staining of Elastin in Different Vessels. Blue represents collagen on the trichrome staining. There is a relative abundance of collagen in large veins such as the vena cava, as well as certain arteries such as the femoral artery, while there is minimal collagen expression in the aorta and carotid. Blackish-brown represents elastin on the van Gieson staining. The aorta and carotid have higher levels of elastin compared to the femoral artery and vena cava. The red arrow also indicates the broken structure of the elastin in the femoral artery specimen, which in this case could be associated with the higher level of expression of MMP-9 in the femoral artery compared to other vessels. (Redrawn from Basu P, Sen U, Tyagi N, Tyagi S. Blood flow interplays with elastin: collagen and MMP: TIMP ratios to maintain healthy vascular structure and function. *Vasc Health Risk Manag*. 2010;6:215–228. Originally published by and used with permission from Dove Medical Press Ltd.)

lineage can vary along a vessel's course and can affect function and response to stimuli during development and in adults. In the embryo, TGF- β 1 induces aortic arch SMC to proliferate whereas abdominal aortic SMC proliferation is inhibited.⁶⁹ Different responses are also seen in the adult aorta with atherosclerosis; the thoracic aorta is generally more resistant to developing atherosclerosis whereas the abdominal aorta is more prone to atherosclerosis. When these segments are experimentally transposed, they retain their atherosclerosis resistance or susceptibility despite their new anatomic location, and these correlate to differences in the SMC origins.⁷⁰ Differences in SMC are believed to be due to HOX gene expression; HOXA9 expression is elevated in the thoracic aorta compared to the abdominal aorta and represses inflammatory responses.⁷¹

Ephrins and Eph Receptors

The determinants of arterial and venous identity are mainly regulated by the erythropoietin-producing hepatocellular

(Eph) receptors. Eph receptors and their ephrin ligands were initially described in the nervous system but have also been found in the vasculature. The Ephrin-B2 ligand and the Eph-B4 receptor are of particular interest in vascular biology. Ephrin-B2 is found almost exclusively in arterial endothelial cells whereas Eph-B4 is found almost exclusively in venous endothelial cells.

Eph receptors and their ephrin ligands have a unique signaling modality known as bidirectional signaling. Typical forward signaling occurs through the Eph receptors whereas reverse signaling can occur in the transmembrane ephrin ligands.⁷² Bidirectional signaling allows simultaneous signal propagation in both ligand and receptor cells and are critical during embryonic development; stimulation of Eph-B4 leads to forward signaling and a venous cell fate while stimulation of Ephrin-B2 leads to reverse signaling and an arterial cell fate. Disruption in the Ephrin-B2 ligand will cause disruption in angiogenesis of both arteries and veins due to this important bidirectional signaling.⁶⁸

TABLE 3.2 Markers of Vessel Identity

Artery	Vein	Lymphatic
Ephrin-B2	Eph-B4	Prox1
Notch-1	COUP-TFII	VEGF-R3
Notch-4	BRG1	Sox18
VEGF-R2	Neuropilin-2	-
Alk1	APJ	-
CD44	Flt4	-
Connexin-37	Tie2	-
Connexin-40	-	-
Delta-Like-4	-	-
Depp	-	-
IGFB-SP	-	-
Neuropilin-1	-	-
Unc5b	-	-
Jag1	-	-
Jag2	-	-
Hey2	-	-
CXCR4	-	-

Arteries

Vascular endothelial growth factor receptor-2 (VEGF-R2) stimulation is required for the expression of Ephrin-B2.⁷³ VEGF-R2 stimulates delta-like ligand-4 (Dll4) that in turn stimulates notch receptors. Notch acts to form arterial identity, and suppresses chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) that induces venous identity. The cleaved end of notch translocates into the nucleus and increases expression of Ephrin-B2, leading to arterial identity. Notch/Dll4 also inhibits COUP-TFII, suppressing venous identity (Fig. 3.6).

Ephrin-B2 in adult endothelial cells plays an important role in inflammation. Ephrin-B2 interacts with circulating monocytes and may promote differentiation into macrophages. Monocytes activated by Ephrin-B2 have an increased expression of proinflammatory cytokines. This interaction with monocytes is believed to be associated with arteriosclerosis as endothelial cells in arteriosclerosis sites have upregulated levels of Ephrin-B2.⁷⁴

Veins

COUP-TFII is a transcription factor that plays a critical role in the formation of venous identity by inducing the expression of Eph-B4. COUP-TFII also suppresses arterial markers by directly inhibiting the notch/Dll4 signaling pathway to suppress Ephrin-B2 expression. Of note, COUP-TFII is likely controlled by many factors but one known factor is brahma-related gene 1 (BRG1). BRG1 will bind the promoter region of COUP-TFII inducing DNA remodeling and transcription of the COUP-TFII gene.

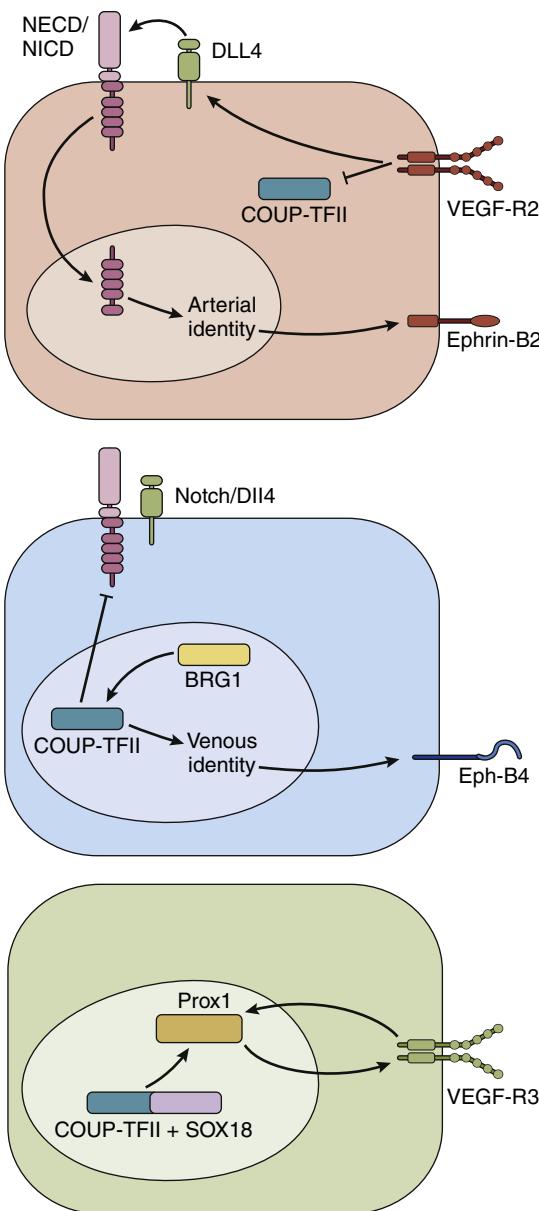


Figure 3.6 Signaling Pathways of Arterial (Ephrin-B2), Venous (Eph-B4) and Lymphatic (Prox1) Endothelial Cells. These pathways are important for development and in adult cells.

Eph-B4 is of particular interest in adult veins in the setting of vein grafts. Maturation of vein grafts involves wall thickening to adapt to the arterial environment; this wall thickening is dependent on the loss of Eph-B4 within the vein graft. Appropriate maturation as well as pathologic wall thickening, which may cause vein graft failure, may also be dependent on Eph-B4 expression.⁷⁵

Lymphatics

Lymphatics sprout from the venous system. Prospero-related homeobox transcription factor (Prox1) is essential to the formation of lymphatics; in the absence of Prox1 there is a complete absence of lymphatic endothelial cells.⁷⁶ Prox1 is regulated by several other proteins which include COUP-TFII, Sox18 and

VEGF-R3, COUP-TFII and Sox18 are required to activate the expression of Prox1. VEGF-R3 has a feedback loop with Prox1 to maintain and regulate lymphatic cell fate.

HEMODYNAMICS AND VASCULAR WALL BIOLOGY

Blood vessels constantly experience the mechanical forces of cyclic strain and shear stress. Blood pressure is the primary determinant of cyclic strain as radial and tangential forces in the vessel wall work to counteract the intraluminal pressure. Hemodynamic forces have long been viewed as important modulators of protein synthesis, cell morphology, migration, differentiation, and proliferation. Shear stress results from the flow of blood against the vessel wall and this frictional force acts parallel to the vessel surface. Cyclic strain affects all cell types in the vessel wall, whereas shear stress principally exerts its frictional forces on EC at the interface of the blood and vessel wall (Fig. 3.7).

Shear Stress

Normal hemodynamics have both pulsatile pressure and flow, which includes changes in blood pressure and velocity throughout the duration of the cardiac cycle. Pulsatile flow induces both long- and short-term reactions, which vary among different regions of the vasculature. Mean physiologic shear stress on the vascular endoluminal surface ranges from 10 to 15 dyne/cm² throughout the arterial network. During changes in stress, the vessel wall compensates to restore the vessel to basal levels of tensile and shear stress. Acute changes in stretch and shear stress induce transient changes, including the release of vasoactive agents and alterations in vessel diameter. Chronic changes in mechanical forces induce vascular remodeling that lead to significant adaptive alterations in vessel wall shape and composition.

Shear stress is a determinant in the release of relaxing factors. Long-term increases in shear stress enhance the L-arginine/nitric oxide (NO) pathway in EC. Marked increases in NO synthase mRNA and cyclic guanosine monophosphate occur with chronic increases in shear stress and, with matrix metalloproteinases (MMP), can lead to apoptosis, enlargement, and vascular remodeling. Increased flow through an isolated artery induces the release of endothelium-derived hyperpolarizing factor, prostaglandin I₂ (PGI₂), and NO, which suggests that flow-induced dilation depends on the release of endothelium-derived factors.^{2,77–79} Long-term exposure to increased shear stress upregulates the expression of type III NO synthase in cultured EC. NO and superoxide anions help activate MMP. Increased shear stress stimulates the production of MMP-2 and MMP-9 in the arterial wall, inducing matrix degradation, tears in the IEL, and increases in arterial diameter and distensibility (Fig. 3.5, red arrow). MMP-induced remodeling will continue until arterial caliber increases to such a point that wall shear stress is normalized. In contrast, restriction of blood flow, and thus a decrease in shear stress, causes a significant delay in the

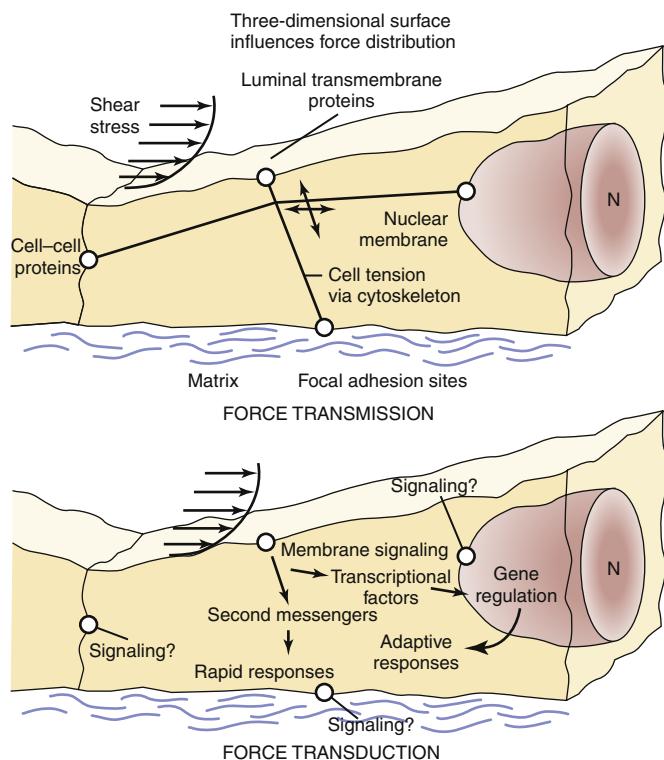


Figure 3.7 Transmission of Mechanical Force to Arterial Wall Surfaces. Extracellular fluid mechanical forces acting on the luminal surface are transmitted by the cytoskeleton to remote intracellular locations, such as cell–cell junctions, focal adhesion sites, and the nucleus (N). (Redrawn from Helmke B, Davie P. Cytoskeleton under external fluid mechanical forces: hemodynamic forces acting on the endothelium. *Ann Biomed Eng*. 2002;30:284–296.)

growth of the carotid artery, a 25% reduction in diameter in the carotid artery, and a decrease in elastin content in young rats.⁸⁰

Endothelium

Shear stress primarily affects EC, although long-term changes in shear stress encourage vascular remodeling throughout the arterial wall. EC align in the direction of stress, and the greater the shear stress, the more elongated the cells.^{81,82} Such changes are correlated with a redistribution of intracellular stress fibers and fiber quantity. In areas of high shear stress, EC express higher amounts of stress fibers, including actin, myosin, and other contractile proteins. Although arterial levels of shear stress induce significant changes in stress fiber expression, venous levels of shear stress induce no change.

It is believed that transient changes in laminar shear stress do not permanently alter protein levels, although they may lead to the reorganization of cytoskeletal proteins and changes in cell shape. Shear stress regulates expression and activity of genes encoding for many growth factors, vasodilators, vasoconstrictors, and adhesion molecules. Cyclic strain interacts with shear stress, in that the strain reduces EC sensitivity to shear stress. *In vivo*, however, it is difficult to distinguish between shear stress and cyclic strain-induced changes in endothelium function.

Mechanotransduction begins on the luminal side of the EC, causing deformation at the level of individual cells. This

deformation is transduced through the cytoskeleton to other points on the cell, including basal adhesion points where the cells are attached to the ECM, cell junctions, and the nuclear membrane. The redistribution of forces stimulates immediate and delayed processes. The cell membrane responds by activation of stretch-induced ion channels, phospholipids, and integrins.⁸³⁻⁸⁴ The nuclei upregulate the production of platelet-derived growth factors α and β , tissue plasminogen activator (tPA), TGF β , endothelin-1, nitric oxide synthase III, and ICAM-1.^{85,86} Increased shear stress upregulates ICAM-1 and vascular cellular adhesion molecule-1 (VCAM-1), and oscillatory stress decreases levels of both molecules.

Shear stress functions as an important regulator of arterial diameter via the release of vasoactive substances by the endothelium. Though EC do not synthesize arachidonic acid, it is a crucial precursor to PGI₂, a vasodilator and platelet inhibitor released by EC. The percentage of arachidonic acid incorporated into diacylglycerol and phosphatidylinositol is significantly increased in EC exposed to arterial levels of shear stress. Shear stress-induced PGI₂ release is significantly dependent on the PLC/diacylglycerol lipase pathway for arachidonic acid supply.⁸⁷⁻⁸⁹

Circumferential Stretch

Cyclic or circumferential stress represents the constant, rhythmic deformation of the vascular wall caused by pulsatile, hydrostatic pressures linked to the oscillations of systole and diastole. Both EC and underlying vascular SMC are subjected to such forces and are susceptible to altered phenotype and changes in morphology.

SMC, like EC, when subjected to cyclic strain, change their orientation. They uniformly align perpendicular to the direction of the strain vector in a circular fashion. When exposed to cyclic strain, SMC increase proliferation but also fail to align. SMC hypertrophy in major arterial trunks tends to develop only when the distending pressure reaches a threshold level.² Cyclic stretch at low intraluminal pressure demonstrates decreased levels of smooth muscle marker proteins h-caldesmon and filamin; physiologic intraluminal pressure, 80 mm Hg, maintains these levels, and further cyclic stretching of cultured SMC increases expression of smooth muscle myosin heavy chains and myosin light chain kinase, augments smooth muscle myosin heavy chain SM-1 and SM-2 protein content, and decreases nonmuscle myosin NM-A.⁹⁰ It is believed that certain levels of stretch are needed to maintain SMC in a quiescent state and that excessive stretching triggers an increase in protein synthesis and hypertrophy.

In summary, increases in circumferential stress induce increases in SMC hypertrophy and in collagen and elastin production. Conversely, decreased circumferential stress causes the wall to atrophy.⁹¹ As the diameter of a blood vessel increases, the number of lamellar units and the overall wall thickness

increase in order to maintain circumferential stress. In elastic large arteries, the adaptive response normalizes tensile stress. Sustained hypertension is associated with an ongoing response in large arteries and arterioles, including increased medial wall thickness, changes in wall composition, altered arterial function, and increased SMC size and number.⁹²

Hemodynamic Effects on Vessel Identity

Hemodynamic factors play an important role in vessel identity, both in development and in adult vessels. Prior to the first heartbeat arterial and venous markers appear on EC but full differentiation is dependent on these markers in addition to hemodynamic forces.^{68,93,94} In embryos, if arterial blood flow is ligated there is a loss of expression of arterial markers, such as Ephrin-B2, but if arterial blood flow is restored these arterial markers return.⁶⁸ This plasticity in vessel identity is also seen in adult vessels of mice, rats and humans. When a vein graft is placed into the arterial environment the endothelial cells lose the expression of Eph-B4 but do not gain expression of Ephrin-B2; that is, venous identity is lost but there is no gain in arterial identity.^{75,95,96} Interestingly, after arteriovenous fistula creation, the vein wall distal to the fistula gains dual identity with increased expression of both Eph-B4 and Ephrin-B2,⁹⁷ suggesting that shear stress and circumferential stretch likely play a critical role in determining loss and gain of vessel identity to adapt to each environment.

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Atherosclerosis

KAREN J. HO

Based on a previous edition chapter by Christopher D. Owens and Karen J. Ho

ATHEROSCLEROTIC LESIONS 41

THEORIES OF PATHOGENESIS 42

Lipid Hypothesis 42

Response-to-Injury Hypothesis 43

Monoclonal Hypothesis 43

ATHEROSCLEROSIS AS A CHRONIC INFLAMMATORY DISEASE 44

Low-Density Lipoprotein Retention 44

Monocytes 44

Macrophages 45

Lymphocytes and Adaptive Immunity 45

Smooth Muscle Cells 46

Calcification 46

Putting It All Together: The Inflammasome 46

C-Reactive Protein 46

LOCALIZATION OF ATHEROSCLEROSIS 47

PROGRESSION/REGRESSION OF PLAQUES 47

THROMBOTIC COMPLICATIONS OF ATHEROSCLEROSIS 48

IDENTIFICATION OF VULNERABLE LESIONS 49

Vascular surgeons care for a diverse set of clinical manifestations related to atherosclerosis, from transient cerebral ischemic attacks, strokes, aortoiliac occlusions, aortic aneurysms, mesenteric ischemia, to lower extremity arterial occlusive disease. However, despite the wide range of manifestations, culprit lesions are more alike than different. Accumulation of large amounts of cholesterol ester in the arterial wall and formation of complex advanced plaque are common to all lesions. Although the formation of atherosclerotic lesions is insidious and spans decades, the lesions can reach a clinical dénouement within minutes and manifest as catastrophic myocardial infarction (MI), stroke, or limb ischemia. Atherosclerosis-related cardiovascular disease (CVD) is the leading cause of death and disability globally: ischemic heart disease and stroke are the world's first and third causes of death, representing 84.5% of cardiovascular deaths and 28.2% of all-cause mortality.¹ However, age-standardized CVD mortality rates have decreased globally in the last two decades, and improved survival has translated into growing disease prevalence and a staggering financial burden.² It is estimated that 121.5 million Americans have CVD, with an estimated annual cost of treatment exceeding \$350 billion.³ Globally, 202 million people have peripheral arterial disease (PAD), which disproportionately affects individuals living in low- to middle-income countries.^{1,3} Particularly troubling is the high prevalence of CVD risk factors in children and young adults.^{4,5} A sedentary lifestyle, abdominal

obesity, and poor diet contribute to dyslipidemia and high blood pressure. Autopsy studies in children and young adults demonstrate a link between these risk factors and early lesions.⁴

This chapter outlines existing theories about the pathophysiology of atherosclerosis and the relationship to traditional and emerging risk factors. It also describes our current conceptual understanding of the fundamental biology of atherosclerotic plaque.

ATHEROSCLEROTIC LESIONS

Lesions of the arterial wall have been divided into eight types (type I to type VIII) based on their histopathologic features.^{6–8} However, while the classification system is useful for comparing pathologic specimens, it has limited clinical practicality.

Autopsy studies from the Bogalusa Heart Study and the Pathobiological Determinants of Atherosclerosis in Youth study demonstrate that atherosclerotic lesions form in early childhood and increase with age.^{4,9–11} In an intravascular ultrasound (IVUS) study of heart donors, 17% of individuals younger than 20 years had evidence of atherosclerosis.¹² Notably, both the Bogalusa Heart Study and the Pathobiological Determinants of Atherosclerosis in Youth study highlight that the number and severity of early lesions are directly related to known CVD risk factors, thus suggesting that the presence of traditional CVD risk factors (such as familial

hypercholesterolemia, hypertension, severe obesity, type 2 diabetes mellitus) and certain medical conditions (such as type 1 diabetes mellitus, chronic kidney disease, chronic inflammatory conditions, and underlying structural or functional heart disease) in childhood warrant early risk stratification, close surveillance, preferential use of pharmacotherapy to reduce CVD risk in selected individuals, and heart-healthy therapeutic life-style behaviors.

Diffuse intimal thickening has been identified in the atherosclerotic-prone areas of coronary arteries as early as 36 weeks of gestation.¹³ Although the fatty streak – dominated by lipid-filled macrophages – is itself benign, it is the precursor of the more clinically relevant late lesion. The fatty streak is the first lesion visible to the naked eye. Its yellow color is attributed to lipid in the form of cholesterol and cholesterol esters within macrophages and smooth muscle cells (SMC).

Advanced lesions, or fibrous plaque, are characterized histologically by the amount of extracellular lipid and fibrous connective tissue. They are whitish in gross appearance and are elevated so that they protrude into the lumen. These fibroatheromas are prone to provoking clinical sequelae by erosion of the surface endothelial cells, rupture of the fibrous cap, erosion of a calcium nodule, or intraplaque hemorrhage (Fig. 4.1).^{6,14} Plaques differ in consistency and may be relatively soft and friable or densely sclerotic and calcific. Likewise, some have well-formed fibrous caps, whereas others are covered by a narrow zone of loose connective tissue or by endothelium alone.

The necrotic core usually occupies the deeper central regions of the plaque and contains amorphous lipid and cholesterol crystals. The term *atheroma* is derived from the Greek word *ather*, meaning “porridge-like gruel.” SMC and inflammatory cells are located adjacent to the necrotic core and at the shoulders of the plaque, where it is most susceptible to rupture.

The fibrous caps contain varied levels of SMC adjacent to the collagen and basement membrane. These cells have reduced proliferative ability and may be regarded as senescent. However, the fibrous cap of ruptured plaque is often infiltrated with foam cells, which are largely of macrophage origin¹⁵ and thus indicative of active inflammation and vulnerability to rupture. A priori identification of these so-called thin-cap fibroatheromas is an active area of cardiovascular imaging research.¹⁶

THEORIES OF PATHOGENESIS

Each of the well-regarded theories of atherosclerosis discussed in this section attempts to explain the underlying pathogenesis of atherosclerotic plaque. Each theory has undergone a steady evolution since the time that it was initially proposed, a consequence of advancements in our scientific inquiry from histologic descriptions to discernment of powerful molecular mechanisms.

Lipid Hypothesis

Cholesterol has been one of the most studied molecules in biomedical research. The modern era of cholesterol research

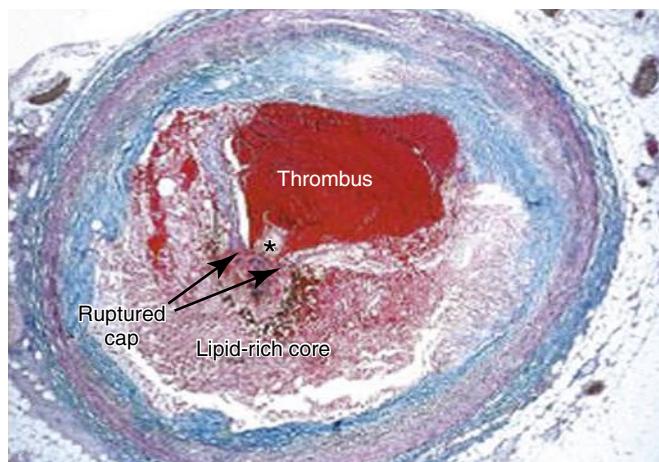


Figure 4.1 Thrombotic Complication of a Fibroatheroma Seen on a Trichrome-Stained Cross-Section of a Human Coronary Artery. Thrombus and intraplaque hemorrhage stain red; collagen stains blue. The fibrous cap has ruptured (*area between the arrows), and the highly thrombotic lipid core is exposed to circulating blood with the subsequent production of acute occlusive thrombosis.

began in St. Petersburg, Russia, at the turn of the 20th century, when Nikolaj Anitschkow produced vascular lesions in rabbits by feeding them purified cholesterol dissolved in sunflower oil.^{17,18} These lesions closely resembled those seen in human atherosclerosis. He hypothesized that they were caused by elevated serum cholesterol and also noted a distinctive pattern consisting of the location of the lesions near arterial branch points and concluded that this was probably determined by hemodynamic factors. Nevertheless, this work was criticized because the levels of cholesterol produced were too high and could not be experimentally reproduced in more conventional animal models such as rats and dogs.^{17,18}

Cholesterol is insoluble in water, and the early work done in Russia provided no clues as to how it was transported to the arterial wall and formed plaques. The discovery that cholesterol was associated with proteins that allowed it to be transported in the aqueous environment led to investigations into lipoproteins. The first investigation in which the lipoprotein content in whole serum was accurately quantified involved analytic ultracentrifugation and was led by John Gofman of the Donner Laboratory at University of California at Berkeley.¹⁹ Lipoprotein fractions were isolated and characterized by their densities and flotation characteristics.¹⁹ Of note, this group noted that it was not simply the total cholesterol that was important but the species of lipoprotein contained within the cholesterol.²⁰

Cholesterol is transported in the aqueous environment by esterification of the sterol of long-chain fatty acids and packaging of these esters with the hydrophobic cores of plasma lipoproteins. With its polar hydroxyl group esterified, cholesterol remains sequestered within this core, which is essentially an oil droplet composed of cholesteryl esters and triglycerides, solubilized by a surface monolayer of phospholipid and unesterified cholesterol and stabilized by protein. In persons who are fasting, lipids circulate in plasma as lipoprotein particles that are defined on the basis of their density as very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein

(HDL). The protein of the VLDL, IDL, and LDL molecule is apoB-100, which has β -electrophoretic mobility.

The major milestone in the lipoprotein field was the discovery of the defective gene associated with familial hypercholesterolemia by Joseph L. Goldstein and Michael S. Brown at The University of Texas Southwestern. In fibroblasts cultured from normal human subjects, the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-controlling enzyme in cholesterol biosynthesis, is regulated by the content of LDL (but not HDL) in the culture medium. Goldstein and Brown found that HMG-CoA was not sensitive to normal feedback regulation by LDL in cultured skin fibroblasts from homozygous familial hypercholesterolemia patients.^{21,22} They then determined that the defect was related to deficient binding of LDL to the cells of patients with familial hypercholesterolemia and that suppression of HMG-CoA activity was related to the amount of bound LDL.²³ Therefore, these patients had overproduction of cholesterol because they lacked the appropriate receptor. They demonstrated that cellular uptake of LDL absolutely requires the LDL receptor, without which the LDL cholesterol concentration builds up to 800 to 1000 mg/dL (20.7–25.9 mmol/L). This important work culminated in a Nobel Prize in Physiology or Medicine for Goldstein and Brown in 1985.

Epidemiologic evidence from numerous studies, including the Japanese migration studies^{24,25} and the Framingham Heart Study,²⁶ demonstrated the association between cholesterol and incident CVD. Other studies showed that diets rich in unsaturated fats resulted in lowered cholesterol and reduced cardiovascular events.²⁷

Widespread acceptance of the causative role of cholesterol began with publication of the Coronary Primary Prevention Trial, sponsored by the National Institutes of Health.^{28,29} This trial demonstrated that lowering cholesterol with the bile acid-binding resin cholestyramine reduced cardiovascular events. A decade later, this trial was reinforced with powerful data that emerged from the statin era.³⁰ Although the primacy of the “cholesterol hypothesis” has been called into question, there is no doubt that lipids play a critical role in the pathogenesis of atherosclerosis. Indeed, recent clinical evidence with the PCSK9 inhibitors (inducing marked reduction of LDL) have suggested a significant benefit in patients with PAD with a 42% reduction in major adverse limb events over 2 years.³¹

Response-to-Injury Hypothesis

The basis of this hypothesis builds on earlier work by Jack Duguid,³² John Poole and Howard Florey,³³ and John French³⁴ and was formally advanced in 1973 by Russell Ross and John Glomset,³⁵ who emphasized the importance of injury to the endothelium as a seminal event in the development of atherosclerosis lesions. Even though the definition of the term *injury* has been modified over the last three decades,³⁶ all response-to-injury hypotheses have emphasized the primacy of the endothelium in stimulating the cascade of events leading to lesion formation.^{35–40}

Endothelial injury may result from mechanical disruption, exposure to toxic or infectious agents, or endogenous inflammatory signals. Injury to the endothelium allows adhesion of platelets and an influx of LDL and other serum factors into the subendothelial space. Platelets release their alpha granules and stimulate migration of SMC into the intima, where they proliferate and form a thickened neointima responsible for narrowing of the arterial lumen. Restoration of a healthy endothelial cell layer abates the process. During work on this hypothesis, Ross et al. performed experiments leading to the discovery of platelet-derived growth factor (PDGF).^{41,42}

Other investigators have countered that it is not injury to the endothelium that is the initiating event but rather retention of inflammatory lipids in the subendothelial space that renders a particular area susceptible to atherosclerosis. The observation that LDL accumulates in the intima within two hours after a bolus infusion⁴³ and that the accumulation occurs before the formation of fatty streaks^{44,45} led to the conclusion that retention of LDL is the initiating event of atherosclerosis. The so-called “response-to-retention” theory purports that retained apoB-containing lipoproteins stimulate a macrophage- and T cell-dominated inflammatory response in the arterial wall.^{46,47}

Monoclonal Hypothesis

This hypothesis suggests that each lesion of atherosclerosis is derived from a single SMC that serves as a precursor for the clonal expansion of proliferating SMC.⁴⁸ The hypothesis put forth by Earl and John Benditt used the concept that in every female cell there is only one active X chromosome and the progeny of that cell will express the same X chromosome as the parent cell. Glucose-6-phosphate dehydrogenase (G6PD) has two isoforms that can be separated by electrophoresis. Its gene is located on the X chromosome and can therefore be used to identify the progeny of a parent cell. This approach was used to determine that uterine leiomyomas are composed of cells with the same active X chromosome, whereas adjacent normal myometrium is composed of a mixture of cells containing both G6PD isoforms, implying that both X chromosomes are active.⁴⁹

The Benditts examined a series of atherosclerotic plaques from four black females and compared them with adjacent normal areas of arterial wall. They determined that SMC from the plaque contained only one G6PD isoform, whereas adjacent control areas contained a mixture of isoforms. This allowed them to conclude that each lesion is a clonal outgrowth derived from a single precursor SMC located in the intima. It is noteworthy that SMC within individual neonatal intimal thickenings are monoclonal in origin, whereas cells from the subjacent media are polyclonal.

Others challenged the hypothesis by noting that identification of a single enzyme phenotype does not necessarily imply clonal origin.^{50,51} Regardless, the work was importantly heuristic and hinted toward the role that modern molecular biology would play in unraveling the genetic basis of atherosclerosis.

ATHEROSCLEROSIS AS A CHRONIC INFLAMMATORY DISEASE

The original version of the response-to-injury hypothesis of atherosclerosis proposed that endothelial denudation was the first step in atherosclerosis.³⁵ Subsequent versions of the hypothesis proposed that an endothelium chronically bathed in serum with high concentrations of LDL or exposed to other cardiovascular risk factors would render susceptible areas of the endothelium dysfunctional or activated.³⁹ Indeed, an intact endothelium may be a necessary factor for lesion progression, and it is now clear that developing atheromas are covered by an intact endothelium throughout most stages of lesion progression.^{7,36,52–54} In humans, only the most advanced ulcerated lesions are focally devoid of endothelium. The injury results in increased adhesiveness and an increase in permeability of the endothelium to inflammatory cells (Fig. 4.2).

Atherosclerosis is now recognized as an inflammatory disease, and components of the innate and adaptive immune system are involved in every step of the atherosclerotic process. Much of our modern understanding comes from examination of human pathology specimens and transgenic animals. Genetic deletion of apolipoprotein E (*ApoE*^{-/-}) or the LDL receptor (*Ldlr*^{-/-}), which produce mice with severe hypercholesterolemia and atherosclerotic lesions with features of mature human atheroma, have become cornerstones in atherosclerosis research laboratories.^{55–57} The importance of the LDL receptor in cholesterol regulation was noted by Goldstein and Brown when studying patients with familial hypercholesterolemia. Apolipoprotein E suppresses atherosclerosis, and *ApoE*^{-/-} mice have very low levels of pre- β HDL and their plasma is poor at promoting the efflux of cholesterol from lipid-laden macrophages.⁵⁸ Cross-breeding these mice with other strains carrying null mutations in immunologically relevant genes produces a robust research tool to dissect out the contribution of individual components of immune pathways in atherosclerosis. For example, some of the earliest approaches using compound mutant mice involved the global loss of the entire adaptive immune system. *Rag1*^{-/-} and *Rag2*^{-/-} mice lack the V(D)J recombinase required to form lymphocyte antigen receptor genes and hence have a complete loss of B and T cells. *ApoE*^{-/-} mice fed a regular chow diet developed plasma cholesterol levels between 390 and 470 mg/dL. Double knockout *ApoE*^{-/-}/*Rag1*^{-/-} mice had a 40% reduction in aortic atherosclerotic lesions compared with immunocompetent animals, thus demonstrating the role cellular immunity plays in the pathogenesis of atherosclerosis.⁵⁹

Low-Density Lipoprotein Retention

Trafficking of circulating LDL into and out of the subendothelial space is probably a function of concentration gradients and endothelial permeability.^{60,61} Normal intima does not retain LDL particles, thus suggesting that most particles return to plasma or are degraded in situ. However, subendothelial retention of apoB-100-containing lipoproteins is an early event in atherosclerosis.⁶¹ Factors favoring net retention include a balance of uptake and degradation of LDL by macrophages,

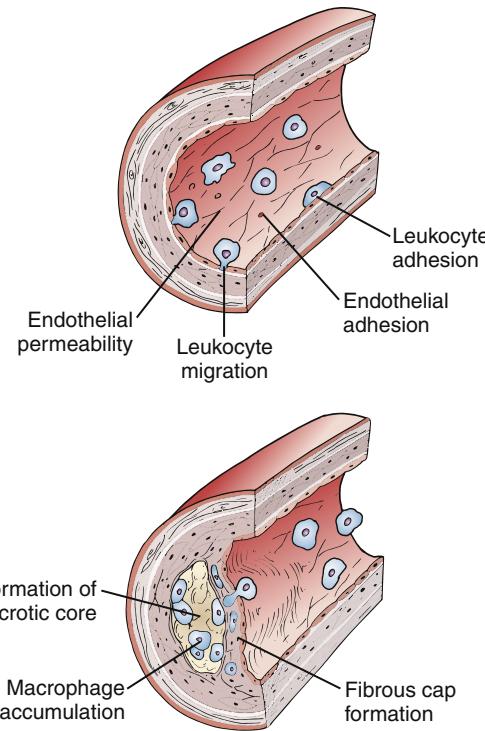


Figure 4.2 Initiation and Progression of Atherosclerotic Plaque. Cardiovascular risk factors, hemodynamic forces, toxins, and infectious agents interact with the vessel at the level of the endothelium to produce injury, resulting in decreased nitric oxide production and increased permeability. Once injured, the endothelium increases the expression of leukocyte adhesion molecules, which increases the adherence of macrophages and other leukocytes. Permeability of the endothelium also permits entry of leukocytes and lipoproteins into the subendothelial space. Chemokines and cytokines such as monocyte chemotactic protein-1 and interleukin-8 further enhance the recruitment of leukocytes and smooth muscle cells (SMC) into the subendothelial space. Lipoproteins retained in the subendothelial space are biochemically modified such that they can be taken up by macrophages and SMC to form foam cells. Foam cells at the central-most position of the developing atheroma become necrotic and form the central lipid core, whereas the shoulder regions contain SMC, macrophages, and other leukocytes. Platelet-derived growth factor and transforming growth factor- β stimulate SMC migration and collagen formation in the subendothelial space, as well as formation of the fibrous cap.

egress of LDL-containing macrophages into the circulation, and complex interaction with proteoglycans such that the LDL particles are sufficiently modified to maintain the gradient. Once bound to the matrix, the lipoprotein is modified so that it becomes oxidized.^{60,62} Oxidized LDL, unlike native LDL, is chemotactic to monocytes and rapidly taken up by macrophages to form foam cells by scavenger receptors.

Monocytes

As early as 1958, Poole and Florey noted that macrophages adhere to the surface of endothelial cells overlying atheroma.³³ In studies of hypercholesterolemic monkeys, monocytes are seen attached to the endothelium within 12 days of initiation of an atherogenic diet.⁶³ It is now apparent that monocytes play a very early role in atheroma formation.^{64,65} Endothelium, which is rendered activated, expresses adhesion molecules that interact with circulating leukocytes, principally monocytes

and lymphocytes. E- and P-selectins slow monocytes, mediate rolling, and loosely tether them to the endothelium.⁶⁶ More permanent fixation is due to members of the immunoglobulin superfamily (vascular cell adhesion molecule-1 [VCAM-1]⁶⁷ and intercellular adhesion molecule-1 [ICAM]), which are upregulated and firmly fix the leukocytes to the wall. The importance of VCAM-1, and ICAM-1 in the pathogenesis of atherosclerosis is evident in either ICAM-1 or VCAM-1 mutant mice exhibiting far less atherosclerosis than wild type counterparts.^{68–70} The adherent leukocytes are stimulated to migrate into the subendothelial space by a number of chemokines, including monocyte chemoattractant protein-1 and oxidized LDL.^{71–73}

Macrophages

Once within the subendothelial space, monocytes undergo a series of phenotypic modulations and become resident tissue macrophages that take up oxidized LDL via the scavenger receptor A (SR-A), as well as CD36.⁷⁴ One of the key signals for macrophage activation, macrophage colony-stimulating factor, can enhance scavenger receptor expression and promote replication of macrophages and their production of proinflammatory cytokines. Experiments in mutant mice deficient in macrophage colony-stimulating factor have shown the importance of this factor in atheroma formation.^{75,76} Although most foam cells are macrophage in origin, SMC also take up lipids via scavenger receptors and CD36.

In mice, proinflammatory M1 macrophages, induced by hyperlipidemia, produce the inflammatory cytokines IL-1 β and TNF- α . The IL-1 gene family encodes three major proteins. The first two, IL-1 α and IL-1 β , exert proinflammatory effects by binding to the IL-1 receptor type 1. The third is IL-1 receptor antagonist (IL-1Ra), an endogenous inhibitor that competitively blocks the binding of IL-1 α and IL-1 β to the IL-1 receptor. Direct evidence for the proatherogenic role of IL-1 was obtained in experiments in which *ApoE*^{-/-} mice received subcutaneous injection of recombinant IL-1 receptor antagonist. Mice injected with IL-1 receptor antagonist displayed a marked reduction in atherosclerotic lesion size. Similarly IL-18, previously called interferon- γ (IFN- γ)-inducing factor, is a Th1-promoting cytokine and therefore has the capacity to promote inflammation through the innate and the adaptive immune pathways.^{77,78} Knockout of IL-18 in atherosclerosis-prone animals reduces aortic atherosclerotic lesion size.

Macrophages also contribute to thrombosis in several pivotal ways. As discussed later, ruptured atherosclerotic lesions characteristically contain abundant macrophages underlying a thin and collagen-poor fibrous cap. Matrix metalloproteinase (MMP)-1, MMP-8, and MMP-13 specifically co-localize with macrophages in human atheroma. Rupture of the fibrous cap may be a balance between collagen synthesis by SMC and collagen breakdown by matrix metalloproteinases generated by activated macrophages. Studies in collagenase-resistant mutant “knock-in” mice crossbred with *ApoE*^{-/-} mice demonstrated increased intimal collagen and SMC content.⁷⁹ Similarly, compound mutant *ApoE*^{-/-} mice crossed with MMP-13/

collagenase-3^{-/-} mice have increased fibrillar collagen that is thicker and more aligned in aortic atherosclerosis.⁸⁰ Hence, interstitial collagenases produced by activated macrophages greatly influence the structure and integrity of the fibrous cap overlying atherosclerotic plaques. Macrophages and SMC within atherosclerotic plaques also overexpress the potent procoagulant tissue factor in response to C-reactive protein or CD40 ligand. Hence, inflammation links atherosclerosis and thrombosis, leading some to refer to it as “atherothrombosis.”

Lymphocytes and Adaptive Immunity

The finding in the early 1980s that macrophages expressed the major histocompatibility class II antigens needed for antigen presentation to CD4 $^+$ T cells suggested that adaptive immunity was involved in the atherosclerotic process.⁸¹ T lymphocytes, which account for as much as 10% to 20% of the leukocyte population, are found most abundantly in the shoulder and fibrous cap region of the atheroma. In advanced lesions, these T cells display markers of chronic activation and produce the prototypical Th1 cytokine, IFN- γ , which further stimulates expression of class II major histocompatibility antigens in SMC and macrophages. The crossbreeding of severe combined immunodeficiency (SCID) mice that lack T and B cells with *ApoE*^{-/-} mice produces offspring that are both hypercholesterolemic and immunodeficient. When lesions in these mice were compared with those in the immunocompetent *ApoE*^{-/-} animals, a dramatic 70% reduction of lesion size was observed. However, transfer of oxidized LDL-reactive T cells to *ApoE*^{-/-}/SCID mice is more efficient at lesion acceleration than the transfer of T cells with no specificity to a plaque-derived antigen, demonstrating the importance of antigen presentation in the pathogenesis of atherosclerosis.⁸²

Because of the participation of all components of the innate and adaptive immune system, atherosclerosis resembles other inflammatory and autoimmune diseases such as rheumatoid arthritis and type 1 diabetes mellitus. Potential endogenous autoantigens that activate T cells include LDL or heat shock protein 60.^{82,83} Cellular and humoral immune responses are mounted toward these antigens in humans and mice, and protective immunization strategies in mice have provided encouraging results.

In mice, functionally distinct T cell subsets appear to exist in atheroma. Natural killer T cells appear to accelerate atherosclerosis when recognizing lipid antigens presented through CD1 molecules.⁸⁴ Conversely, regulatory T cells may suppress Th1 effector cells through production of anti-inflammatory cytokines such as IL-10 and transforming growth factor- β (TGF- β).⁸⁵ T cells, although far fewer in number than macrophages, likely serve as key regulators of the concerted immune response.

Mast cells are located at the shoulder and more central area of the cap, where they may participate in rupture or erosion of the cap.⁸⁶ Once recruited to the subendothelial space in the intima, they can perpetuate and amplify the ongoing inflammatory response that led to their recruitment.⁸⁷ The CD40 receptor and CD40 ligand are expressed by several inflammatory cells, including macrophages and B and T lymphocytes.⁸⁸ It

is thought that this system contributes to leukocyte adhesion, matrix degradation, and cytokine-induced inflammation. Interruption of the CD40 signaling pathway reduces progression of atherosclerosis in experimental models.

Smooth Muscle Cells

Proinflammatory mediators can stimulate the migration of SMC from the tunica media into the intima. Growth factors produced locally provide a paracrine stimulus for SMC proliferation and activation. Activated SMC appear capable of producing growth factors (e.g., PDGF, fibroblast growth factors) that can stimulate their own proliferation and that of their neighbors in an autocrine and paracrine fashion.⁸⁹ TGF- β stimulates the production of matrix and collagen in the subendothelial space. As the plaque matures, necrotic foam cells contribute to the central lipid core, whereas collagen contributes to the overlying fibrous cap of a mature fibroatheroma.

Calcification

Bone morphogenetic protein-2 (BMP-2), a member of the TGF- β family, and inorganic phosphate induce the osteochondrogenic phenotype in SMC.⁹⁰ BMP-2, in turn, is produced by endothelial cells exposed to hypoxia, reactive oxygen species, turbulent flow, high pressure, or inflammation.⁹⁰ Atherosclerotic calcification proceeds through a process similar to chondrogenesis, whereby cartilaginous metaplasia precedes osteoblast induction. This is distinct from medial artery calcification, which proceeds through a process similar to intramembranous bone formation. The latter is common in patients with diabetes or chronic kidney disease. The reason that some plaques undergo calcific changes whereas others do not is not clear.

Thus, the inflammatory reaction stimulated by modified LDL and white blood cells in the subendothelial space provides a nidus for the subsequent events leading to the formation of mature fibrocalcific atherosclerotic plaque. Notably, this process, which probably begins in childhood, is silent until sufficient arterial stenosis exists or until a catastrophic plaque destabilizing event occurs and produces a clinical symptom.

Putting It All Together: The Inflammasome

As discussed earlier, IL-1 β has several layers of regulation and is synthesized as pro-IL-1 β and requires activated caspase-1 to cleave pro-IL-1 β and IL-18 into their active and secreted form. Caspase-1 activation requires a second signal mediated through a complex of intracellular proteins known as inflammasomes. The nucleotide-binding domain leucine-rich (NLR) family pyrin domain-containing 3 (NLRP3) inflammasome has been most extensively studied. When NLRP3 receptors are activated, they oligomerize and recruit caspase-1 through the adapter protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and autocatalytically activate caspase-1, which cleaves pro-IL-1 β into its mature forms.⁹¹ NLRP3 inflammasome activation historically

included pore-forming toxins, extracellular adenosine triphosphate, viral DNA, and gout-associated uric acid crystals. However, recent evidence demonstrates that cholesterol crystals can also activate the inflammasome and therefore increase secretion of the potent proinflammatory cytokines IL-1 β and IL-18. These data argue that cholesterol crystals are not solely an inert byproduct within the wall of the artery but an active contributor to atherosclerosis.^{92,93}

Moreover, hypercholesterolemic *Ldlr*^{-/-} mice reconstituted with bone marrow from mice deficient in NLRP3, ASC, or IL-1 β have significantly reduced aortic lesion sizes compared with those reconstituted with wild-type bone marrow.⁹² These mice showed significantly lowered levels of IL-1 β and IL-18. The implication of caspase-1 was evaluated recently in atherosclerosis as a pathogenic enzyme because this deficiency decreases atherosclerosis in apoE-deficient mice.⁹⁴ Hence, within the vascular wall, lipids and inflammation appear to be inextricably linked. Indeed, the independent role of IL-1 β mediated actions on atherosclerosis progression has been confirmed in humans in the CANTOS trial, whereby anti-IL-1 β monoclonal antibody therapy (canakinumab) was associated with a significant reduction in morbid cardiovascular outcomes.⁹⁵

C-Reactive Protein

Many prospective studies have established an association between biomarkers of inflammation and first and recurrent cardiovascular events. For example, studies have noted that the circulating soluble ICAM-1 and VCAM-1 are elevated in the plasma of patients before the development of peripheral arterial disease.⁹⁶ Similarly, the inflammatory cytokines IL-6 and IL-1 β , as well as acute-phase reactants C-reactive protein (CRP), fibrinogen, and serum amyloid A, have all been associated with peripheral arterial disease and its progression.^{97,98} Among the many inflammatory biomarkers evaluated, high-sensitivity C-reactive protein (hsCRP) has emerged as the leading biomarker for clinical application. This is because CRP is a very stable analyte over time, has a relatively long half-life, has no diurnal variation, and requires no special processing for sampling.⁹⁹ In addition, very low (<0.5 mg/L) and very high (>10 mg/L) values of hsCRP both provide important prognostic information on cardiovascular risk.^{100,101}

Many studies have confirmed the use of hsCRP as a useful biomarker. For example, in 28,000 apparently healthy women, hsCRP was a stronger predictor of cardiovascular events than LDL, total cholesterol, or other markers of inflammation and thrombosis.¹⁰²⁻¹⁰⁷ Prediction models for future cardiovascular risk demonstrate that hsCRP enters the model just behind hypertension but ahead of smoking and total and LDL cholesterol.¹⁰⁸ The predictive value of hsCRP for cardiovascular risk is similar in the United States and Europe, thus demonstrating consistency in different populations.¹⁰⁹ In the Pravastatin or Atorvastatin Evaluation and Infection Therapy—Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) study, patients with acute coronary syndromes and the fewest subsequent events were those who reached LDL cholesterol levels of less than 70 mg/dL (1.81 mmol/L) and those who reduced

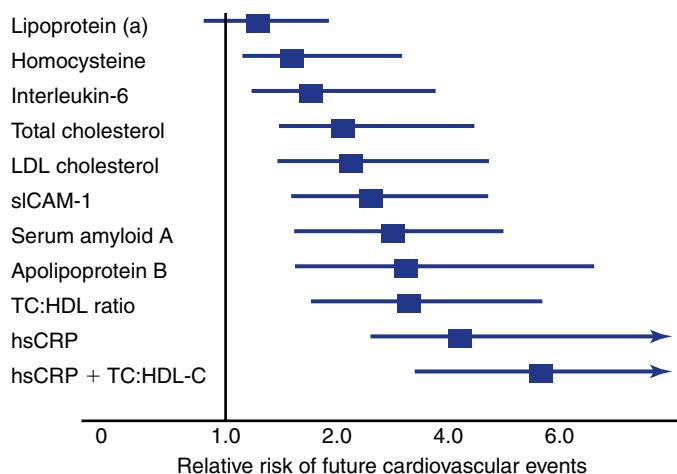


Figure 4.3 Comparison of lipid and nonlipid risk factors for cardiovascular disease (CVD) in 28,000 women enrolled in a nested case-control study from the Women's Health Study cohort of postmenopausal, apparently healthy women. hsCRP adds to the total predictive value of cholesterol. Note that lipoprotein (a) and homocysteine are relatively weak predictors of CVD. HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; sICAM-1, soluble intercellular adhesion molecule-1; TC, total cholesterol.

their CRP levels to less than 2 mg/L (19.1 mmol/L).^{110,111} Accordingly, hsCRP is at least as robust a biomarker for predicting future cardiovascular events as traditional biomarkers (Fig. 4.3).

The Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) adds further evidence of the role inflammation plays in atherosclerosis. The primary objective of JUPITER was to determine whether treatment with rosuvastatin would reduce the rate of first major cardiovascular event in patients with normal cholesterol levels but high levels of inflammation.¹¹² The trial was terminated early because rosuvastatin resulted in a 44% reduction in the composite primary endpoint of MI, stroke, hospitalization for unstable angina, arterial revascularization, and cardiovascular death. Of particular interest to the surgical community was the 46% reduction in arterial revascularization and 48% reduction in stroke in patients allocated to rosuvastatin. The latter is particularly intriguing given the favorable data for statins in patients with carotid stenosis.^{113–115} Thus, individuals with elevated levels of inflammatory biomarkers are at high vascular risk even when other risk factors are acceptable, and individuals at increased risk due to inflammation benefit from statin therapy they otherwise would not have received.^{116,117}

LOCALIZATION OF ATHEROSCLEROSIS

The propensity of atherosclerosis to occur at specific sites in the arterial tree has been observed since the time of Nikolaj Anitschkow's cholesterol-fed rabbits.¹¹⁵ Other investigators noted increased incorporation of ³H-thymidine into endothelial cells at branch sites in animals not exposed to injury; this was thought to be due to hemodynamic consequences.^{118–120} William Insull and colleagues found that atherosclerosis was

predominantly located at intercostal branch points of the human thoracic aorta.¹²¹

Areas of well-developed laminar shear stress are relatively resistant to atheroma formation, whereas areas of turbulent or low shear stress (such as the carotid bifurcation) are more susceptible to atherosclerosis. Shear stress response elements occur in the promoter regions of a number of atheroprotective genes. Endothelial nitric oxide synthase (eNOS) is the product of one such gene. Laminar shear increases eNOS activity and thus nitric oxide (NO) production, which renders endothelial cells more thromboresistant and results in less adhesion molecule expression and decreased SMC migration. NO reduces VCAM-1 gene expression through a novel pathway involving the inhibition of nuclear factor κB (NF-κB). Superoxide dismutase is expressed at higher shear stress and may reduce oxidative stress by catabolizing the highly reactive superoxide anion. Thus, areas of high laminar shear stress have anti-inflammatory and antioxidant properties and exhibit less adhesion to circulating leukocytes.

Branch points, bifurcations, and major curvatures disrupt laminar flow and cause boundary layer separation, flow reversal, and shifting stagnation points. Such areas are characterized by increased particle contact time with the luminal surface, which may favor lipid deposition.

Hence, fluid mechanical forces influence endothelial gene expression through certain response elements sensitive to shear stress. Endothelial dysfunction, characterized by decreased NO production, promotes vessel wall entry and modification of circulating LDL. Therefore, it is possible that the differential regulation of endothelial genes by distinct flow profiles allows certain areas of the vasculature to more effectively resist the influence of risk factors such as hyperlipidemia and diabetes.

PROGRESSION/REGRESSION OF PLAQUES

Our current understanding of atherosclerotic plaque emphasizes a dynamic evolution. Serial angiographic studies of human coronary arteries demonstrate periods of intermittent growth spurts followed by relative quiescence.^{102,122} What may account for the non-uniform progression of these atherosclerotic lesions? One prevailing theory suggests that most plaque disruptions with *in situ* thrombus formation do not always proceed to total occlusion and clinical sequelae, but instead are clinically silent (Fig. 4.4). Though unnoticed by the patient or clinician, they are far from benign. The local nonocclusive platelet thrombus induces a healing response with local inflammatory cytokine and growth factor production. TGF-β and PDGF stimulate SMC collagen production and migration. Thrombin production accelerates SMC migration and proliferation, and fibrin stimulates wound contraction and progressive luminal narrowing.^{123,124} Healed fibrous cap ruptures can be detected microscopically by the identification of breaks in the fibrous cap with a surrounding repair reaction consisting of a proteoglycan-rich mass or collagen-rich scar.¹²⁵ Thus, in contrast to slow steady plaque growth in which compensatory

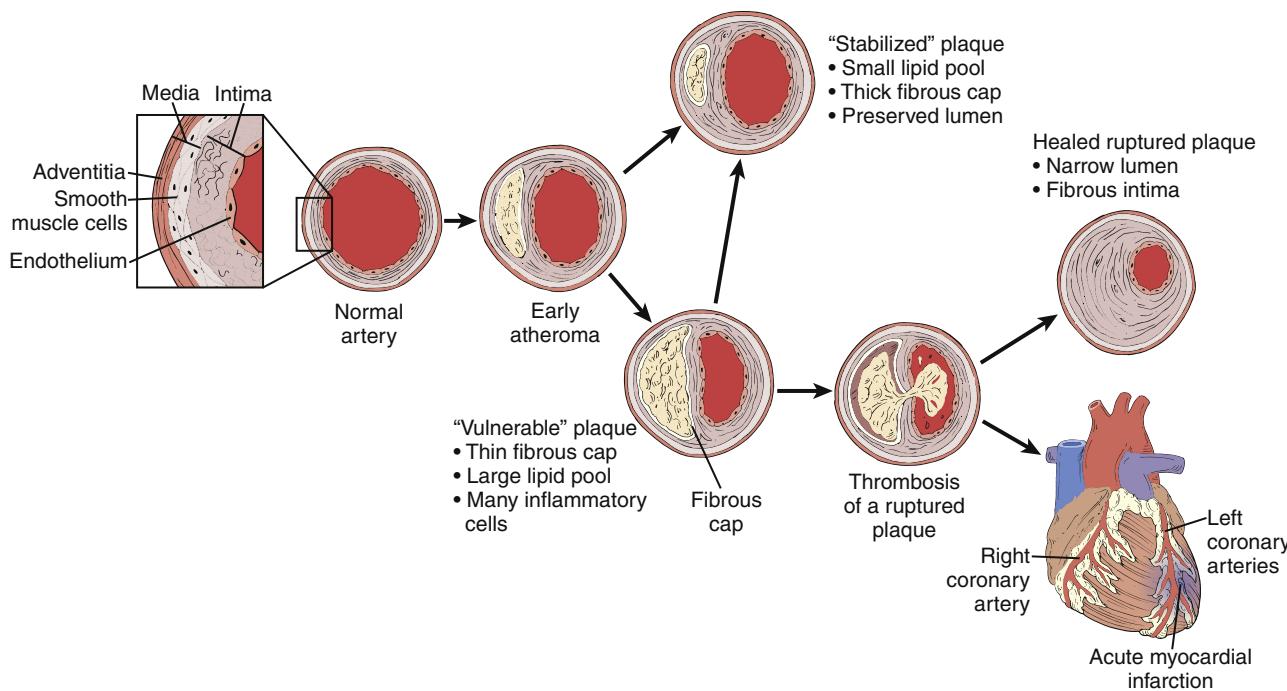


Figure 4.4 Life Cycle of Human Atherosclerotic Plaque. Rather than relentless progressive enlargement, human atherosclerotic plaque may undergo periods of progression, regression, and growth spurts. As the plaque extrudes into the lumen, the vessel undergoes compensatory enlargement, which preserves the lumen. The so-called vulnerable plaque consists of a relatively large lipid core and a thin ($<100\text{ }\mu\text{m}$) fibrous cap. Thrombotic complications can occur as a result of cap rupture, superficial endothelial erosion, intraplaque hemorrhage, or erosion of a calcified nodule in which circulating blood elements come in contact with the thrombogenic lipid core. The fate of such an event may be manifested as a myocardial infarction, transient ischemic attack, or in situ thrombosis of a peripheral artery. Far more commonly, however, they result in nonocclusive thrombus that incites a healing response. These healed ruptures result in a fibrous plaque with a narrowed lumen. (From Fowkes FG, Rudan D, Rudan I, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet.* 2013;382:1329–1340.)

enlargement of the vessel may protect from luminal encroachment, acute plaque rupture with nonocclusive thrombosis may signal a cascade of events leading to a fibrous atheroma and constrictive remodeling.

The use of cross-sectional images by IVUS reveals that segments of arteries that appear normal by angiography may nonetheless harbor substantial atherosclerotic disease.¹²⁶ Through morphometric studies of nonhuman primates and detailed autopsy studies of human coronary arteries, we understand that vessels undergo compensatory enlargement (remodeling) and recognize that lumen encroachment is a relatively late occurrence in the evolution of atherosclerotic plaque.^{127,128}

More recently, IVUS has been used to document plaque progression/regression in human coronary arteries with intensive statin treatment.^{129,130} The Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial measured the rate of disease progression in patients treated with either 40 mg of pravastatin or 80 mg of atorvastatin. LDL was reduced to 79 mg/dL (2.05 mmol/L) in the atorvastatin arm versus 110 mg/dL (2.85 mmol/L) in the pravastatin arm, and CRP was reduced 36.4% versus 5.2%. This resulted in a significant change in the primary endpoint of total atheroma progression of -0.4% in the atorvastatin arm versus 2.7% in the pravastatin arm.¹³⁰ In A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound to EvAtheroma Burden

(ASTEROID), 80 mg of rosuvastatin resulted in a change in atheroma volume of -0.98% over a 24-month treatment period. Collectively, these trials suggest that atherosclerotic plaque, far from being fixed, is a dynamic and mutable lesion that may regress in response to intensive hypolipidemic therapy. These trials also reflect the clinical benefit seen in the intensive treatment arms in trials such as PROVE IT-TIMI 22 and provide further rationale for recommendations of reducing LDL cholesterol below 100 mg/dL (2.59 mmol/L).¹³¹ Further investigation is needed to determine whether intensive statin therapy can reduce the progression of atherosclerotic disease in other vascular territories, such as the femoral arteries.

THROMBOTIC COMPLICATIONS OF ATHEROSCLEROSIS

Significant resources in vascular surgery are expended in caring for the acute thrombotic events complicating atherosclerosis and in situ thrombosis of the peripheral circulation. These catastrophic clinical syndromes often occur suddenly and without warning.

In the coronary circulation, various lines of evidence reveal that generally the culprit lesions are not necessarily those associated with the tightest stenosis.^{132–134} This is not to say

that high-grade stenosis does not undergo thrombotic complications; on a per-lesion basis, the individual probability of a complication from a high-grade lesion is higher than the probability from a less severe lesion.¹³⁵ However, because noncritical stenoses outnumber lesions producing critical stenosis, the total probability of a thrombotic complication attributable to the noncritical stenosis is higher. It must also be remembered that these assessments are based on standard planar angiography and do not take into account compensatory enlargement. Thus, low-grade stenosis does not necessarily equate with smaller lesions.

Physical disruption of the atherosclerotic plaque rather than critical stenosis can commonly precipitate arterial thrombosis. Four mechanisms of plaque disruption may cause thrombosis or rapid plaque expansion.¹⁴ Each may be operative in different vascular territories or with a different constellation of risk factors. For example, complete fracture of the plaque's fibrous cap causes most cases of fatal coronary thrombosis.¹³⁶ Plaque rupture is probably caused by both mechanical and biologic factors.^{15,137} Physical forces acting on the shoulder region of the plaque, where circumferential wall stress and cap fatigue are the greatest, render this area particularly vulnerable, especially when the central lipid core accounts for greater than 40% of the total lesion area and the cap is relatively thin (less than 100 µm).¹³⁸ Inflammation within the plaque may destabilize the cap and potentiate injury by hemodynamic factors. Macrophages and mast cells degrade the extracellular matrix by phagocytosis and secretion of proteolytic enzymes.¹³⁹ SMC and macrophages, when stimulated by inflammatory cytokines, produce MMPs including collagenases, elastases, gelatinases, and stromelysins, which degrade the matrix of the fibrous cap and result in thinning and weakening.^{140–145} It is now apparent that endothelial cells, SMC, and macrophages all increase the expression of the CD40 ligand and its receptor under the direction of the inflammatory cytokines. Ligation of CD40 in turn upregulates the production of MMPs, inflammatory cytokines, and tissue factor, thus emphasizing the autocrine and paracrine nature of the local inflammatory response of the fibrous cap.^{142–145}

Once the plaque is fractured, blood is exposed to the underlying thrombogenic substrate and thrombosis ensues. Tissue factor initiates the extrinsic clotting cascade and is a major regulator of coagulation and thrombosis. Thrombus formation and platelet adhesion create further stenosis and thrombotic occlusion. As previously mentioned, most thrombotic complications of plaque lead to progression of stenosis rather than occlusion of the artery. Cigarette smoking, hyperglycemia, and elevated LDL cholesterol all increase blood thrombogenicity.¹⁴⁶ These same risk factors are characterized by endothelial abnormalities such as increased generation of superoxide anion and decreased endothelium-derived NO.^{147–150} Thus risk factors are linked to progression of atherosclerosis and to its thrombotic complications at susceptible areas in the vascular tree through abnormalities in blood coagulation, as well as endothelial function.

Although complete fracture of the fibrous cap may be the most common cause underlying coronary thrombosis, other mechanisms may be more important in the periphery. Intraplaque hemorrhage can transform an asymptomatic carotid

plaque into a symptomatic lesion and produce transient ischemic attacks or stroke. Neovascularization and proliferation of the vasa vasorum, as in diabetic retinopathy, may produce a local microvascular network that is fragile and friable. Intraplaque hemorrhage can cause rapid plaque expansion and thinning or disruption of the cap. Superficial plaque erosion producing *in situ* thrombus without plaque rupture is prevalent in patients with diabetes and in women.^{14,151} Apoptosis of endothelial cells may promote superficial erosion, and various inflammatory stimuli may promote apoptosis.¹⁵² In particular, macrophage-derived myeloperoxidase may be operative in that it promotes both tissue factor generation and endothelial cell apoptosis, thus linking superficial erosion and *in situ* thrombosis.¹⁵³ Finally, erosion through the intima of a calcified nodule represents another less common form of atherosclerotic thrombosis that may be relevant in both the coronary and peripheral circulations.

Distinct artery-dependent patterns of atherosclerosis probably account for differences in the pathogenesis of thrombotic-related complications.¹⁵⁴ Both coronary and carotid atherosclerotic plaques appear to be laden with foam cells and to have large lipid cores; plaque rupture or intraplaque hemorrhage is common. In the femoral artery, plaque is more commonly fibrous without extensive foam cells. Here, it is likely that superficial plaque erosion and erosion of a calcific nodule more commonly lead to thrombosis. Evidence for the role of thrombosis in atherosclerotic associated morbidities is the benefit of low dose anticoagulation with factor Xa inhibition, highlighted in the COMPASS and VOYAGER trials.^{155,156}

IDENTIFICATION OF VULNERABLE LESIONS

How does one predict *a priori* which patients may be more likely to progress from an atherosclerotic lesion to one with symptoms? As discussed previously, IVUS evaluation determines the true extent of the size of the atherosclerotic plaque and can detect the degree to which an artery has remodeled in response to a plaque. For instance, it has been determined that excessive expansive remodeling may result in a thin vulnerable plaque.¹⁵⁷

Impaired endothelial vasodilator function is seen in patients with CVD risk factors. Brachial artery flow-mediated vasodilation has been used as a surrogate for the more relevant coronary circulation functional risk.^{158,159} Impaired flow-mediated vasodilation is seen in patients with inflammation and in those with classic risk factors,^{160,161} and it is often present before symptoms of peripheral or coronary arterial disease are evident.¹⁶² Impaired flow-mediated vasodilation has also been demonstrated to predict adverse events in patients undergoing vascular surgery.^{163–165} Hence, the classic Framingham risk factors, as well as inflammation, appear to impair endothelial function and promote atherosclerosis.

Finally, molecular imaging is on the immediate horizon to help identify vulnerable plaque. For example, plaque with active inflammation may be identified by extensive accumulation

of macrophages. Successful detection of plaque inflammation with magnetic resonance imaging was possible with gadolinium-loaded micelles coupled with antibodies to the scavenger receptor.¹⁶⁶ Alternatively, computed tomography may be used with a novel iodinated nanoparticulate contrast agent to detect inflammation in atherosclerotic plaque.¹⁶⁷ Contrast-enhanced ultrasound may also be used by attaching VCAM-1 to microbubbles and identifying areas of active adhesion molecule expression.¹⁶⁸

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Intimal Hyperplasia

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Based on a previous edition chapter by Mark G. Davies

BIOLOGY OF INTIMAL HYPERPLASIA	51
Adaptive Intimal Hyperplasia	51
Arterial Healing Response	52
CLINICAL FACTORS IN THE DEVELOPMENT OF INTIMAL HYPERPLASIA	54
CLINICAL SCENARIOS OF INTIMAL HYPERPLASIA	55
Arterial Response to Therapeutic Injury	55

Drug-Coated Balloons	56
Drug-Eluting Stents	56
Autogenous Vein Grafts	57
Healing Response of Prosthetic Grafts	59
Dialysis Access	60
CONCLUSION	61

Intimal hyperplasia represents the healing response to vascular injury as well as the adaptive response to developmental or physiologic events. Injury induces a progressive structural change within the blood vessel lumen that begins with the disruption of endothelial barrier function and damage to the underlying medial smooth muscle cells, initiating platelet deposition and inflammatory changes. These changes begin a cascade of events that lead to smooth muscle cell migration and proliferation with associated extracellular matrix deposition that ultimately results in neointimal lesion formation that compromises blood vessel patency. Its development can be chronologically subdivided into hyperacute, acute, and chronic stages, reflecting the timelines of classical wound healing (Table 5.1). Macroscopically, this lesion appears firm, pale, and homogeneous and is located in the subintimal location between the endothelium and the internal elastic lamina of an artery. Intimal hyperplasia may be focal at a site of injury or at an anastomosis or it can be diffuse in nature. While clearly adverse in the setting of vascular injury, intimal hyperplasia and vascular remodeling are necessary responses during development and growth.¹ It plays an essential role in important developmental events including the closure of the ductus arteriosus and the ductus venosus after birth. It is also important in the normal growth of arteries. Similarly, vein bypass grafts undergo adaptive remodeling when taken out of the venous circulation and placed into the arterial system. The biology of intimal hyperplasia is complex and it still remains unclear how an adaptive response becomes pathologic. Adaptive and pathologic intimal hyperplasia, such as following therapeutic

injury and bypass surgery, share many common mechanisms (coagulation, inflammation, cell proliferation, cell migration, proteases and extracellular matrix [ECM], and remodeling) but also have unique characteristics.

BIOLOGY OF INTIMAL HYPERPLASIA

Adaptive Intimal Hyperplasia

Vascular remodeling that occurs as normal adaptive responses is evident during the developmental process. It is essential to change fetal circulation to the post-fetal configuration. The functional change that directs blood through the pulmonary and systemic circulation is driven by changes in vascular resistance and hypoxia that leads to vasoconstriction of the ductus arteriosus and ductus venosus.² These changes occur rapidly to eliminate blood flow through these vessels. The anatomic closure of these structures occurs over the subsequent few weeks. The hypoxia in these excluded vessels stimulates medial smooth muscle cell apoptosis followed by the release of growth factors that simulate intimal hyperplasia and fibrosis that result in the obliteration of these vessels.

During growth and aging, arteries increase in diameter and wall thickness. Between the ages of 20 and 90 years, the arterial intimal medial thickness increases nearly threefold even in the absence of atherosclerosis.^{3,4} While medial thickness remains stable during the aging process, the intimal thickness gradually increases with age. Outward remodeling results in growth in lumen size despite the intimal hyperplasia, adapting to the

changes associated with aging. It is thought that lower rates of intimal hyperplasia are consistent with less aging while higher rates suggest accelerated aging.⁵ In the presence of disease such as hypertension and diabetes, the intimal thickness and composition of the arterial wall change to include more extracellular matrix and result in increased stiffness.

TABLE 5.1 Stages of Intimal Hyperplasia

	Vessel Lumen	Vessel Wall
Stage 1		
Hyperacute (minutes–hours)	Endothelial cell denudation Platelet aggregation Release of growth promoters	SMC injury Activation of SMC Proto-oncogene expression Release of growth promoters
Stage 2		
Acute (hours–weeks)	Organization of thrombosis Endothelial cell ingrowth Release of growth inhibitors Progenitor cell deposition	Medial SMC replication Medial SMC migration Infiltration of leukocytes Infiltration of adventitial cells Infiltration of progenitor cells Synthesis of growth promoters Synthesis of growth inhibitors
Stage 3		
Chronic (weeks–months)	Reendothelialization change of luminal dimensions	Intimal SMC replication Intimal SMC synthesis of ECM Remodeling of ECM Synthesis of growth inhibitors Vessel remodeling

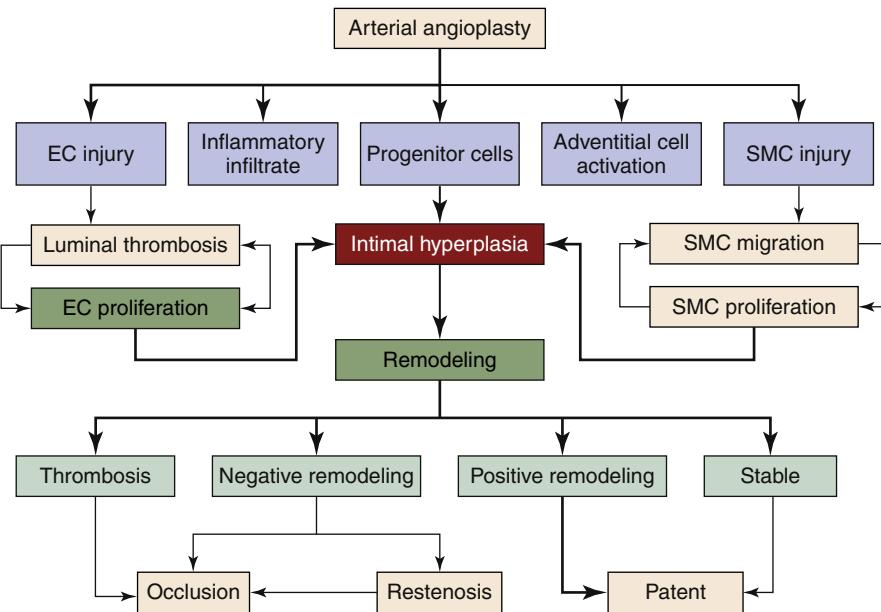
ECM, extracellular matrix; SMC, smooth muscle cell.

Figure 5.1 Pathobiology of the Injury Response After Angioplasty. Flow diagram demonstrating the key elements in the arterial response to injury. Endothelial cell (EC) injury leads to luminal thrombosis, inflammatory cell infiltration, cellular proliferation, and clearance of the thrombotic material on the surface with development of a neoendothelium. Injury to smooth muscle cells (SM) and adventitial cells leads to cell proliferation and migration. Progenitor cells are recruited to the vessel wall. With the migration and proliferation of SMC, adventitial cells, and progenitor cells as well as the deposition of extracellular matrix, intimal hyperplasia develops. Over time this lesion remodels, either remaining stable, demonstrating positive remodeling with an increase in luminal diameter, or negative remodeling with a decrease in luminal diameter. These chronic changes in the intimal lesion can lead to continued patency, restenosis, or occlusion.

Arterial Healing Response

Most forms of arterial injury arise from therapeutic interventions such as angioplasty or arterial bypass, which create local areas of vessel wall injury. Angioplasty creates a controlled focal injury to the vessel wall (Figs. 5.1 and 5.2) and is the basis of a popular experimental model of intimal hyperplasia. The immediate response of the vessel to injury is to achieve hemostasis. Endothelial injury and denudation expose the subendothelial matrix which leads to platelet adherence and aggregation. Platelet accumulation occurs rapidly for approximately 8–10 hours after injury.⁶ The use of aspirin and other antiplatelet agents following therapeutic vascular interventions is aimed at reducing this early platelet response. Platelet levels on the vessel surface decrease from days 3 to 7. Platelets interact with subendothelial collagen through platelet membrane glycoprotein receptors (GPIb, GPIc/GPIIa, and GPIa/GPIIa), plasma von Willebrand factor, and fibronectin. Glycoprotein IIb/IIIa inhibitor drugs are used to reduce platelet aggregation following percutaneous coronary interventions.^{7,8} The coagulation cascade (tissue factor, factor V, factor VIIa, factor Xa, or α -thrombin) is also activated and contributes to the initiation of intimal hyperplasia. Controlling the coagulation cascade early is key to preventing early stent thrombosis. Following injury, apoptosis (programmed cell death) can be identified in the cells in the arterial intima and media within 1 to 2 hours⁹ and abates after 4 hours. It then increases again by day 7 with 50% of medial cells undergoing apoptosis, potentially linked to increased proliferation at that time. By day 14, apoptosis is again markedly decreased.

Following these early events, a robust inflammatory response ensues with the recruitment of polymorphonucleocytes (PMNs) and monocytes to the site of injury by the adherent platelets, activated endothelial cells, and the exposed matrix and smooth muscle cells. There is a sequential expression of inducible cell surface molecules in both endothelial cells and smooth



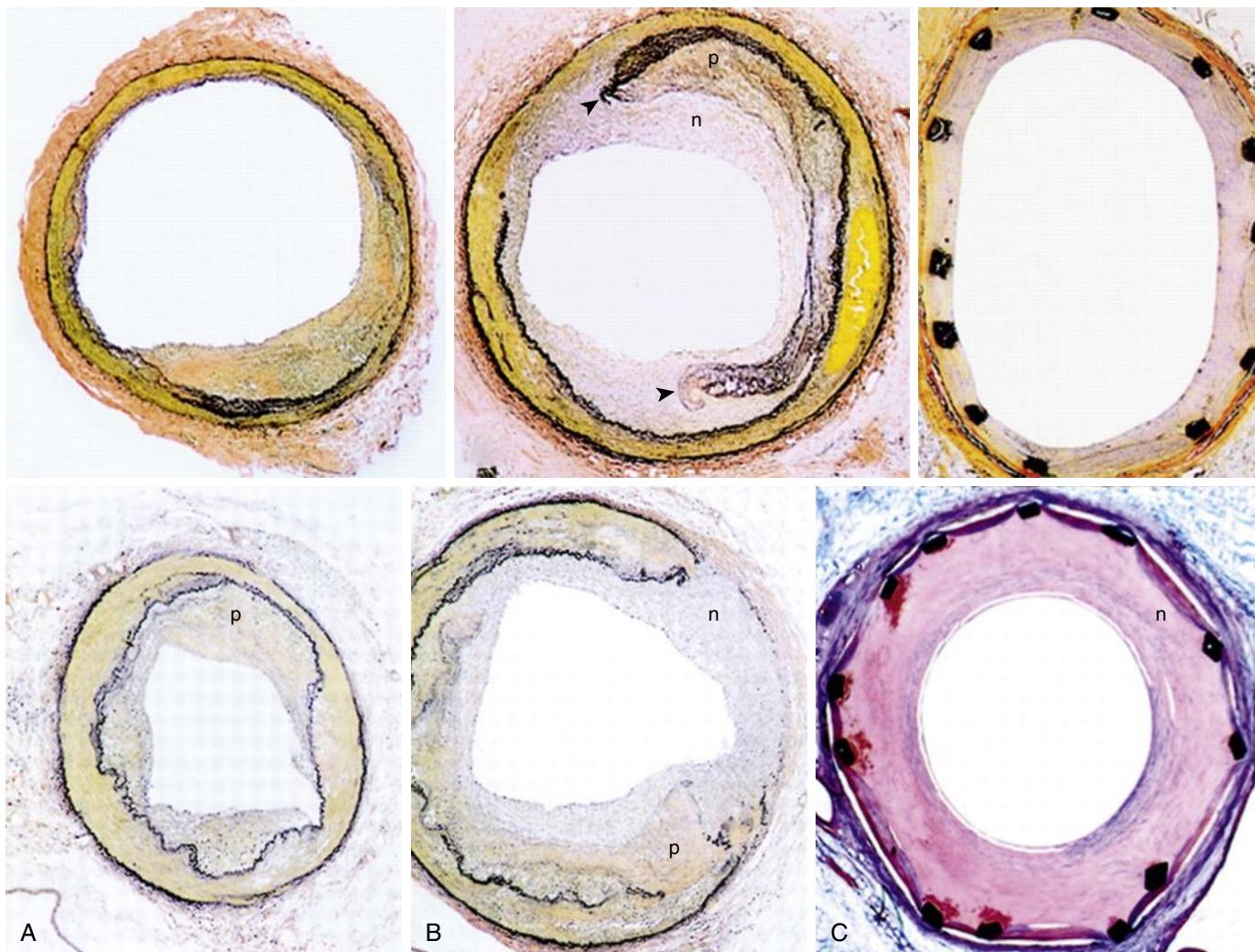


Figure 5.2 Photomicrographs of Stented Vessel. **Upper Panel:** Atherogenic diet induced complex, atheromatous intimal lesions in an uninjured primate iliac artery (A). Contralateral iliac artery was removed 35 days after angioplasty (B). Angioplasty fractured the preexisting plaque (*p*; arrowheads) and injured the underlying media, and stimulated neointimal lesion formation (*n*). Palmaz stents were deployed in the proximal left subclavian artery, and after 35 days, neointimal growth uniformly covered the underlying plaque and stent struts (C). (Original magnification: A and B, $\times 40$; C, $\times 100$. All panels, Verhoeff-van Gieson stain). **Lower Panel:** Composite photomicrograph demonstrates a typical response 4 weeks after angioplasty and stenting in atherosclerotic primates. Primates consumed an atherogenic diet for 2.5 years which resulted in the formation of complex plaques (*p*) in uninjured common iliac arteries (A). Following balloon injury, arteries demonstrated fracture of the plaque (*p*) and the underlying media with the growth of neointima (*n*) arising from the site of fracture (B). External iliac arteries treated with stenting (C) developed a typical neointimal lesion (*n*). Vessels were from a single treated animal. A and B, Verhoeff-van Gieson stain; C, trichrome stain; original magnification all panels, $\times 40$. (Upper Panel: Reproduced with permission from: Deitch JS, Williams JK, Adams MR, et al. Effects of beta3-integrin blockade (c7E3) on the response to angioplasty and intra-arterial stenting in atherosclerotic nonhuman primates. *Arterioscler Thromb Vasc Biol.* 18(11);1998:1730–1737. Lower Panel: Reproduced with permission from: Cherr GS, Motew SJ, Travis JA, et al. Metalloproteinase inhibition and the response to angioplasty and stenting in atherosclerotic primates. *Arterioscler Thromb Vasc Biol.* 2002;22(1):161–166.)

muscle cells after experimental angioplasty.^{10,11} Compared with the normal endothelium, injured and activated endothelial cells express high levels of vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM). Within 10 days, smooth muscle cells express both ICAM and MHC class II antigens. The upregulation of these adhesion molecules returns to baseline levels by 30 days post-injury. Chemokines and their receptors participate at every step of the vascular remodeling process.¹² These chemokines signal monocytes to infiltrate into

the injured vessel wall, further stimulating inflammation and smooth muscle cell proliferation. The monocyte chemotactic protein (MCP)-1/CC motif receptor 2 (CCR2) axis induces monocyte infiltration and smooth muscle cell proliferation. The RANTES (regulated upon activation, normally T-cell expressed, and presumably secreted) receptors CCR1 and CCR5 also regulate monocyte infiltration and neointimal growth. Re-endothelialization and intimal growth is mediated by the chemokine CXCL1, which is augmented by stromal cell-derived

factor-1 alpha (SDF-1 α) and its receptor CXCR4 that recruit circulating progenitor cells as well as medial smooth muscle cell progenitors to the subintimal location.¹³

Cytokines including tumor necrosis factors (TNF), interleukins (IL), lymphokines, monokines, interferons, colony-stimulating factors, and transforming growth factors (TGFs) are produced by both infiltrating inflammatory cells and injured cells of the vessel wall.¹⁴ These cytokines propagate the inflammatory response, cell adhesion, proliferation, migration, and apoptosis. Cytokines are also linked to increased mitochondrial reactive oxygen species production, activation of Ca²⁺, and multiple intracellular protein kinase pathways. Cytokines interact with integrins and matrix metalloproteinases (MMPs) to modify extracellular matrix composition, a key component of the developing neointima.¹⁵ The inflammatory response to arterial injury can be modulated by inducing changes in macrophage phenotype with a reduction in intimal hyperplasia.¹⁶ Administration of low-dose inhaled carbon monoxide (CO) for a brief exposure prior to angioplasty injury results in a significantly attenuated intimal hyperplastic response by modifying leukocyte function.¹⁷ In humans, targeted therapy against IL1 α showed a trend toward reduced restenosis following SFA interventions.¹⁸ Exogenous granulocyte colony-stimulating factor (GF) increased circulating endothelial progenitor cells (EP) and increased reendothelialization, resulting in reduced vascular inflammation and neointima size.¹⁹

Medial smooth muscle cells are normally quiescent with <1% in a proliferative state. This increases to >20% within 48 hours after injury.^{20–24} The exact mechanisms by which vascular injury induces and promotes smooth muscle cell proliferation remains an area of investigation. The first phase of smooth muscle cell proliferation appears to be driven by basic fibroblast growth factor (bFGF) released from dead and damaged cells in the injured vessel. Extracellular matrix degradation by matrix metalloproteinases (MMPs) allows the medial smooth muscle cells to migrate into the subintimal space. Smooth muscle cell migration is regulated by receptor tyrosine kinase-linked agonists (PDGF, bFGF, and hepatocyte growth factor) and G-protein coupled receptor agonists (vascular endothelial cell growth factor, chemokines, LPA, thrombin, and uPA). Inhibition of the PDGF receptor PDGFR- β/β with blocking antibodies inhibited intimal hyperplasia in animal models but was not effective in human trials.^{25–27} Circulating mesenchymal stem cells also contribute to the subintimal cells,^{28–30} differentiating into smooth muscle cells.^{28–31}

Once within the subintima, the smooth muscle cells begin to proliferate around day 7 and reach a peak at 14 days before returning to baseline by 28 days when vascular healing is complete.²⁴ However, proliferation may continue for up to 12 weeks in areas where reendothelialization is delayed. While the total number of neointimal smooth muscle cells stabilizes after 12 weeks, the neointima continues to grow through the elaboration of extracellular matrix (collagen and proteoglycans) by the smooth muscle cells. In human intimal lesions, hyaluron content was inversely related to collagen I and III staining. Little matrix accumulation occurs over the first 1 to 2 months following balloon injury but dramatically increases from 3 to 6 months in restenotic human coronary arteries.³² As collagen increases in the ECM, the intimal and adventitial smooth

muscle cells shift to a contractile phenotype, leading to arterial contraction and “negative” remodeling.

Myofibroblasts represent an important cell population in intimal hyperplasia. A marked infiltrate of myofibroblasts, some derived from circulating mesenchymal stem cells, is observed by day 2 and may represent up to 50% of cells within the intima by day 14.^{33,34} The presence of myofibroblasts is common in wound healing and contributes to wound contraction. A similar phenomenon may occur in the healing vessel. Injured vessels may undergo chronic elastic recoil or negative remodeling, reducing lumen size without increasing neointimal area. Retrieved atherectomy specimens from restenotic lesions showed low proliferative activity³⁵ but the smooth muscle cells from these lesions exhibited elevated migratory activity and collagen synthesis. These findings support the important role of intimal remodeling in the final determination of luminal diameter.^{36,37}

Traditionally, the regulation of vascular development and response to injury has been attributed to the endothelial cells and the medial smooth muscle cells in an “inside-out” fashion. The adventitia was believed to serve as structural support and transporting nutrients as well as maintaining sympathetic innervation to the vessel wall.³⁸ It is now recognized as a source of cells that regulate all the layers of the vessel wall, supporting an “outside-in” hypothesis of vascular inflammation and healing. The cells in the adventitia include fibroblasts, smooth muscle cells, adipocytes, pericytes, and resident inflammatory cells. It is rich in stem and progenitor cells that can migrate and differentiate into a variety of cells in the media and neointima during vascular healing and disease. The adventitia is also home to the vasa vasorum, lymphatics and perivascular nerves. These cells and structures are embedded in an extensive network of extracellular matrix rich in collagen, elastic fiber nets, proteoglycans, fibronectins, and tenascin-c.³⁹ The adventitial collagen fibers protect the vessel from over distention at high pressures while physiologic responses are mediated by the medial elastin layers.⁴⁰ Evidence supporting outside-in healing is that early after injury, adventitial myofibroblasts express high levels of signals including monocyte chemoattractant protein-1 (MCP-1) that recruit and activate monocytes to the adventitia.⁴¹ Following arterial injury, adventitial fibroblasts become activated through sonic hedgehog signaling and increased reactive oxygen species production from upregulated NADPH oxidase and leads to increased neointima formation.⁴² The adventitial myofibroblasts, while contributing to the neointimal cells, are also important for negative remodeling through vascular contraction. Finally, aging results in increased resident inflammatory cells in the adventitia and may explain the greater susceptibility of aged arteries to atherosclerosis and intimal hyperplasia.

CLINICAL FACTORS IN THE DEVELOPMENT OF INTIMAL HYPERPLASIA

The degree of intimal hyperplasia that develops in a vessel is dependent on the degree of injury.⁴³ Intimal proliferation is minimal when the media is uninjured but increases in proportion

to the depth of the medial injury, indicating that the severity of smooth muscle cell injury regulates the magnitude of the proliferative response.^{44,45} Further evidence suggests that smooth muscle cell distention without endothelial cell injury can also stimulate smooth muscle cell proliferation. The length of the injury correlates with the extent of endothelial injury. Reendothelialization occurs from the edge of the denuded area and possibly from the endothelial cells of the vasa vasorum. Until reendothelialization is complete, the underlying smooth muscle cells are without the modulating influence of homeostatic endothelium derived factors such as nitric oxide and prostacyclin.^{46,47} After severe arterial wall injury, luminal compromise results from neointima formation as well as negative remodeling.⁴⁸ Medial damage is accompanied by massive proliferation of adventitial myofibroblasts⁴⁹ that mediate negative remodeling.³⁸

Changes in hemodynamic parameters affect both normal and diseased vessels.⁵⁰ Clinical studies suggest that femoral angioplasty in patients with compromised outflow is associated with increased restenosis. Hehrlein et al. confirmed that reduced vascular runoff increased intimal hyperplasia following angioplasty.⁵¹ Blood flow and shear stress are best associated with the development of intimal hyperplasia, whereas circumferential deformation of the vessel wall correlates with medial thickening.⁵² Kohler^{53,54} reported reduced intimal thickness with increased flow while increased intimal hyperplasia occurred with decreased flows, suggesting a direct impact of flow on smooth muscle cell function. However, they did not detect an ability of flow changes to alter established neointimal lesions.

The development of intimal hyperplasia in diseased blood vessels is exaggerated compared to normal arteries subjected to injury. In animal models of hyperlipidemia,^{55–57} balloon injury increased vascular inflammation and intimal hyperplasia. The resultant neointimal lesions had significant components of atheroma formation. Clinically, similar associations between hyperlipidemia with higher rates of restenosis have been reported.^{58,59} Hyperlipidemia may result in the expansion of a subset of CD14/CD16 rich monocytes that may target sites of vascular injury more significantly. These cells have increased myeloperoxidase activity that contributes to increased oxidative stress and promotes further inflammation and the proliferation and migration of smooth muscle cells.⁵⁸ Risk factors that contribute to atherogenesis also increase intimal hyperplasia. Cigarette smoke increases experimental intimal hyperplasia by twofold⁶⁰ while cholesterol reduction with statins has been shown in some studies to reduce restenosis.^{61,62} However, there is also evidence that statins improve outcomes through other mechanisms but do not impact rate of restenosis.⁶³

Diabetes is a predictor for augmented intimal hyperplasia and restenosis in response to vascular injury. In animal models, diabetes is associated with increased inflammation and intimal hyperplasia following injury.^{64–66} In the setting of metabolic syndrome or pre-diabetes, arterial injury upregulated adhesion molecules ICAM-1 and P-selectin with increased macrophage infiltration of the arterial wall and increased neointima

formation.⁶⁷ The expression of oxidized LDL receptor was also upregulated. Advanced glycosylation end products (AGEs) accumulate in blood vessels during aging and is further enhanced in diabetes.⁶⁸ AGEs are particularly abundant at atherosclerotic lesions.⁶⁹ AGEs interact with specific receptors (RAGEs) present on inflammatory cells and smooth muscle cells to stimulate inflammation, smooth muscle cell proliferation and migration, and ECM production. RAGE expression is upregulated in diabetes as well as following arterial injury. RAGE is also a receptor for other ligands released from injured cells including damaged associated proteins such as HMGB1 and S100. Inhibition of RAGE reduced neointima formation in nondiabetic and diabetic animals.^{68,70} Higher rates of restenosis are observed in diabetic humans.^{71–73} Diabetic patients with restenosis have a higher rate of subsequent in-stent restenosis.⁷⁴ Atherectomy specimens collected from restenosis lesions from diabetic patients had lower cellularity and increased collagen rich matrix than nondiabetic patients.⁷⁵ These findings suggest that negative remodeling and recoil may contribute to restenosis in diabetes.

CLINICAL SCENARIOS OF INTIMAL HYPERPLASIA

Arterial Response to Therapeutic Injury

After balloon angioplasty, platelet aggregation, intimal hyperplasia, elastic recoil, and negative remodeling all contribute to restenosis. In contrast, after stent placement, elastic recoil and negative remodeling are minimized⁷⁶ and platelet aggregation, endothelial injury and intimal hyperplasia are the main contributors to in-stent restenosis.^{77,78} Thus, the biology of in-stent restenosis is different from that seen after balloon angioplasty alone.⁷⁹ A stent is generally used if the result of angioplasty alone is technically unsatisfactory or in the setting of arterial occlusion, immediate elastic recoil, dissection, or restenosis (Fig. 5.3). Intravascular ultrasound has demonstrated that stents do not completely appose the vessel wall along their entire length, leading to areas of uneven injury.⁷⁶ Thus, stents result in generalized injury to the treated vessel with focal areas of increased injury where the struts appose the wall.

After stent placement, the stent is rapidly covered by a strongly adherent layer of protein, predominantly fibrinogen, after 1 minute.⁸⁰ The stent interstices are filled with thrombus, and the adherence of platelets and leukocytes is enhanced by disturbance of electrostatic equilibrium.^{81,82} The mechanisms of smooth muscle cell proliferation and migration after stent placement are similar to after balloon injury⁸³ but the process is more prolonged and robust secondary to the inflammatory response induced by the stent.⁸⁴ This response is often more significant at the edges of the stent than in the body (Fig. 5.2). The adventitial response is also prolonged with adventitial giant cell body formation being noted. Stents prevent elastic recoil and cause progressive atrophy of the media.⁸⁵

Early after stenting in humans (≤ 11 days), fibrin, platelets, and acute inflammatory cells are always present around the stent struts.⁸⁶ The stent–arterial wall interface influences the

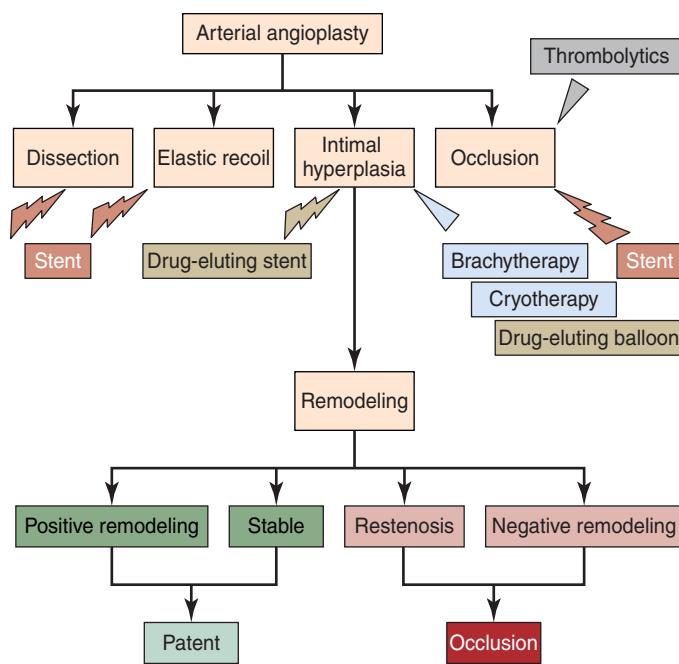


Figure 5.3 Consequences and Treatment of Angioplasty-Induced Injury. Flow diagram demonstrating the outcomes of a vessel's response to balloon angioplasty and the therapeutic maneuvers to correct the adverse outcomes. If a technical failure occurs after angioplasty due to elastic recoil or dissection, a stent is placed. If there is concern for the development of intimal hyperplasia, brachytherapy or cryotherapy may be applied. Sudden occlusion is corrected with thrombolysis or primary stenting. Remodeling will be influenced by placement of stents, drug-eluting stents, brachytherapy, or cryotherapy.

severity of associated inflammation with increased inflammatory infiltrates when the stent is adjacent to injured media or lipid core compared with fibrous plaque. Even after the acute injury associated with stent placement has resolved, chronic inflammatory changes persist. Plaque compressed by stent struts is seen in 91% of vessel sections with penetration of the stent struts into the lipid core being a common event.⁸⁶ Neointima develops within 2 weeks, and histologic success or failure of the stent is determined by neointimal growth within the stent and is not influenced by artery or stent size. Neointima thickness is increased when medial damage is present compared with struts in contact with atherosclerotic plaque or an intact media. Furthermore, increased in-stent neointimal growth correlates with stent size relative to the arterial lumen with stent oversizing contributing to increased in-stent restenosis. Despite these histologic changes, neointimal cell density and proteoglycan deposition in coronary stents were similar to coronary vessels treated with angioplasty alone.^{86,87}

Drug-Coated Balloons

Drug-coated balloons (DCBs) utilize the pressure and contact of an inflated angioplasty balloon to deliver a therapeutic cytotoxic drug (e.g., paclitaxel) to the arterial wall to reduce local intimal hyperplasia and restenosis.⁸⁸ This technology was designed to improve vessel patency without the dependence on a stent which itself can stimulate inflammation and promote intimal proliferation while hindering vascular healing.

Scheller et al. reported >60% reduction in neointimal area following angioplasty with paclitaxel-coated balloons in pigs in 2004.⁸⁹ DCBs were quickly developed and were FDA approved by 2014. While many DCBs exist on the world market, only three are available in the US and they all deliver paclitaxel. Paclitaxel is a chemotherapeutic drug commonly used to treat a variety of cancers including ovarian and breast cancers. It stabilizes microtubule structure, preventing it from disassembling, thus preventing mitosis and proliferation. Paclitaxel is indiscriminate and targets any proliferating cell. Thus, DCB-treated vessels exhibit reduced intimal hyperplasia as well as delayed healing characterized by increased fibrin deposition, delayed reendothelialization, and increased medial smooth muscle loss.^{90–92} The impact on endothelial healing may explain the observation that DCB-treated arteries are slower to regain endothelium-dependent vasodilation compared to vessels treated with uncoated balloons.⁹¹ The use of DCB in the femoropopliteal arterial segment has improved primary patency and decreased target lesion revascularization at 5 years.⁹³ Similar outcomes have not been reported for infrapopliteal target arteries.⁹⁴ Currently, major controversy exists regarding potential mortality associated with DCB use, although no clear mechanism has been identified.⁹⁵ Some suspect that this mortality may be related to higher concentrations of paclitaxel used in DCBs compared to drug-eluting stents (DESs) in the coronary circulation. While current FDA-approved DCBs rely on paclitaxel as the active agent, rapamycin-based therapies are in development. Rapamycin, or sirolimus, is an immunosuppressant used in transplant therapy. In addition, it inhibits cell proliferation through effects on the mammalian target of rapamycin (mTOR) that blocks cell cycle progression. Windecker et al. reported improved patency and safety profiles with sirolimus compared to paclitaxel-eluting stents.⁹⁶

Drug-Eluting Stents

Drug-eluting stents (DESs) have been used for coronary revascularization since 2002 and for the peripheral circulation since 2012. The early coronary DESs delivered either sirolimus or paclitaxel with both drugs significantly reducing restenosis. Because the sirolimus family of drugs have improved pharmacokinetics and wider therapeutic windows, current generations of coronary DES favor these agents over paclitaxel. DES improves stent patency through the inhibition of smooth muscle cell proliferation. However, because paclitaxel and sirolimus have no cell specificity, endothelial cell migration and proliferation are also impacted. The presence of a DES decreases homing, proliferation and differentiation of endothelial progenitor cells which contribute to reendothelialization.⁹⁷ Furthermore, both sirolimus and paclitaxel can induce endothelial dysfunction in the coronary vasculature distal to the stent.⁹⁸ Both drugs induce endothelial tissue factor expression,^{99,100} which may contribute to the increased thrombosis observed with DESs.¹⁰¹ A number of the currently successful DESs require a polymer coating for drug delivery.¹⁰² The polymer can be associated with hypersensitivity reactions that, in some cases, may lead to stent thrombosis.¹⁰³ Efforts have been made to address this

in later formulations using more lipophilic derivatives of sirolimus that improve tissue penetration and drug delivery.¹⁰⁴ As a result, these later generations of DES have lower risks of late stent thrombosis.¹⁰⁵

In the coronary circulation, the DESs more commonly utilize sirolimus or its related compounds such as everolimus. Paclitaxel stents are more commonly used in the peripheral circulation. This is due to a lack of commercially available sirolimus-eluting stents for peripheral use, although these devices are currently in development. Cardiology literature reports superior outcomes in the coronary circulation using sirolimus-analog stents.^{106–108} The more lipophilic analogs of sirolimus exhibit improved safety profiles with significantly reduced rates of stent thrombosis compared with paclitaxel and even sirolimus DESs.¹⁰⁹ As with paclitaxel DCBs, peripheral arterial paclitaxel DESs are under scrutiny due to reported increases in patient mortality.¹¹⁰

Autogenous Vein Grafts

Saphenous veins are the preferred conduit for infrainguinal revascularization. They can exhibit a spectrum of preexisting pathologic conditions ranging from thickened walls to post-phlebitic changes and varicosities. Histologically, 91% of saphenous veins have moderate to severe fibrosis in the vein wall. In one study, 2% to 5% of veins were unusable and up to 12% were considered “diseased.”¹¹¹ These “diseased” veins have a patency rate one-half that of “nondiseased” veins. The etiology of the observed venous diseases are multifactorial in origin, and without gross morphologic evidence of disease, there is currently no clear prognostic indicator to distinguish the veins that should not be used for conduit.^{111,112}

Veins implanted into the arterial circulation adapt to the increased flows and pressures through a process called arterialization. This adaptation is characterized by an intact endothelium with the formation of increased layers of medial smooth muscle cells.¹¹³ Early changes include expression of endothelial E-selectin and VCAM-1 with the recruitment of neutrophils and monocytes/macrophages. While the vein wall becomes thicker in response to arterial hemodynamics, the vein undergoes dilation over the first 12 months due to the drop in shear stress encountered in the arterial circulation and preserves the flow lumen in face of vein wall thickening.^{114,115} Focal areas of vein disease result in focal areas of intimal hyperplasia that are maladaptive and can lead to stenosis on top of this adaptive arterialization process.

Acute thrombosis causes immediate graft failure, whereas intimal hyperplasia represents the mode of failure in the months to years after vein graft insertion. Whether the same series of events occur in an “arterializing” vein graft (summarized in Figure 5.4) as in an injured artery, remains to be determined. Progenitor cells may play a more significant role in vein graft remodeling than in the artery. In experimental models, many endothelial cells in the vein grafts are derived from circulating progenitor cells and up to one-third appear to be derived from bone marrow progenitor cells.¹¹⁶ In general, adaptive intimal hyperplasia is a self-limited process that does not produce

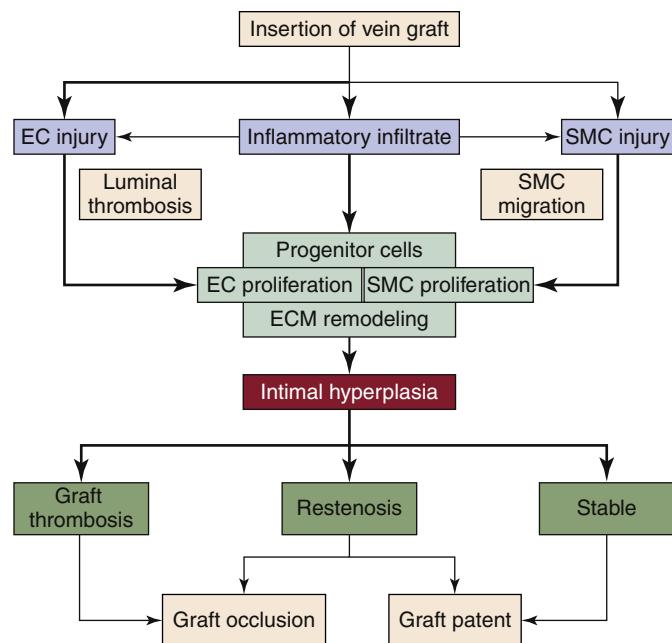


Figure 5.4 Pathobiology of the Vein Graft Response to Implantation. Flow diagram demonstrating the key elements in the vein's response to insertion into the arterial circulation. Denudation of the endothelium is dependent on the degree of implantation injury. Endothelial cell (EC) injury leads to luminal thrombosis, inflammatory cell infiltration, cellular proliferation, and clearance of the thrombotic material on the surface with restoration of the endothelium. If this fails to progress adequately, graft thrombosis may occur. Injury to smooth muscle cells (SM) leads to cell proliferation and migration. Progenitor cells are recruited to the vessel wall. With the migration of proliferation of SMC, the appearance of progenitor cells and the deposition of extracellular matrix (ECM), intimal hyperplasia develops to reestablish the tangential stress across the wall. Over time this lesion remodels and may produce a stenotic lesion due to the bulk of neointima or due to negative remodeling restenosis. This may result in graft occlusion.

luminal compromise due to outward remodeling and usually becomes quiescent within 2 years of graft insertion. In focal areas, however, the intimal hyperplastic process can persist and lead to a significant stenosis.^{117–120} Studies of peripheral vein grafts have documented that the majority of stenotic lesions that develop in the graft are composed of neointimal tissue.^{119,120} Graft stenoses develop more often at sites of unrecognized defects or early appearing conduit abnormalities¹²¹ and less at the sites of valves or tributary ligation.¹²²

Surgical preparation of veins for use as conduit produces significant tissue damage. Such implantation injury leads to endothelial injury and denudation and smooth muscle cell injury, all of which initiate intimal hyperplasia. Every effort should be made to reduce injury to the vein during harvest.^{123–126} There appears to be a direct relationship between the morphologic integrity of the vein graft prior to implantation and its later histopathologic appearance and function.^{124,126} Poorly prepared vein grafts develop significantly greater intimal hyperplasia and increased smooth muscle cell contractility compared with carefully handled vein grafts (Fig. 5.5).^{124,126} Damage to the adventitia during harvesting disrupts the vasa vasorum, leading to vessel wall hypoxia that initiates inflammation and cytokine release.¹¹⁵ The adventitia is an important reservoir of mesenchymal stem cells that contribute to endothelial healing. The

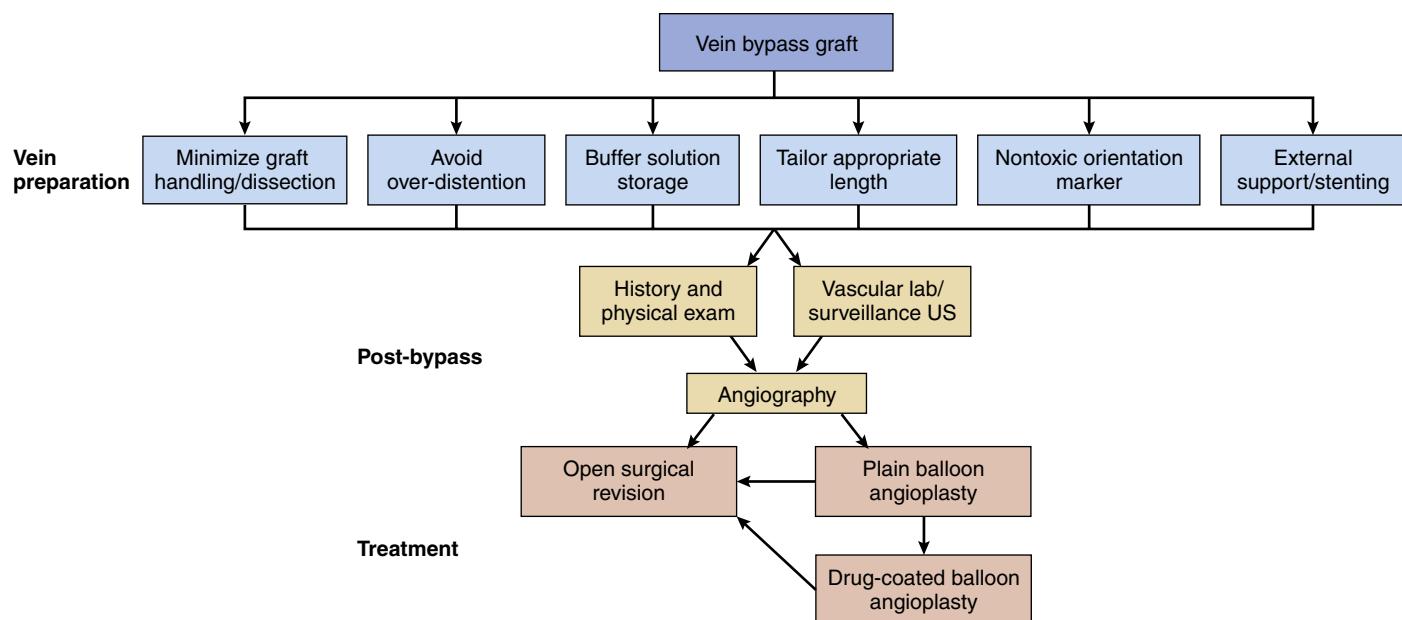


Figure 5.5 Prevention of Vein Bypass Intimal Hyperplasia. Vein graft susceptibility to developing intima hyperplasia starts with the handling of the vein during harvesting. Careful handling and treatment of the vein can reduce vein wall injury and intimal hyperplasia. After implantation, careful evaluation of the bypass through physical examination and through ultrasound (US) surveillance can help to detect limiting lesions. Flow-limiting lesions can be treated endovascularly or through surgical revision.

perivascular adipose tissue is a location for resting inflammatory cells that are activated in response to injury. It is thought that the “no-touch” technique of vein graft harvesting can reduce injury to the layers of the vein wall and preserve the adventitial environment to limit the drive for intimal hyperplasia.¹²⁷ However, methods of reducing adventitial injury including in situ bypass grafting¹²⁸ and endoscopic harvesting techniques¹²⁹ have not improved graft performance. Overdistention of vein grafts (>150 mm Hg) increases risk of graft failure likely due to endothelial denudation at high pressure.¹³⁰ Storage media or pharmacologic preparation has been proposed as adjunctive therapy when preparing vein grafts.¹³¹ Even the skin marker that is used to orient the vein can damage the endothelium as evidenced by the loss of endothelium-dependent relaxation.¹³² Evidence also suggests that arterial hemodynamics can activate protein tyrosine kinases in the medial smooth muscle cells to initiate proliferation.¹³³ Vein grafts in vascular beds with lower flows are associated with greater intimal thickening.¹³⁴ For example, a 50% reduction in arterial blood flow increased intimal hyperplasia by 60% and medial hypertrophy by 17% in vein grafts after 4 weeks.¹³⁵ Other studies have suggested a role for increased wall tension in the development of intimal hyperplasia.^{136,137}

With few exceptions, patients who undergo vein bypass grafting have a significant degree of arteriopathy and concomitantly have one or more atherogenic risk factors present. Hypertension in both human and experimental models does not affect the development of intimal hyperplasia or vein graft atherosclerosis, at least in coronary vein grafts.^{138–141} In contrast, both experimental and clinical studies show an association between hyperlipidemia with vein graft disease and with higher

failure rates.^{138,142,143} Clinically, diabetes does not significantly impact vein graft patency but it does enhance short-term intimal hyperplasia development secondary to an exaggerated inflammatory response in experimental models.^{138,144} The combination of diabetes and hyperlipidemia has a significant additive effect on experimental vein graft intimal hyperplasia. The neointimal lesions of human vein grafts retrieved 1 month after coronary bypass consisted of proliferating smooth muscle cells with only scattered macrophages in the subintima.¹⁴⁵ No association has been found between vein graft disease and patient age, sex, presenting symptoms, hypertension, diabetes, or the condition of the outflow vessel. The incidence of stenosis appears to be increased the more distal in the arterial tree the anastomosis is placed.¹⁴⁶

Several peripheral factors may also play a role. Increased plasma fibrinogen and homocysteine concentrations have been identified as potent risk factors for vein graft stenosis.^{147,148} Antibodies to cardiolipin are associated with infrainguinal vein bypass failure.¹⁴⁹ Studies suggest that platelet dysfunction, lipoprotein(a), smoking, and serotonin concentrations are all associated with the development of bypass graft stenosis^{146,150–152} while others showed no association between pre-operative serum lipoprotein(a) and homocysteine levels with 1-year graft occlusions.¹⁵³

Lower serum cholesterol levels correlated with reduced vein graft occlusive disease for up to 7 years,¹⁵⁴ and high patency rates were achieved in familial hypercholesterolemia with aggressive lipid-lowering therapy.¹⁵⁵ The Post-CABG NHLBI study reported that while vein graft atherosclerosis worsens with age, lipid-lowering therapy significantly reduced vein graft disease regardless of when the therapy was initiated.¹⁵⁶

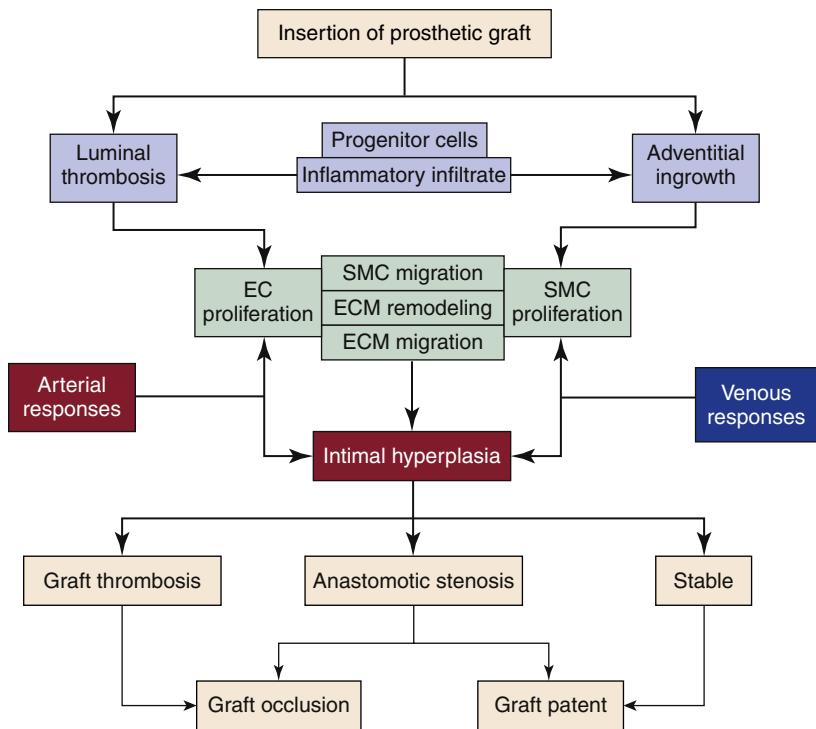


Figure 5.6 Pathobiology of the Prosthetic Graft Response After Implantation. Flow diagram demonstrating the key elements of the body's response to implantation of a prosthetic graft into the arterial or venous circulation. Luminal thrombosis and adventitial infiltration are associated with an inflammatory response and recruitment of progenitor cells. Injury to the endothelial cells (EC) and smooth muscle cells (SMC) at either the venous or arterial anastomosis leads to the development of intimal hyperplasia within the native vessel and ingrowth into the prosthetic conduit as a result of EC and SMC migration and proliferation occurs with the laying down of an extracellular matrix (ECM). Over time these lesions remodel and may produce a multifocal stenotic lesion at the anastomoses and in the floor of the recipient vessel.

Gemfibrozil delayed coronary atherosclerosis and the formation of bypass graft lesions after CABG in men with low HDL cholesterol.¹

Vein grafts retrieved from patients with angiographic evidence of occlusive disease exhibited histologic features of atherosclerosis^{139,158–161} as early as 6 months after implantation. Thus, late vein bypass graft occlusion may be due to the development of a rapidly progressive and structurally distinct form of atherosclerosis termed “accelerated atherosclerosis.”¹¹⁸ Accelerated atherosclerosis is morphologically different from spontaneous atherosclerosis in that lesions are diffuse and more concentric with greater cellularity and varying degrees of lipid accumulation and mononuclear cell infiltration. Accelerated atherosclerosis shares many of the pathophysiologic features of intimal hyperplasia; however, the prime mediators of this type of atherosclerosis are likely to be derived from the macrophage. In addition, the endothelium overlying accelerated atherosclerotic lesions expresses MHC class II antigens that are not observed in spontaneous atherosclerosis.

A number of investigators have attempted to limit intimal hyperplasia in vein grafts. The PREVENT III study evaluated the ability of a decoy oligonucleotide that blocks the transcriptional factor E2F, important in the expression of cell cycle proteins, to prevent vein graft failure in lower extremity bypass surgery.¹⁶² Unfortunately, the treatment of vein grafts with the E2F decoy prior to implantation did not improve vein graft performance as compared with placebo. PREVENT IV also failed to show benefit of the treatment in vein grafts used in coronary bypass.¹⁶³ Animal studies suggest other translational interventions may be effective. Treatment of vein grafts with preservation media infused with carbon monoxide gas prior to implantation attenuated intimal hyperplasia.^{164,165} Trocha et al. recently reported that perioperative protein restriction in

rodents undergoing vein bypass surgery increased endogenous hydrogen sulfide production, decreased early graft inflammation, and attenuated intimal hyperplasia. Altering the mechanics of saphenous vein graft through external stenting decreased intimal hyperplasia in patients undergoing coronary artery bypass grafting,¹⁶⁶ presumably by promoting a more consistent lumen diameter and decreasing shear stress in the grafted vein. Therefore, both mechanical and pharmacological modification of vein grafts may improve performance.

Healing Response of Prosthetic Grafts

Prosthetic grafts were first developed in the 1950s to use for the repair of aortic aneurysms. Materials used in these synthetic grafts are predominantly polyesters and polytetrafluoroethylene and very little change in these materials has occurred through the decades. While effective in large caliber artery repair, they perform poorly in infrapopliteal reconstruction^{167,168} due predominantly to thrombosis and intimal hyperplasia^{169,170} (Fig. 5.6). Polytetrafluoroethylene (PTFE) bypass grafts explanted from humans showed that between 5 and 24 days after implantation, red blood cells, fibrin, and scattered macrophages cover the anastomoses and some of the luminal surface. Between 11 and 48 months, a single layer of endothelial cells and a thin layer of fibrin are detected covering the anastomotic segments of the graft. In addition, there is evidence of developing intimal hyperplasia at the anastomosis with collections of smooth muscle cells and collagen. From 94 to 149 months after implantation, the anastomotic intimal hyperplasia remains relatively unchanged and looks similar to that seen in grafts at 11–36 months. Chronic inflammatory cells (macrophages, lymphocytes, monocytes, and giant cells) can be identified in incorporated ePTFE grafts.¹⁷¹ Perigraft tissue infiltration and

encapsulation forms around the length of the prosthetic graft over time. The presence of external reinforcement does not affect this tissue infiltration.¹⁷² The luminal surface is covered with connective tissue matrix with scattered thrombi¹⁷³ in grafts explanted at 94–149 months. There is no evidence that prosthetic grafts can become endothelialized over the entire luminal surface in humans, which is in contrast to experimental models.¹⁷² Instead, endothelial cells populate the luminal surface for just a few centimeters beyond the anastomoses while the remainder of the graft is covered with a layer of organized fibrin, platelets, and leukocytes.^{172,174} The incidence of lipid and cholesterol deposits is high,¹⁷⁵ and atheromatous changes have also been detected in PTFE grafts.¹⁷⁶ Collagen type III is predominant, although collagen subtypes I, IV, and V can also be identified. Elastin fibrils are seen within the anastomotic neointimal lesion.¹⁷⁷

A neo-endothelium can form in a prosthetic graft by transmural endothelialization through capillary ingrowth and by endothelial migration from the anastomoses. Capillary ingrowth through the graft wall depends upon graft porosity. In low-porosity grafts (10- and 30-μm internodal distance) placed in the aortoiliac position in baboons, luminal endothelial coverage was limited to short segments near the anastomoses, resulting from migration from the adjacent artery.¹⁷⁸ In higher-porosity grafts (60 and 90 μm), endothelial coverage was complete.^{179,180} Evidence of transmural endothelialization was provided by Graham et al.¹⁸¹ who demonstrated that polyethylene terephthalate (PET) grafts wrapped with vein and then implanted into dogs developed a uniform endothelial coverage of the wrapped portion of the graft and not of the uncovered portion. PET graft impervious to transmural or edge migration implanted into canine aorta showed endothelial islands within the graft, supporting fallout endothelialization from the blood stream as a third possible mode of graft healing.¹⁸² The process of healing in a prosthetic graft is shown in Figure 5.6. Aspirin administration can prevent early graft thrombosis but does not reduce anastomotic intimal hyperplasia.¹⁸³

It is well known that prosthetic grafts fail commonly at the distal anastomosis where intimal hyperplasia forms. The etiology of this response is a combination of arterial injury due to the surgical anastomosis, flow disturbances related to the configuration of the anastomosis, and to the compliance mismatch created by the transition between the stiff graft to the more compliant artery. The method of suturing also contributes to the decreased compliance of the anastomosis itself with continuous suturing resulting in greater stiffness^{184,185} compared to interrupted sutures. Other approaches include anastomotic cuffs and patches that include the Linton patch, Miller cuff, St. Mary's boot, and Taylor patch, which rely upon the addition of a vein segment between the graft and the artery to provide a gentle transition in compliance between the graft and the artery as well as to create a gentle geometry of an end to side anastomosis.¹⁸⁵ These adjuncts have resulted in variable improvement in prosthetic graft patency through reduced intimal hyperplasia.

Dialysis Access

Dialysis access grafts reflect an amalgamation of arterial, prosthetic, and venous intimal hyperplasia. Needle puncture sites initially seal by thrombus formation but are then filled in by surrounding connective tissue.¹⁸⁶ Within the fistula, circumferential and valvular stenoses, and mural thrombus at puncture sites can be identified angiographically.¹⁸⁷ The anastomoses are the sites of maximal intimal hyperplasia, resulting from surgical trauma and flow disturbance. Two distinct regions of intimal thickening have been noted: one at the anastomotic site, which is greater in PTFE grafts than in native vein, and the second in the floor of the artery, which is the same for both PTFE and native vessels.¹⁸⁸ The high failure rate of PTFE dialysis grafts is ascribed to intimal hyperplasia at the venous anastomoses.¹⁸⁹ A significant mismatch in elastic properties or compliance mismatch between the vein and the graft and elevated local peak systolic velocity at the anastomoses leads to the development of anastomotic stenosis within the first 2 years.^{190,191}

The geometry of the venous anastomosis, either end-to-end or end-to-side, does not impact intimal hyperplasia because flow stability, turbulence, and kinetic energy transfer are equivalent.¹⁹² Venous anastomotic intimal hyperplasia develops in unbanded grafts, where flow is 100% greater than a banded equivalent, with a direct correlation between the Reynolds number and subsequent intimal hyperplasia development in the outflow vein.¹⁹³ The neointimal lesions at the venous anastomoses consist of smooth muscle cells with extensive ECM. Proteoglycan was identified in the ECM close to the lumen, whereas collagen and elastin predominate deeper in the wall of the vein.¹⁷³ These lesions have significant neovascularization, and perivascular macrophages can be readily identified. There is intense proliferative activity in the neointimal lesions involving both the neovascular endothelial and smooth muscle cells as well as neointimal smooth muscle cells.¹⁹⁴ In areas with high proliferation rates within the neointima, there is intense perivascular staining for tenascin, suggesting that neovascularization is associated with intimal hyperplasia.¹⁹⁵

Treatment of AV graft intimal hyperplasia is typically focused on the venous anastomosis. Standard treatment through the years involved surgical revision with patch angioplasty across the venous anastomosis but is subject to significant recurrence. While it alleviates the stenosis and improves flow, the signals for ongoing intimal hyperplasia persist. Currently, balloon angioplasty of the venous anastomosis offers an easy method of dilating the stenosis and is now the first-line treatment for AV graft intimal hyperplasia. Similar to surgical revision, the patency achieved with balloon angioplasty is often short-lived due to continued cell proliferation and matrix deposition aggravated by the angioplasty injury.¹⁹⁶ Adjunctive maneuvers to improve the durability of angioplasty includes the placement of stents across the anastomosis as well as the use of DCB. The use of bare metal stents across the venous anastomosis, however, did not prove to be more effective than angioplasty alone.¹⁹⁷ While effective at reducing recoil after

angioplasty, the recurrence of intimal hyperplasia through the stent struts resulted in early restenosis. In contrast, Haskal et al.¹⁹⁸ reported on a multicenter trial evaluating stent grafts versus angioplasty alone in AV grafts that showed improved, but still poor, patency with the stent grafts at 6 months (51% vs. 23%). Unfortunately, these findings reinforce the aggressive nature of AV graft intimal hyperplasia. DCBs have been applied to these lesions with mixed results. Small clinical trials in AV fistula¹⁹⁹ and in AV grafts²⁰⁰ demonstrated some early improvement in target lesion patency without an impact on AV access patency at 1 year. Further investigation is needed to really define the role of DCB in AV access. DES use for this application has been minimal to date. One investigational therapy used paclitaxel-coated grafts and found reduced anastomotic intimal hyperplasia in a pig model and the benefit was proportional to the dose of paclitaxel delivered.²⁰¹ Another approach targeted the compliance mismatch at the venous anastomosis with a PTFE stretch cuff,²⁰² which reduced intimal hyperplasia in a pig model.

CONCLUSION

Intimal hyperplasia is an adaptive response of arteries and veins to development and remodeling. It is also a pathophysiologic response to therapeutic vascular injury that contributes to the failure of surgical and endovascular interventions despite substantial advances in our understanding of vessel wall physiology, biology, pharmacology, and pathology. Numerous therapeutic modalities have been proposed and tested through

the decades in an effort to curb this over-exuberant healing response. While DCB and DES offer some hope, the search for an optimal therapy continues.

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Ischemia-Reperfusion

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HISTORICAL BACKGROUND	62
IMPACT OF ISCHEMIA-REPERFUSION INJURY ON THE VASCULATURE	62
Microvascular Dysfunction	62
Arterioles	63
Capillaries	63
Venules	63
Leukocytes and Endothelial Interactions	63
VASCULAR ISCHEMIA-REPERFUSION INJURY SCENARIOS AND CLINICAL TRIALS	64
Myocardial Stunning and Hibernation	64
Acute Limb Ischemia	64
Compartment Syndrome	65

Ischemia-Reperfusion Injury from Aortic Clamping	66
Mesenteric Ischemia	66
Hemorrhagic Shock	66
Multiorgan Dysfunction Syndrome	66
A Role for the Youth	66
PATHOPHYSIOLOGY OF ISCHEMIA-REPERFUSION INJURY	67
Cellular Changes During Ischemia-Reperfusion Injury	67
Programmed Cell Death in Ischemia-Reperfusion Injury	67
Circulating Mediators of Ischemia-Reperfusion Injury	68
FUTURE WORK	68

Ischemia-reperfusion injury (IRI) is the biological equivalent of a well-executed 1-2 punch in boxing. It is best prevented in the first place; the less impactful the first punch, the better. Since the second punch builds on damage of the first punch, limiting ischemia time is mission critical. In IRI, the cells in the affected tissue beds are stunned and then starved by an ischemic state. The initial stunning is a form of resiliency and elicits preconditioning pathways that may be favorable for cell survival, but this is time-sensitive and quickly progresses into cellular starvation and cell death signaling pathways. The vascular surgeon's role is to limit the ischemic time and provide sustained revascularization of the tissue bed. This reperfusion elicits a chemokine surge from resident and migratory cells that evokes both local and systemic damage to this tissue and to the patient as a whole. Both necrotic and apoptotic signaling pathways are evoked locally, but they also drive systemic organ failure that can be lethal.

In this chapter we begin with a brief historical background, and then proceed to discussing how IRI affects the vascular system, and provide a state of the state clinically for vascular patients. We then describe the pathophysiology of IRI at the cellular and protein level and the impact of these pathways on vessel homeostasis. Finally, we provide a brief description of some future directions that may enable a safer transition during reperfusion for patients in the future.

HISTORICAL BACKGROUND

Early work in the myocardium identified two important scenarios related to IRI that deserve attention. First, the “no-reflow phenomenon” was identified. This describes the ischemic time at which not only are the surrounding tissues no longer viable but that the vasculature responsible for perfusion of these tissues can no longer accept blood flow.^{1,2} Second, it was demonstrated that short bouts of ischemia and reperfusion in the heart resulted in subsequent infarct-sparing effects.³ Most recently it has been noted that the structure of the vasculature is disrupted by IRI,⁴ and that this structure may be critical to surviving both the first and second hit. Together these two extremes have led to a robust body of work trying to find the Goldilocks recipe of restoring blood to ischemic tissues and organs.⁵ Unfortunately, this ideal therapy remains a distant goal.⁶ Fortunately for our younger colleagues the field of IRI remains under-developed and is a ripe and important field of primary investigation for vascular surgeons.⁷

IMPACT OF ISCHEMIA-REPERFUSION INJURY ON THE VASCULATURE

Microvascular Dysfunction

The vascular endothelium is an early target of the IRI storm. The endothelium provides an anti-thrombotic barrier for blood

flow and protects against permeability, aided by the endothelial glycocalyx.⁸ The endothelial cell is particularly vulnerable to the deleterious effects of IRI. During ischemia, the lack of oxygen depletes energy stores and alters the endothelial membrane potential leading to cellular stress and, if not corrected, death.⁹ The release of proinflammatory mediators disturbs both tight junctions (occludins and claudin) and adherens junctions (cadherins).¹⁰ The resulting increased vascular permeability and altered vascular tone sets the stage for the systemic spread of IRI. With reperfusion, nitric oxide production decreases and reactive oxygen species (ROS) production increases, contributing to an activated and pro-thrombotic endothelium.⁹ The end response to IRI is reduced blood flow and subsequent perfusion throughout capillary beds and the initiation of robust inflammatory responses.¹¹

Arterioles

In IRI, arterioles have impaired nitric oxide (NO)-mediated vasodilation and an increased reactivity leading to vasoconstriction. In a 1987 landmark study NO was found to be responsible for impaired vasodilation during IRI.¹² Endothelial cells contain both constitutively expressed endothelial NO synthase (eNOS) and inducible NOS (iNOS). In IRI, increased arginase activity exhausts the L-arginine pool critical to NO synthesis.¹³ Impaired vasodilation contributes to vascular smooth muscle impairment and further vasoconstriction. The resultant sluggish flows reduce shear forces within the microvasculature creating a vicious cycle of malperfusion.^{14,15} This transmural vascular dysfunction can be systemic involving key organ systems (lung, liver, intestines) and large volumes of tissues (skeletal muscle and skin).¹⁶⁻¹⁸

Capillaries

Endothelial barrier dysfunction in capillaries is occlusive in nature, either through narrowing or leukocyte plugging. The vessel narrows in response to interstitial edema. Leukocyte plugging occurs in response to platelets and leukocytes attaching to the activated endothelium. Leukocytes are not deformable and can become trapped in the narrowed capillary channels. Some promising therapeutic targets have been discovered in mice that are genetically deficient in leukocyte or endothelial adhesion factors.¹⁹ Stal dynamism, arising from the plugging of capillaries with leukocytes, can be modulated by the injection of anti-Ly6 antibodies that target neutrophils and improve penumbral blood flow.²⁰ Additionally, animals that express superoxide dismutase in excess demonstrate improved microvascular perfusion in the capillaries post-IRI.²¹ Reduced damage at the capillary level may attenuate the inflammatory response both upstream in the arterioles and downstream in the venules, making this a therapeutically attractive target. This location is also the origin of the no-reflow phenomenon.

Venules

The canonical inflammatory response associated with IRI peaks in the venules. As in the capillaries, the endothelial glycocalyx

is damaged early, promoting neutrophil extravasation.⁸ This is mediated by xanthine oxidase and MMP-2 and -9.²² Mast cells and macrophage residing in the interstitial spaces are activated by inflammation and reperfusion and migrate toward the venular endothelium, eliciting leukocyte migration and venule permeability, followed by platelet adhesion and aggregation.^{23,24} Systemically, diffuse edema contributes to hemodynamic instability and volume depletion.

Leukocytes and Endothelial Interactions

Activated neutrophils play a major role in innate immune system-mediated tissue damage as a part of IRI. They are a major source of ROS, generated through two main pathways: (1) the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex and (2) the production of myeloperoxidase from azurophilic granules.²⁵⁻²⁷ In addition to producing damaging O₂⁻ and hypochlorous acid, neutrophils produce membrane-degrading proteases, including matrix metalloproteinases (MMPs), that severely damage the basement membrane and other functional barrier proteins. This inflammatory response, termed “sterile inflammation” because of the lack of microorganisms, is essential for wound repair, but in IRI it is a major source of proinflammatory stimuli and tissue damage.^{26,28}

Transendothelial migration occurs primarily during reperfusion and the restoration of oxygen and nutrients to the tissues. This coordinated passage is accompanied by a significant amount of plasma fluid and protein leakage, attributing to the mechanical disruption of the endothelial barrier. Upregulation of ICAM-1, VCAM-1 and E-selectin allow for margination and rolling, followed by tight adhesion and diapedesis.²⁹ Once across the barrier and inside the tissues, chemotactic factors guide the migration process. TNF-alpha (TNF- α) is one of the best studied endothelial-barrier cytokines. It directly modulates endothelial permeability by regulating the expression of proinflammatory agonist-1 (PAR-1) and the TRPC1 Ca²⁺ channel.³⁰ IL-1, along with TNF- α , is a NF- κ B dependent proinflammatory cytokine that works to upregulate E-selectin and the ICAM-1 pathway of leukocyte extravasation.³¹

The chemokine arsenal that neutrophils secrete is stored in azurophilic and specific granules. Azurophilic granules are lysosome-like organelles that contain myeloperoxidase (MPO), elastase, glycoproteins and neutral proteases.²⁷ MPO catalyzes hydrogen peroxide and chloride ions to produce hypochlorous acid (HOCl), which reacts to form singlet oxygen ($^1\text{O}_2$). ROS initiate lipid peroxidation and leaky membrane bilayers. HOCl also produces hydroxyl radicals through a reaction with superoxide and ferrous iron via the Fenton reaction. These extremely destructive radicals modify DNA through strand breaks and base modifications.³² Specific granules are released from neutrophils during an inflammatory response and proteases, like MMP-8, are released from these organelles further degrading the extracellular membrane and remodel connective tissues.³³ Neutrophils are known to cause remote organ injury, with increased levels seen after aortic occlusion and SIRS.³⁴⁻³⁶

VASCULAR ISCHEMIA-REPERFUSION INJURY SCENARIOS AND CLINICAL TRIALS

Myocardial Stunning and Hibernation

Myocardial IRI has been described in a number of clinical settings including thrombolysis, percutaneous coronary intervention (PCI) and coronary bypass grafting.³⁷ The consequences of IRI in the myocardium include: reperfusion-induced arrhythmias, myocardial stunning, and myocyte death.

After a period of prolonged ischemia, the inner mitochondrial membrane loses the ability to maintain the anion channel that normally stabilizes the myocyte action potential, triggering arrhythmias.³⁸ Up to 50% of the size of an infarct can be attributed to reperfusion injury and a significant amount of viable myocardium immediately post-reperfusion may not be functional 3 hours later.^{39,40} Myocardial stunning is a cell's adaptive survival response to ischemia. Stunning is reversible and the myocardium must exhibit normal or near normal blood flow. This differentiates stunning from hibernation, in which the blood flow is reduced.⁴¹ Although transient, stunning is a prolonged abnormality in myocyte contractility that can result in 24 hours of dysfunctional contractility in response to 15 minutes of ischemia.⁴² Physiologically, it is a consequence of oxidative stress and intracellular calcium overload, leading to decreased responsiveness of contractile elements.⁴³ Both myocardial stunning and myocardial hibernation result in upregulation of myocardial survival gene expression and protein production. Where myocardial stunning represents an absence of myocardial function, hibernation represents a decline in function from baseline. This quasi-stable state persists with contractile dysfunction until revascularization is achieved.

The concept of hibernation is closely linked to ischemic preconditioning, since chronic intermittent ischemia primes the myocardium to adapt to extended ischemic events. Resistance to damage in preconditioning is characterized by both a "first window that confers protection for up to 2 hours" and a "second window that buffers for up to 72 hours" as a result of altered gene expression through heat shock proteins, anti-oxidant enzymes and inducible forms of nitric oxide synthase.^{44–46} These discoveries have resulted in a volume of research that have identified therapeutic strategies capitalizing on infarct-sparing opportunities.

Preconditioning was first shown to reduce infarct size in canines by Zhao, and Staat et al. translated those findings into a successful human trial.^{47,48} The ongoing DANAMI-3 trial, a randomized controlled trial, is investigating two methods to reduce reperfusion injury in patients undergoing multi-vessel intervention for STEMI.⁴⁹ One method aims to perform post-conditioning while the other delays stent placement until 1–3 days after reopening an occlusive coronary artery.^{49,50} In a more focused method, remote ischemic preconditioning (RIPC) through upper arm induction of ischemic cycles has shown some benefit in reducing perioperative myocardial injury in

patients undergoing coronary artery bypass grafting (CABG), despite no improvement in clinical outcomes.⁵¹ While there are reports that RIPC leads to a decrease in myocardial infarction or their markers and renal failure and shorter hospital stays,^{52,53} this was not so in ruptured abdominal aortic aneurysm (AAA) patients,⁵⁴ coronary artery bypass grafting (CABG), or aortic valve operations.⁵⁵ RIPC in cardiac or vascular surgery patients has been used with mixed results and without clear benefit.^{56,57} In fact, the protective effects of RIPC may be more relevant for patients undergoing endovascular interventions,^{58,59} supporting the belief that the ischemic time is the most important contributor to IRI.

Infusion of cardioprotective agents that target specific myocardial biochemical pathways is an established manner of reducing the effects of IRI. Infusion of adenosine proved to reduce infarct size significantly in patients with an anterior MI in the AMISTAD I and AMISTAD II trials.⁶⁰ The protective effects of adenosine are thought to promote post-reperfusion vasodilation and inhibit the inflammatory response in damaged tissues.⁶¹

Acute Limb Ischemia

Acute limb ischemia (ALI) is another complication of IRI with both local and systemic consequences.⁶² ALI involves disruption of normal blood flow to an extremity (usually embolic or thrombotic), and it usually occurs acutely but by definition includes ischemia out to 14 days from symptom onset.^{63,64} Intraluminal shunting can be useful for long transports or other delays to revascularization. After reperfusion, there is massive muscle swelling with substantial release of intracellular contents including myoglobin and potassium that can lead to acute renal failure and hemodynamic compromise. Fasciotomies can protect the peroneal nerve but may not protect against skeletal muscle death, and in the face of unrelenting myonecrosis an urgent amputation may be warranted. In more favorable scenarios volume resuscitation may be able to maintain urine output and prevent renal failure. Over time there can be a significant, sustained, and detrimental inflammatory cell reaction in this tissue that may require urgent amputation to save the patient's life.⁶⁵

A recent review has demonstrated that revascularization of chronic limb ischemia eliciting an IRI is largely driven by oxidative stress damage to the skeletal muscle cell and mitochondria, which is time sensitive and can be mediated to a degree on a biomarker level by certain anti-oxidants.^{66,67} The role of soluble gases like nitric oxide have also been demonstrated to have protective effects.⁶⁸ However, preclinical benefits have been found to be modest.^{69–71}

Best medical therapy for patients who present with acute limb ischemia remains the administration of anticoagulation, in the form of heparin or its derivatives, to inhibit further clot propagation. Multiple trials, including TOPAS, STILE, DUET and others have aimed to surmise which form of intervention is best fitted to ameliorating the IRI, but these are beyond the scope of this chapter.^{72–76} The CRAIL (Controlled

Reperfusion vs. Conventional Treatment of the Acutely Ischemic Limb) trial studied the effects of controlled reperfusion on ALI patients who traditionally would have received conventional thrombectomy with normal restoration of blood flow.⁷⁷ The methodology involved filling a series of blood bags with a combination of oxygenated blood and protective crystalloid solution (ratio of 6:1) containing a dihydrogen phosphate buffer, allopurinol and sodium citrate. The resulting solution is then reperfused into the limb at a constant perfusion pressure of 60 mm Hg for 30 minutes post-thrombectomy. No difference in amputation-free survival was noted at 4-week or 1-year time points.⁷⁷

The clinical trial data to date has been recently summarized⁷⁸ but select trials are summarized in Table 6.1.

Compartment Syndrome

Acute compartment syndrome results from muscle swelling that is restrained from expanding by the fascia. This leads to increased pressures contained to a closed anatomical compartment in the body.⁸¹ The rising intramuscular pressures decrease blood flow to the microvasculature while negatively altering environmental oxygenation and pH.^{82,83} Edema, a sequelae of increased fluid extravasation and interstitial pressures, reduces perfusion to the point of hypoxemia. This decrease in oxygen and increase in oxidant stress leads to the

cascade of channel dysregulation and microvascular dysfunction, cardinal features in IRI.⁸⁴ This process was first outlined by Matsen and Krugmire with the arteriovenous pressure gradient theory that described increased intraluminal venous pressure leading to a decreased pressure gradient between the inflow and outflow.⁸⁵

Without intervention, compartment syndrome culminates in catastrophic downstream effects including muscle necrosis, ischemic contracture, delayed fracture healing, neurologic deficit and acute renal failure.^{86,87} A “two-hit inflammatory model” has been described with a large focus on the surges of TNF- α that occur in compartment syndrome, initially during the inciting events and later after fasciotomy. The second peak causes a deluge of cellular debris and inflammatory mediators to flood the systemic circulation and induce an inflammatory response.⁸⁸ Despite this expected response, timely fasciotomy is the treatment of choice and delay increases 30-day amputation rates.⁸⁹

A high suspicion should be maintained for compartment syndrome, with special attention to the anterior compartment because it hosts the common peroneal nerve, which is important to eversion and dorsiflexion.⁶³ The timetable for nerve damage begins with neuropraxia at 1 hour and, if left to persist past 6 hours, can result in tissue necrosis and impaired limb function, however this may be dependent on degree of intra-compartmental pressures.⁹⁰

TABLE 6.1 Relevant Clinical Trials for IRI

Trial	Hypothesis	Patients (n, male vs. female)	Outcomes
DANAMI-3; NCT01435408 ⁴⁹	1. Ischemic post-conditioning will reduce infarct size 2. Deferred stent implantation post-PCI will reduce IRI	Ongoing	Ongoing/primary end point: all-cause mortality or hospitalization for heart failure (2 years out)
Cardioprotective and B1:G10 Effects of Remote Ischemic Preconditioning in Patients Undergoing Coronary Artery Bypass Surgery; NCT01406678 ⁵¹	Remote ischemic preconditioning (RIPC) would reduce risk of myocardial injury after CABG	RIPC (n = 162; 134 male and 28 female) vs. Control (n = 167; 135 male and 32 female)	Lower concentrations of cardiac troponins in RIPC group; lower all-cause mortality in RIPC group
CIRCUS: Cyclosporine before PCI in Patients with Acute Myocardial Infarction; NCT01502774 ⁷⁹	Cyclosporine will improve outcomes and prevent adverse LV remodeling	970 patients: 474 cyclosporine, 495 control/80% male cyclosporine, 84% male control	No significant benefit
CYCLE: Cyclosporine A in Reperfused Myocardial Infarction: The Multicenter, Controlled, Open-Label CYCLE Trial; NCT01650662 ⁸⁰	Will cyclosporine improve ST-segment resolution?	410 patients (79% male)	No effect on clinical outcomes or ST-segment resolutions at 6 months
CRAIL: Controlled reperfusion versus conventional treatment of the acutely ischemic limb: results of a randomized, open-label, multicenter trial; NCT00567801 ⁷⁷	Controlled reperfusion may have protective effects on ischemic tissue	174 patients	No significant benefit on amputation-free survival at 4 weeks or mortality
Heart			
Limb			

Ischemia-Reperfusion Injury from Aortic Clamping

The aorta is the major artery of the body, and is responsible for accepting and propagating the fluid bolus from each heartbeat for distribution to the organs and tissue of most of the human body. Aortic clamping is required for certain vascular operations, and upon release of the clamp induces a powerful IRI.⁹¹ The initial clamp increases the systemic vascular resistance (SVR) and releases vasoactive catecholamines and angiotensin that further increase SVR.⁹² The removal of the clamp decreases the SVR and initiates a relative hypovolemia due to vessel dilation downstream of the clamp and the subsequent release of vasodilators like adenosine, lactate and CO₂ that perpetuate the IRI.^{93,94} To lessen this response, it is common to slowly come off the clamp and to coordinate unclamping with volume support from anesthesia.

The proximity and duration of the aortic clamp towards the heart significantly increases the risk of IRI and spinal cord injury. There is a relatively recent review of how best to prevent damage to the spinal cord, including the use of hypothermia.⁹⁵ The pathophysiology of IRI is similar for neurons and skeletal muscle cells (ATP depletion, Na⁺ overload leading to cellular edema, followed by necroptotic/apoptotic pathways), but since neurons do not survive prolonged ischemic events, the oxidative stress from reperfusion is not as well understood in neurons. As such, current experimental approaches target neuronal resilience.⁹⁶ Endovascular approaches may limit ischemia time to the cord and visceral vessels. Adjunctive limb revascularization (when able) may also be useful in limiting IRI in other territories as a cause of spinal cord morbidity in select patients.^{97,98} One promising pharmacologic approach utilizes a PARP-1 inhibitor (INO-1001) that helped with hemodynamic support after 45 minutes of aortic cross-clamping a porcine aorta.⁹⁹ The same drug later proved to reduce spinal cord damage after aortic occlusion induced IRI.¹⁰⁰

Mesenteric Ischemia

IRI can occur after revascularizing patients with both chronic and acute mesenteric ischemia (AMI).

The intestines have an exceptional amount of surface area and are home to a rich microbiome that make the intestine very sensitive to IRI. The gravity of reperfusion injury in the gut was demonstrated by Parks and Granger when they showed that 3 hours of ischemia and 1 hour of reperfusion resulted in greater mucosal damage than 4 hours of ischemia alone.¹⁰¹ During ischemia, the intestinal barrier integrity is injured, allowing for bacterial translocation through the mucosa and into surrounding lymph nodes and tissues.¹⁰² As aerobic metabolism slows and ATP is depleted, the integrity of the mitochondrial membrane is again compromised.¹⁰³ If ischemia persists past 45 minutes, cell death ensues.¹⁰⁴ After reperfusion, energetic metabolism is re-established, and

inflammatory cells are recruited to sites of epithelial disruption.^{105,106} The complement cascade deposits activated C3 throughout the damaged lumen and IL-6, IL-8 and TNF- α (among others) are released.¹⁰⁴ The Paneth cell is found in the crypts of Lieberkun.¹⁰⁷ In a healthy gut, the Paneth cell secretes antimicrobial proteins into the lumen and reinforces the microbiome-gut barrier. Under IRI Paneth cells experience heightened endoplasmic reticulum stress, compromising their ability to prevent bacterial translocation.¹⁰⁸

Hemorrhagic Shock

Hemorrhagic shock produces global ischemia and often requires large volume resuscitations that result in massive third spacing and edema. In the abdomen, this results in intestinal edema and abdominal compartment syndrome, both of which create an ischemic state for the gut and intra-abdominal organs. Abdominal compartment syndrome requires surgical decompression to relieve the intra-abdominal pressure.^{109,110} As with other forms of IRI, survivors have their edema improve over time, and in this scenario, the edema typically allows abdominal closure in 5–7 days.¹¹¹ Resuscitative endovascular balloon occlusion of the aorta (REBOA) has been developed as a tool for improving survivability of non-compressible vascular injuries. While this is a new technique, and there remains hope for utility with REBOA, the prolonged ischemic times and variability of testing in large animal models limit our ability to bring meaningful data to this chapter.^{112–114}

Multiorgan Dysfunction Syndrome

MODS is a particularly devastating consequence of IRI and is the endgame of an untampered chemokine storm. MODS is a leading cause of death in critically ill patients and a recognized outcome of reperfusion injury. The number of involved organ systems is closely associated with the danger of mortality and often begins with deterioration of the pulmonary system, trailed closely by hepatic, renal and GI dysfunction.¹¹⁵ Respiratory failure progresses to acute respiratory distress syndrome, a rapidly progressive exacerbation of pulmonary edema and infiltration, within 72 hours. Intestine and liver IRI leaves the lungs especially vulnerable, since they are the first capillary bed encountered by the post-ischemic surge of blood.¹¹⁶ MODS is a complicated combination of insults that manifests with venous and arterial thrombosis, disseminated intravascular coagulation and, ultimately, death.

A Role for the Youth

Anecdotally, the young tolerate IRI better than adult patients. The benefits of youth on IRI have been demonstrated experimentally in pigs,¹¹⁷ guinea pigs,¹¹⁸ rabbits,¹¹⁹ dogs,¹²⁰ and mice.¹²¹ Since vascular disease (outside of trauma) is rare in the young, the mechanistic resiliency identified clinically in young patients may be of great use in identifying translatable pathways that can then be tested in the above animal models.

Diabetes is known to accelerate cellular aging; as such it is not surprising that IRI in diabetic patients is exacerbated through many molecular pathways.¹²²

PATOPHYSIOLOGY OF ISCHEMIA-REPERFUSION INJURY

Each organ or tissue can endure a different degree of ischemia, with human skeletal muscle lasting up to 2 hours before irreparable injury and the small intestine experiencing irreversible damage in just 30 minutes.^{104,123,124} IRI pathophysiology occurs within and outside of the cells in these organs and tissues. In this section we group the discussion in this manner.

Cellular Changes During Ischemia-Reperfusion Injury

Both metabolic dysfunction and alterations in gene expression occur as a result of ischemic injury. Decreased oxygen delivery depletes intracellular stores of adenosine triphosphate (ATP) and upregulates anaerobic metabolism via glycolysis. As a part of glycolytic ATP production, lactic acid byproducts are released, leading to a drop in intracellular pH and accelerating Na^+/H^+ exchange at the cell membrane. This influx of sodium ions acts as a buffer for rising H^+ concentrations inside the cell.^{125,126} Sodium draws water with it causing intracytoplasmic accumulation of water and hydropic swelling. As anoxia becomes more prolonged, ATPases are inactivated and ATP-dependent Ca^{2+} reuptake is interrupted at the cell membrane, allowing Ca^{2+} to build up intracellularly.¹²⁷ The Na^+/K^+ ATP-dependent ionic pump fails without sufficient substrate, further disrupting the transmembrane gradient. Calcium accumulation leads to the activation of intracellular proteases and activation of calpain. Once activated, calpain cleaves Bid, which targets the mitochondria to release pro-apoptotic factors. Calpain directly injures the contractile apparatus through hyper-contracture and contraction band necrosis via its substrates, troponin and tropomyosin.¹²⁸ Calcium-mediated activation of proteases also triggers conversion of xanthine dehydrogenase to xanthine oxidase. Xanthine oxidase acts as a significant source of superoxide and free radical production used in IRI.^{129,130}

Under ischemic conditions, hypoxia inducible factor-1a (HIF-1a) levels increase dramatically, allowing dimerization with HIF-1b and transcriptional activation of vascular endothelial growth factor (VEGF).⁷⁰ Despite an overall inhibition of protein synthesis during hypoxic conditions, VEGF mRNA expression is increased.^{131,132} Hypoxic stress also upregulates the expression of NF- κ B, a pro-inflammatory response element that stimulates COX-2 and the arachidonic acid pathway. Other genes that are upregulated during hypoxia include: (1) activating protein-1 (AP-1), a pleiotropic transcription factor involved in apoptosis, proliferation and inflammation; (2) p53, the “guardian of the genome” involved in cell cycle arrest and apoptosis; (3) SP-1 and SP3, house-keeping genes; and (4) early growth response-1 (Egr-1),

a zinc finger transcription factor that modulates extracellular matrix remodeling, cell survival and thrombosis.^{133,134} As these complex cellular processes are occurring, low pH acts protectively to slow them, further crediting the observation that irreversible damage may be avoided if reperfusion occurs within a short timeframe.^{135,136}

Paradoxically, the restoration of blood flow restores substrates needed for ATP generation, instantaneously increases the oxygen supply and normalizes the pH through washout.^{126,137} This is accompanied by a burst of ROS production via the mitochondrial electron transport chain. ROS initiates cell death primarily through apoptosis and necrosis, two forms of programmed cell demise seen during IRI.¹²⁵

Programmed Cell Death in Ischemia-Reperfusion Injury

Programmed cell death encompasses multiple mechanisms of orchestrated cellular signaling cascades: apoptosis, autophagy, necrosis and necroptosis. These cell death-related processes, specifically apoptosis and necrosis, are seen in IRI and are classified according to morphological appearance, involved enzymes and immune characteristics. Apoptosis is a gene-directed cell death cascade while necrosis is a more passive form.¹³⁸

Apoptosis is an orderly form of programmed cell death seen in IRI. It is primarily activated in response to hypoxic stress and ROS production and is mediated through a mitochondrial response to Bcl-2 proteins. Apoptotic mechanisms are traditionally divided into two major branches that overlap significantly: the extrinsic death receptor pathway and the intrinsic mitochondrial pathway. While the intrinsic pathway transmits most apoptotic stimuli and is the chosen pathway of cell death in ischemia-reperfusion injury, the extrinsic pathway is specialized to transduce signals from binding of specific cell-death ligands. The intrinsic pathway is largely dependent on the activation of proapoptotic proteins Bax and Bak and their effects on the mitochondrial membrane. In IRI, cytoplasmic antiapoptotic Bcl-2 and Bcl-xL are activated by cytoplasmic Bad. In parallel, Bax and Bak proteins undergo a conformational change, translocate into the outer layer of the mitochondria and permeabilize the membrane, activating proapoptotic proteins like cytochrome c, Smac/Diablo and endonuclease G.¹³⁹ Thus it is the blocking of Bcl-2 and Bcl-xL or the activation of Bax and Bak and opening of the mitochondrial permeability transition port (MPTP) that initiate apoptosis. The released proapoptotic factors use caspases-9 and -3 to create an “apoptosome” that stimulates the apoptotic phenotype.^{139,140} The extrinsic pathway is initiated by the binding of the Fas ligand (FasL) to the Fas receptor. This triggers recruitment and formation of a death-inducing signaling complex (DISC) that initiates downstream events to commence DNA injury and cell death.¹⁴¹ Both pathways involve sophisticated and orderly processes that are designed to discard redundant or damaged cells, making this coordinated effort the hallmark of apoptosis. Cells display distinct morphology with shrinkage, condensation and margination

of chromatin, late stage nuclear fragmentation, the formation of apoptotic bodies and plasma blebbing. Biochemically, phosphatidyl-serine is exposed on the outer leaflet of the plasma membrane, marking the cell for removal by phagocytes.^{142,143}

Necrosis is a disorderly cell death that is mediated through necroptotic pathways. Hallmarks of necrosis include cell disintegration, organelle swelling, plasma membrane permeation and loss of mitochondrial function. Previously regarded as unregulated and passive, a subset termed necroptosis is initiated by death receptor activation.¹⁴⁴ Programmed necrotic cell death requires both calcium and ROS and is mediated by (1) TNF-receptor signaling and necrosome formation; (2) direct opening of the mitochondrial MPTP and subsequent uncoupling.^{144,145} The collective endpoint of regulated necrosis is cell membrane fragmentation and release of cytoplasmic enzymes that induce an immune response. The molecular pathway of necroptosis is embodied by the TNF signaling pathway. It begins with stimulation of TNFR1 (TNF receptor 1) and the formation of a TNFR1-associated death domain-dependent (TRADD) receptor-bound complex.¹⁴⁶ The mitochondrial pathway is driven by the opening of the mitochondrial permeability transition pore (MPTP). This is dependent on the MPTPs direct interaction with cyclophilin-D (CyP-D), a prolyl isomerase in the mitochondrial matrix.¹⁴⁴ During reperfusion, ROS flood the cellular environment and the MPTP opens, permitting the loss of water and solutes along with NAD⁺ and leading to impaired mitochondrial uncoupling and decreased electrical potential.^{144,145} Necroptotic pathways are druggable^{147,148} and hold promise for the hibernation of tissue during the ischemic prodrome of IRI.^{149,150} This is discussed in more detail in “Future Work”, below.

Circulating Mediators of Ischemia-Reperfusion Injury

As ischemic tissue is reperfused by an influx of oxygen, xanthine oxidase is degraded to liberate the superoxide anion (O_2^-) and superoxide anion (OH^{\bullet}).¹²⁹ These derived oxidants travel systemically, attaching to endothelial cells and mediating cytotoxicity throughout the body's vascular network.¹⁵¹ Hydroxyl radicals act on the lipid bilayer to release proinflammatory eicosanoids through peroxidation.¹⁵² Xanthine oxidase derivatives also increase expression of cell adhesion molecules, like E-selectin, intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and other chemotactic factors such as plasminogen activator inhibitor-1 (PAI-1), interleukin-1, and tissue factor for the enlistment of neutrophils to locally inflamed tissue.¹⁵³ Given the smaller scale but more frequently repeated IRI that occurs in claudication, it is not surprising that ROS drives many of the pathologic skeletal muscle and neural fibrosis that occurs in peripheral arterial disease, where there is chronic oxidative stress (Fig. 6.1).^{154–158}

The complement system is activated by the classical, alternative or mannose-binding lectin pathways. It is composed of

roughly 30 soluble and membrane-bound proteins and notable markers include C3 and C5 convertase.¹⁵⁹ Regardless of which pathway is induced, the complement system culminates in the formation of the membrane attack complex which injures tissues through opsonization and direct cell lysis. The first description of the complement system's involvement in ischemia-reperfusion injury was in ischemic rat myocardium in 1971. Hill and Ward demonstrated the “bypass method,” describing an enzyme released in ischemic myocardium that “cleaved the third component of complement” (C3) and caused an acute inflammatory response to C3 fragments.¹⁶⁰ Later, mRNA and proteins for complement system components were noted to be increased in ischemic tissues, confirming local production of these inflammatory mediators.¹⁵⁹ Since then, multiple studies have demonstrated the importance of the complement cascade in ischemia-induced cell necrosis.^{161–163} Additionally, actin-mediated aggregation of the cytoskeleton induces neoantigen expression on the cell surface, acting as a substrate for the complement system and deposition of IgM and C3. Complement is activated in IRI. Blockade of complement receptor type one (sCRI or TP10) inhibits neutrophil–endothelial–selectin interactions in animal models of ischemic skeletal muscle, but it had a limited effect in clinical trials.^{164,165} The MAC complex and its major components, C5 and C9, are viable targets for biologics and many have been developed to inhibit their function or formation, including eculizumab and Mubodina.¹⁶⁶

Finally, neutrophil-mediated tissue injury (NMTI) is largely a result of protease enzymes, including cathepsin G, elastase and trypsin.¹⁶⁷ In IRI, these enzymes contribute to tissue necrosis and functional impairment.

In summary a variety of chemokines are elicited by IRI, and there are robust reviews out there with much of the data linked to the liver or other solid organs and pathology outside of the current purview of vascular surgery (Table 6.2).¹⁶⁸

FUTURE WORK

IRI provides rich fodder for basic and translational science. For example, one promising area of study is the necroptotic pathways. Caspase 8 (Casp8) drives extrinsic apoptosis and prevents unleashed receptor-interacting serine/threonine-protein kinase 3 (RIP3) necrosis in mice. In humans, Casp8 and Casp10 functions overlap. These self-activating caspases lie downstream of TNF family death receptor signal transduction. Casp8 (together with Casp10 in humans) and RIP3 (together with RIP1 in mice or humans) are now recognized as entwined components of innate host defense system common to all mammals that can trigger apoptosis or necrosis. These mechanisms appear to regulate cell necrosis in general. Thus, this pathway may be targeted to limit cell necrosis in IRI. Kaiser et al. have shown that double knockout mice that lack both apoptotic and necrotic pathways, are insensitive to inflammatory insult compared to wild type (WT) mice due to reduced death.^{189,190}

Ideas that link the inflammatory cells with limiting the exuberant response to reperfusion hold great promise in our march to novel solutions for IRI.

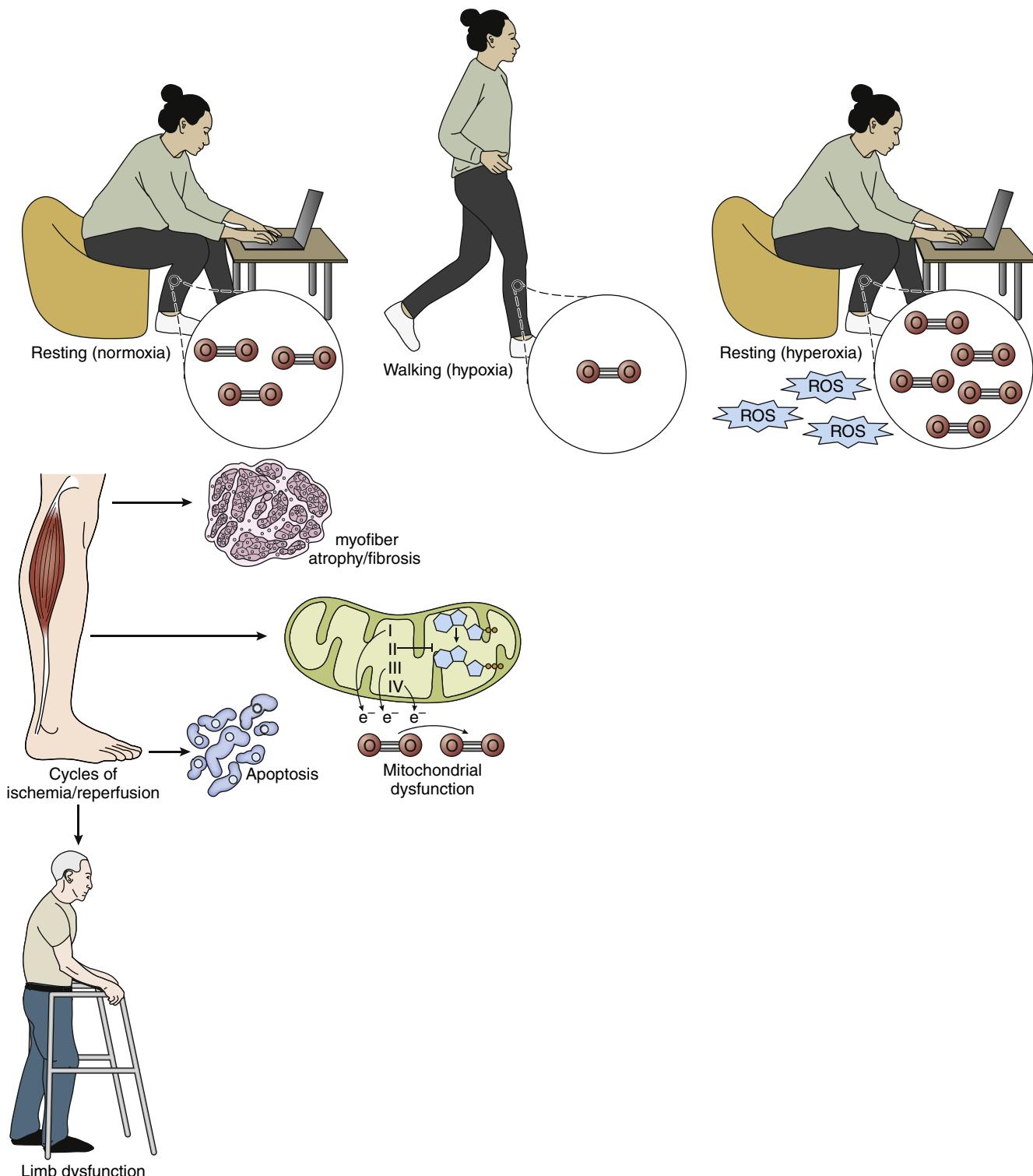


Figure 6.1 Oxidative Stress-Induced Pathology for Peripheral Arterial Disease Patients. (Provided by Dr. Panos Koutakis, Baylor University.)

TABLE 6.2 Summary of IRI Mediators Holding Clinical Importance to Patients

Molecule	Description	Produced or Induced by	Function	Data Derived in Human or Animal	Critical studies/relevance to IRI
Endothelin ^{169,170}	21-amino acid peptide	Endothelial cells of the cardiovascular system	Potent vasoconstrictor (ET _A , vascular specific subtype)	Animal (2005), Human (2016)	Inhibited by bosentan in the treatment of pulmonary hypertension Inhibition by BQ-123 (ET _A) in MI patients resulted in decreased lipid peroxidation Increased NO activity and a decreased ratio of infarcted area to area at risk
Adenosine ^{171–173}	Endogenous nucleotide	Degradation of ATP	Endogenous vasodilation, neutrophil inhibition of ROS and intracellular calcium levels (via K ⁺ ATPase activation)	Animal	Phospholipase C and A3 receptor-induced cytoprotection of skeletal muscle in murine model Improved murine hind-limb function at 48 hours post-IRI
Adreno-medullin ^{174–176}	Vasodilatory peptide	Isolated from human pheochromocytoma Levels increase significantly after reperfusion	Vasodilation (mediated by NO and cAMP) Inhibition of alternative complement pathway (AMBP-1 binding)	Animal	Infusion attenuated myocardial ischemia/reperfusion injury at 24-hours through reduced infarct size, left ventricular end-diastolic pressure and apoptosis Administration after gut IRI downregulated levels of TNF- α , IL-1 β , IL-6, IL-10, AST, ALT, lactate and creatinine Ameliorate lung injury after IR
Heme Oxygenase System and Carbon Monoxide ^{177–179}	Heat-shock protein (HSP-32); HO-1 = inducible form	Present throughout the body (endothelial, epithelial and smooth muscle cells) Increased activity in the spleen during erythrocyte turnover	Catalyzes the degradation of heme to produce biliverdin, ferrous iron and carbon monoxide Downregulation of pro-inflammatory cytokines HO-1 is rapidly transcribed under times of stress by Kupffer cells	Animal	Anti-inflammatory, antiapoptotic, and anti-proliferative actions Promotes CD4 $^+$ T cell death through activation induced cell death (AICD) Acts as cytoprotective component in states of hemorrhagic shock Protects against free radical induced cellular damage
Anticoagulant (heparin, LMWH) ^{180–182}	Naturally occurring GAG anti-coagulant	Stored within mast cells; released in response to inflammation	Binds to antithrombin III (AT III) to induce conformational change; AT III then inactivates thrombin and factor Xa Defense against bacterial invasion or foreign materials	Human Cell Line (2017)/ Animal (2014, 2012, 2008, 2019)	LMWH enhances endothelial barrier function and inhibits transendothelial migration Decreases remote organ injury in the heart, lung and kidney post-IR Enoxaparin (LMWH) is debatably believed to ameliorate tissue thrombosis and muscle necrosis in models of hind-limb ischemia
Hypo-thermia ^{183–186}	27–34°C	Physiologic response to injury	Slows tissue metabolism Decreases tissue edema	Animal	Reduces myocardial infarct size Protects skeletal muscle ischemia in early-reperfusion IR model; decreased CD11b an neutrophil recruitment Reduced oxygen consumption, tissue damage and size of infarct after 6 hours of ischemia in the canine gracilis muscle
Neurogenic ^{187,188}	CN X	Parasympathetic control of the heart, lungs and GI tract	Slows heart rate through decreased force of ventricular contraction and decreased myocardial oxygen demand	Animal	Suppressed cytochrome-c release, caspase-3 activation and MMP-1, MMP-9 release in myocardial reperfusion injury model Improves left ventricular EDP and reduced infarct size; ameliorates LV remodeling Inhibits mitochondrial permeability transition pore (MPTP) opening

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Arteriogenesis and Angiogenesis

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Based on a previous edition chapter by Adam Strickland and Paul DiMuzio

NEOVASCULARIZATION 72

ARTERIOGENESIS 73

Cardiovascular Risk Factors Associated with Impaired Arteriogenesis 76

ANGIOGENESIS 76

Sprouting Angiogenesis 77

Regulation of Angiogenesis 77

Lumen Formation 79

Intussusceptive Angiogenesis 79

Remodeling and Pruning 80

ROLE OF NONCODING RNAs IN NEOVASCULARIZATION 80

CLINICAL TRIALS 82

Gene and Protein-Based Therapies 82

Vascular Endothelial Growth Factor 82

Fibroblast Growth Factor 82

Hepatocyte Growth Factor 82

Developmentally Regulated Endothelial Locus-1 85

Hypoxia-Inducible Factor 85

Cell-Based Therapies 85

Endothelial Progenitor Cells 85

Bone Marrow-Derived Cells 85

Fully Differentiated Cells 86

Peripheral arterial disease (PAD) of the limbs can progress to critical limb ischemia (CLI), characterized by rest pain and tissue loss, including nonhealing ulceration and gangrene.^{1,2} The body compensates via neovascularization, either the formation of collateral circulation to bypass the obstructed vessel (arteriogenesis) or increasing capillary density (angiogenesis) to deliver oxygen and nutrients to ischemic tissue. Despite these responses, disease progression can lead to amputation, decreased quality of life, comorbidity, and death and is an economic burden to the healthcare system.

Traditional treatment of PAD includes risk factor modification (tobacco abuse, diabetes, hypertension, and hyperlipidemia), exercise programs, and medical therapy (antiplatelet agents, anticoagulants, and phosphodiesterase inhibitors).^{1–3} As atherosclerosis progresses, more invasive intervention may be necessary, including endovascular and open surgical therapies. Patients with CLI may not be candidates for these interventions because of severe medical comorbidity or nonreconstructable vascular disease. This patient population may be candidates for biologic treatments, including gene-based, molecular, and cell-based therapies designed to promote healing and prevent amputation.

Advances in basic science research have developed these biologic therapies over the past two decades. Early clinical trials

focusing on gene and molecular therapies, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), have demonstrated limited benefit. More promising results have been observed using cell-based therapies, including endothelial progenitor cells (EPCs) and bone marrow-derived mononuclear cells (BM-MNCs).

Herein, the basic science and processes involved in neovascularization (specifically arteriogenesis and angiogenesis), as well as recent human clinical trials designed to promote neovascularization for CLI, are discussed.

NEOVASCULARIZATION

Neovascularization refers to the formation of new blood vessels and includes vasculogenesis, arteriogenesis, and angiogenesis.⁴

Vasculogenesis is the de novo formation of embryonic blood vessels from vascular progenitor cells or hemangioblasts, which develop into hematopoietic precursors and endothelial cells.⁵ These cells induce differentiation of discrete vascular layers as cells transform into endothelial cells, smooth muscle cells (SMCs), and adventitial pericytes. Although vasculogenesis primarily occurs during the embryonic stages of development, postembryonic adaptive vasculogenesis results from either arteriogenesis or angiogenesis.^{6,7}

Arteriogenesis involves growth of collateral vessels and remodeling from preexisting arterial–arteriolar connections.^{6,8} Arteriogenesis is induced by a change in hemodynamic forces (fluid shear stress [FSS]) resulting from pressure differences within an artery narrowed by atherosclerosis. FSS activates the endothelium, leading to increased transcription of the promoter regions of a variety of proteins contributing to vessel growth (Table 7.1).⁴ With progressive arterial stenosis, blood follows the path of least resistance and is shunted into collateral vessels.⁴ Collateral vessel formation maintains a degree of flow beyond the obstruction.

Angiogenesis, which refers to the sprouting of new capillaries from preexisting ones, is stimulated by decreases in oxygen tension secondary to reduced tissue perfusion. Local ischemia stimulates an increase in hypoxia-inducible factor-1 (HIF-1), which results in increased production of VEGF, a potent angiogenic factor. VEGF induces endothelial cell proliferation and increases endothelial permeability.⁸ Matrix metalloproteinases (MMPs) locally degrade basement membrane and extracellular matrix (ECM), allowing vessel growth and increased tissue perfusion.^{9–11} Although this increased flow provides greater delivery of oxygen and nutrients to ischemic tissues, it is often not sufficient to overcome major arterial obstruction.^{8,11,12}

Postembryonic arteriogenesis and angiogenesis occur over a continuum of vascular adaptations (Fig. 7.1).⁷

ARTERIOGENESIS

Chronic, progressive arterial stenosis leads to generation of a collateral network by remodeling preexisting arterial–arteriolar connections (Fig. 7.2). The formation of this collateral circulation is primarily in response to an increase in FSS with a contribution from increased circumferential wall stress. As an example, femoral artery occlusion results in up to a 200-fold increase in shear stress in the arteriolar network.⁵

FSS is proportional to flow velocity and inversely proportional to the radius cubed, whereby small changes in radius can normalize shear stress.¹¹ Increased FSS activates the endothelium.^{5,8,13} Nitric oxide (NO) is liberated by endothelial cells (via endothelial NO synthase [eNOS]) as well as by macrophages and SMCs in the adventitia (via inducible NOS). NO induces SMC relaxation and vasodilatation beyond the arterial occlusion, thereby improving blood flow.^{12,14,15} NO also stimulates endothelial VEGF secretion, leading to the release of endothelial cell adhesion molecules (CAMs) and monocyte chemotactic protein-1 (MCP-1) by endothelial and SMCs.^{5,6,15} Both

TABLE 7.1 Transcription and Growth Factors Influencing Arteriogenesis and Angiogenesis

Factor	Characteristics	Role in Arteriogenesis and Angiogenesis	Induced by
Transcription Factors			
HIF-1 ^{121–129}	Helix:loop:helix structure Mainly involved in angiogenesis but possible role in VEGF stimulation in arteriogenesis	Induces gene transcription to promote angiogenesis in ischemic environment Is involved in hypoxic vasodilation, cell growth, proliferation, migration, sprouting, recruitment of pericytes/SMCs, vascular remodeling, mobilization of angiogenic cells and EPCs Also induces MMPs for ECM digestion	Hypoxia
EGR-1 ^{148–153}	Increased expression in endothelial cells, SMC, fibroblasts, leukocytes	Required for expression of cyclin D1, a regulator of the cell cycle in vascular cells	Shear stress, mechanical injury, hypoxia, PDGF, FGF-1, FGF-2
Post-Transcriptional Regulators			
MicroRNAs ^{74,80,82,86,154–157}	~22 nucleotides in length, binds to 3' UTR of target mRNA, single stranded RNA Post-transcriptional gene regulation	Promote and/or inhibit angiogenesis and arteriogenesis through interactions with various transcription factors, cytokines, and cell adhesion molecules	Varies with each miRNA
Growth Factors/Cytokines			
VEGF ^{88–97}	Survival factor for endothelial cells Involved in angiogenesis and arteriogenesis	Promotes proliferation, migration, lumen formation Induces monocyte chemotaxis via binding to VEGFR1 on monocytes	Hypoxia and shear stress
FGF ^{88,90–95,103–105}	Perivascular macrophages are source of FGF-2 during collateral growth ⁷ Role in arteriogenesis and angiogenesis	Induces endothelial/SMC proliferation Stimulates endothelial cell migration/differentiation, and increases EGR-1 expression Potentiates VEGF and may be synergistic with VEGF-B	Shear stress, endothelial activation

Continued

TABLE 7.1 Transcription and Growth Factors Influencing Arteriogenesis and Angiogenesis—cont'd

Factor	Characteristics	Role in Arteriogenesis and Angiogenesis	Induced by
TGF- β ^{11,16,126,158–160}	Expressed in areas of collateral formation	Expressed in developing collateral arteries Stimulates arteriogenesis by effects on endothelial cells, vascular SMCs, monocytes, macrophages	Shear stress, endothelial activation
TNF α ^{161–164}	Proximal mediator of inflammation	Angiogenic effects from TNFR2R Enhances activation and adhesion of monocytes by upregulating cell adhesion molecules Upregulates GM-CSF Necessary for migration/adhesion of BM-hematopoietic cells to endothelium via NO synthase-dependent mechanisms	Stimulated by endothelial activation by shear stress and LPS Lipopolysaccharide Ischemia stimulated TNFR2 expression
GM-CSF ^{6,11,165,166}	Enhances arteriogenesis through effects on circulating cells	Enhances release, proliferation, and differentiation of hematopoietic stem cells, mobilization of endothelial progenitor cells Amplifies effects of MCP-1, ¹¹ promotes survival of monocytes and macrophages	Stimulated by endothelial activation by shear stress
HGF ^{109–112}	Augments arteriogenesis by enhancing endothelial cell function Role in angiogenesis and arteriogenesis	Activates Dll4-Notch-Hey2 pathway for inducing proliferation and migration of endothelial cells	Hypoxia, shear stress
ECM Proteins			
Del-1 ^{112,118–120}	Involved with angiogenesis		Ischemia
Chemokines and Chemokine Receptors			
MCP-1 (or CCR2) ^{6,167–170}	CCR2 axis: MCP-1 binds CCR2 receptor on monocytes Potent stimulator of arteriogenesis and angiogenesis	Increases attraction/adherence of monocytes and tube formation. May attract endothelial progenitor cells to sites of vascular injury	Hypoxia and shear stress
ELR-containing CXC ^{167–169,171}	Binds receptors on CXCR1/2/3 Potent stimulator of angiogenesis, some forms inhibit angiogenesis via inhibition of VEGF/FGF	Binds chemokines MIG, IP-10, I-TAC, and PF-4 Decreases formation of collaterals and restoration of perfusion in knockout mice Perfusion improved with infusion of bone marrow mononuclear cells	Hypoxia
Cell Adhesion Molecules			
ICAM, VCAM-1, PECAM-1 ^{172–177}	Enhance attraction and adhesion of monocytes	Support diapedesis of monocytes, while enhancing cell signaling and activation of mechanosensory complexes that activate intracellular changes in response to shear stress	Shear stress
Proteases			
MMPs ^{910,173,178–186}	Macrophages and monocytes are source of MMPs in ischemic/nonischemic tissue Involved in angiogenesis and arteriogenesis	Allow for ECM remodeling via proteolytic degradation of ECM/BM, enabling collateral vessel/capillary growth and endothelial cell migration Liberate growth factors and stimulate endothelial proliferation ECM/BM breakdown by MMPs promotes SMC proliferation and migration	Shear stress and hypoxia; MCP-1 activates macrophages to secrete MMPs MMPs activate release of more MMPs from macrophages

Continued

TABLE 7.1 Transcription and Growth Factors Influencing Arteriogenesis and Angiogenesis—cont'd

Factor	Characteristics	Role in Arteriogenesis and Angiogenesis	Induced by
Immune Cells			
Monocytes/ Macrophages ^{6,9,48,161,173,174,183,184,187–194}	Induce vascular cell proliferation and wall remodeling via paracrine effects	Promote vascular growth and secrete growth factors (MMP, NO, VEGF, FGF-2, GM-CSF, TGF- β , TNF- α) that stimulate arteriogenesis Monocytes accumulate in wall of growing collateral and differentiate into macrophages, liberating MMPs to digest ECM; encourages migration and proliferation of endothelial cells and SMCs	Shear stress-activated endothelium expresses MCP-1, leading to adhesion of monocytes
T cells/NK cells ^{4,176–206}	Immune cells	CD4 and CD8 mononuclear cells migrate to collateral vessel, initiating arteriogenesis/angiogenesis by cytokine activation Athymic mice have higher rates of autoamputation than heterozygotes NK cell deficient mice are unable to form collaterals	Shear stress
Mast cells ^{10,11,206–208}	Present in adventitia of collateral arteries	Release TGF- β , VEGF, FGF-2, MMPs, histamine, serotonin	
Other Cells			
BM-EPCs ^{166,201–203,209–217}	BM-derived cells	Attracted to sites of neovascularization, differentiate into endothelial cells	Shear stress, ischemia Release stimulated by VEGF, SDF-1
Pericytes ^{217–219}	BM-derived cells	Release VEGF, FGF-2, MCP-1 and MMPs, promoting endothelial cell migration, proliferation, and survival	Shear stress
Vascular SMCs ^{11,219–222}	Derived from endothelial cells, mesenchymal cells, and BM-derived cells	Collateral vessel stabilization via ECM production	SMCs attracted by PDGF/VEGF Proliferation stimulated by ECM breakdown

BM, bone marrow; BM-EPCs, bone marrow-derived endothelial progenitor cell; CCR2, CC chemokine receptor 2; CXCR, CXC chemokine receptor; Del-1, developmental endothelial locus-1; ECM, extracellular matrix; EGR-1, early growth response protein-1; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; FGF, fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGF, hepatocyte growth factor; HIF-1, hypoxia-inducible factor-1; ICAM, intracellular adhesion molecule; IL, interleukin; IP-10, interferon gamma-induced protein 10; I-TAC, interferon-inducible T-cell alpha chemoattractant; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MIG, monokine inducible gamma-interferon; MMP, matrix metalloproteinase; NK, natural killer; NO, nitric oxide; PDGF, platelet-derived growth factor; PECAm, platelet endothelial cell adhesion molecule; PF-4, platelet factor 4; SDF-1, stromal cell-derived factor-1; SMC, smooth muscle cell; TGF- β 1, transforming growth factor- β 1; TNF- α , tumor necrosis factor- α ; TNFR2R, tumor necrosis factor receptor 2R; UTR, untranslated region; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

Adapted from Huang P, Li S, Han M, et al. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. *Diabetes Care*. 2005;28:2155–2160.

molecules mobilize to the cell surface, generating a “sticky” endothelium that enhances leukocyte attraction, adhesion, and invasion of arteriolar collaterals and periadventitia.^{6,8}

Monocytes either attach to CAMs to be incorporated into the lumen of the developing vessel or accumulate in the adventitia.¹⁵ Activated monocytes release tumor necrosis factor- α (TNF- α), further enhancing monocyte attraction. Platelet adherence and activation stimulates growth factor and interleukin-4 production, enhancing monocyte adhesion. Monocytes stimulate production of growth factors, chemokines, and cytokines, in addition to immune cells, leading to the proliferation of collateral arterioles (Fig. 7.3).⁵ Macrophages contribute to remodeling of the collateral vessel by liberating proteases.^{6,8,9}

Endothelial cell proliferation and endothelial permeability increase, while vascular SMCs proliferate and change from a contractile to a proliferative phenotype.

Circumferential wall stress also plays a role in inducing arteriogenesis. As part of a second phase of arteriogenesis, vascular SMC growth is induced by circumferential wall stress. Increased intravascular pressure leads to SMC proliferation and increased vessel thickness. Increased vessel thickness enables normalization of circumferential wall stress at low blood pressure, which can lead to cessation of collateral vessel growth prior to the complete resolution of ischemia.¹¹

The final phase of arteriogenesis involves “pruning” or regression of vessels. Poiseuille’s law states that flow is proportional to

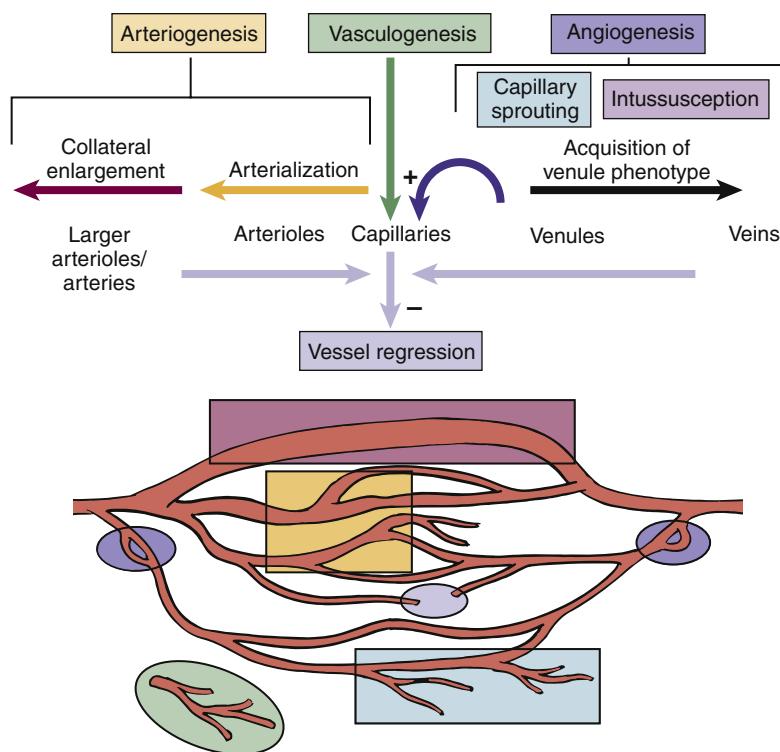


Figure 7.1 Vascular Development. Postembryonic vascular remodeling occurs in response to hypoxia and fluid shear stress and involves a complex continuum of vascular adaptations. (Redrawn from Peirce SM, Skalak TC. Microvascular remodeling: A complex continuum spanning angiogenesis to arteriogenesis. *Microcirculation*. 2003;10:99–111, with permission from Taylor & Francis Ltd. Available at <http://www.tandf.co.uk/journals>.)

radius and predicts that greater flow is observed within fewer larger arterioles rather than many smaller ones. As such, the process of “pruning” leads to regression of smaller collaterals, with persistence of a few larger collateral vessels. Vessel regression is the result of proliferation of the intima that leads to occlusion of the collateral.¹¹

Cardiovascular Risk Factors Associated with Impaired Arteriogenesis

A variety of disease states perturb arteriogenesis, leading to impairment of endothelial or macrophage function. The recruitment of EPCs or growth factor production may also be impaired (Fig. 7.4). Aging itself affects arteriogenesis secondary to an overall decrease in endothelial production of NO¹⁶ and by a higher rate of HIF degradation, along with decreased levels of VEGF, platelet-derived growth factor (PDGF), FGF-2, and chemokine signaling. Growth factor release is also impaired with aging.^{16,17}

Diabetes mellitus retards vessel formation in relation to an attenuated response to the mobilization of mononuclear cells induced by granulocyte-macrophage colony-stimulating factor (GM-CSF)¹⁸ and to impaired monocyte chemotaxis in response to VEGF.^{16,17,19,20} This overall decrease in circulating EPCs leads to endothelial dysfunction and poor collateral artery formation.^{16,20} In addition, eNOS is inhibited by an increase in free radicals related to diabetes.¹⁷

Hypertension can have a variety of effects on arteriogenesis. Elevated blood pressure can cause an increase in FSS, which stimulates arteriogenesis. Hypertension is also associated with activation of the renin-angiotensin system and

subsequent activation of arteriogenesis. Angiotensin’s role in regulating the inflammatory response can lead to initiation of arteriogenesis induced by inflammation. Angiotensin also stimulates increases in circulating VEGF, PDGF, and FGF, all of which stimulate arteriogenesis. Conversely, activation of angiotensin by hypertension is associated with endothelial cell dysfunction as a result of increased oxidative stress due to activation of NADPH (reduced nicotinamide adenine dinucleotide phosphate) oxidase activity. The increase in oxidative stress increases reactive oxygen and superoxide levels. These oxygen radicals uncouple eNOS, thereby reducing the availability of NO.^{16,21,22}

Hyperlipidemia affects various steps in arteriogenesis and has direct toxic effect on both endothelial cells and vascular SMCs.¹⁷ Oxidized low-density lipoprotein (LDL) cholesterol interferes with VEGF function, leading to disordered endothelial cell migration via eNOS inhibition.^{17,22} In addition, endothelial cell FGF and T-lymphocyte migration are reduced, as is endothelial cell replication. Expression of FGF receptors, HIF-1, and VCAM-1 is impaired, leading to impairment of monocyte chemotaxis.²³

Lastly, tobacco abuse impairs EPC number, function, migration, and adherence. Monocyte migration in response to VEGF is disordered with smoking. Decreased levels of VEGF and HIF-1 further impair EPC function.^{16,17,22}

ANGIOGENESIS

Angiogenesis involves new capillary formation induced by distal tissue ischemia via sprouting and nonsprouting (intussusceptive microvascular growth [IMG]) mechanisms.^{24–26}

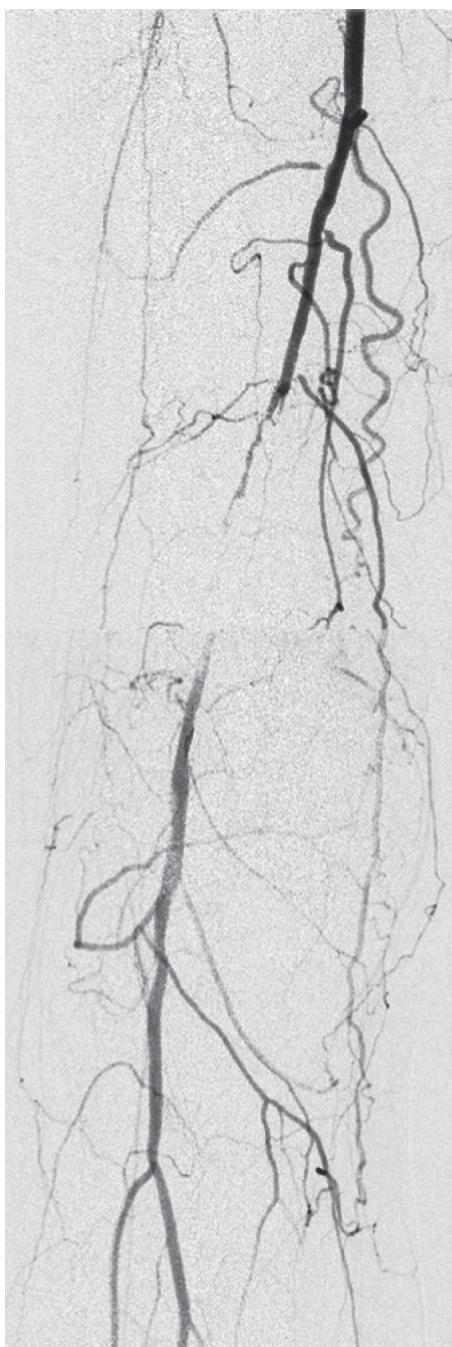


Figure 7.2 Clinical Example of Arteriogenesis. Digital subtraction arteriography of the right lower extremity in a patient with claudication. A distal superficial femoral artery (SFA) occlusion with reconstitution via collateral circulation is demonstrated. These collaterals formed via arteriogenesis in response to progressive SFA stenosis, allowing blood flow beyond the occlusion. The patient underwent successful angioplasty with stent placement, as these collaterals were insufficient to prevent symptomatic claudication (not shown). (Courtesy of Paul DiMuzio, MD, Division of Vascular and Endovascular Surgery, Thomas Jefferson University Hospital, Philadelphia, Pennsylvania.)

Sprouting Angiogenesis

Sprouting angiogenesis involves endothelial cell projections into surrounding connective tissue. Breakdown of the basement membrane occurs along with inter-endothelial junction formation, enabling endothelial cell projection.^{26–28} Endothelial cells

migrate along the projection's front with further sprouting, ultimately developing a complex capillary meshwork.

Sprouting angiogenesis requires specialization of cells along the migrating projection into “tip,” “stalk,” and “phalanx” cell phenotypes on the basis of the interaction of factors promoting or inhibiting angiogenesis.^{29–34} Tip cells are polarized migratory cells that are at the forefront of the endothelial sprout. These cells branch at the tip of the stalk as they extend filopodia toward the stimulus; this is accomplished with minimal proliferation. Stalk cells conversely exhibit a proliferative phenotype responsible for the lengthening of the endothelial sprout. These cells are also responsible for secretion of basement membrane along the stalk and formation of vascular lumina from the initial luminal slit.^{32,34,35} Additional stability to the proliferating stalk is provided by pericytes, which surround the basement membrane and provide further vessel coverage and decrease leakage from the vessel.^{35–37} Initially, the process of sprouting requires minimal endothelial cell proliferation, although this demand increases with continued sprouting.^{29,30,35}

Phalanx cells are endothelial cells that become quiescent after completion of the vascular branch. These cells deposit basement membrane and form tight cellular junctions via increased expression of vascular endothelial cadherin (VE-cadherin). These cells are ultimately responsible for delivery of oxygen and nutrients to surrounding tissues.^{35,38}

Regulation of Angiogenesis

Angiogenesis is initiated by ischemia, leading to increased VEGF expression. VEGF is a potent angiogenic factor, serving to encourage endothelial cell binding to VEGF receptor 2 (VEGFR-2), which promotes endothelial chemotaxis. VEGF expression induces extension of tip cells and proliferation of stalk cells with concomitant synthesis of basement membrane components.^{39–41} In addition, pericytes are attracted and contribute to capillary network formation. Whereas VEGF induces sprouting, Notch signaling pathways function to limit tip migration. Notch signaling occurs by increasing expression of VEGFR-1, competitively binding VEGF and thereby limiting its availability.^{25,31–33,42} The balance of VEGF and Notch signaling therefore regulates sprouting-related vessel development.

Transformation to a tip cell phenotype is induced by exposure of endothelial cells to VEGF. Delta-like ligand 4 (Dll4), a Notch binding ligand, is highly expressed by tip cells, increasing sensitivity to VEGF and binding to VEGFR-2.^{31,32,39,43–46} Increased VEGF–VEGFR-2 binding up-regulates Dll4, leading to downregulation of VEGFR-2 on adjacent endothelial cells. This process allows the tip cell to competitively maintain its position.^{29,33,41,47} These adjacent cells transform into a stalk cell phenotype and express Notch, which is induced by Dll4.^{31,46,48} Whereas tip cells have low Notch signaling, stalk cells express higher Notch signaling and higher expression of the jagged protein-1 (JAG-1), counteracting Notch-Dll4 activity and limiting tip cell migration (Fig. 7.5).^{31,39,47–50}

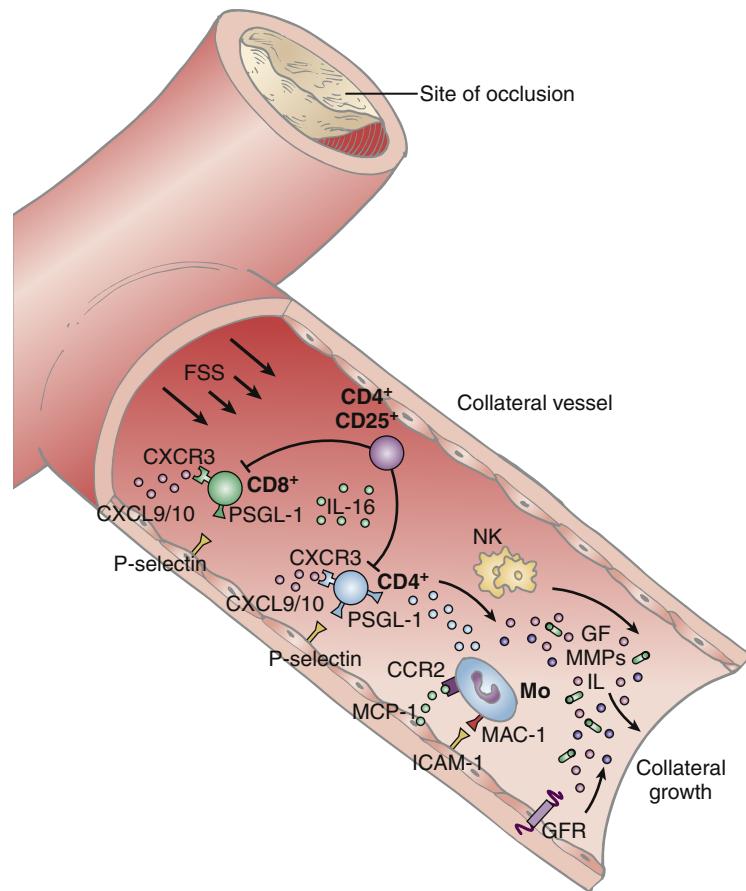


Figure 7.3 Role of Immune Cells in Arteriogenesis. With ischemia, CD8⁺ T cells are attracted to the growing collateral vessel and recruit CD4⁺ cells, which subsequently augment arteriogenesis in association with natural killer (NK) cells. Monocytes are recruited, initiating vessel growth by synthesis of angiogenic/arteriogenic cytokines. *CCR2*, CC chemokine receptor 2; *CXCL 9/10*, CXC chemokine ligand 9/10; *CXCR3*, CXC chemokine receptor 3; *FSS*, fluid shear stress; *GF*, growth factor; *GFR*, growth factor receptor; *ICAM-1*, intercellular adhesion molecule-1; *IL*, interleukin; *MAC-1*, membrane attack complex-1; *MCP-1*, monocyte chemotactic factor-1; *MMPs*, matrix metalloproteinases; *Mo*, monocytes; *PSGL-1*, P-selectin glycoprotein ligand 1. (Redrawn from Silvestre JS, Mallat Z, Tedgui A, Lévy BI. Post-ischaemic neovascularization and inflammation. *Cardiovasc Res*. 2008;78:242–249, with permission from Oxford University Press.)

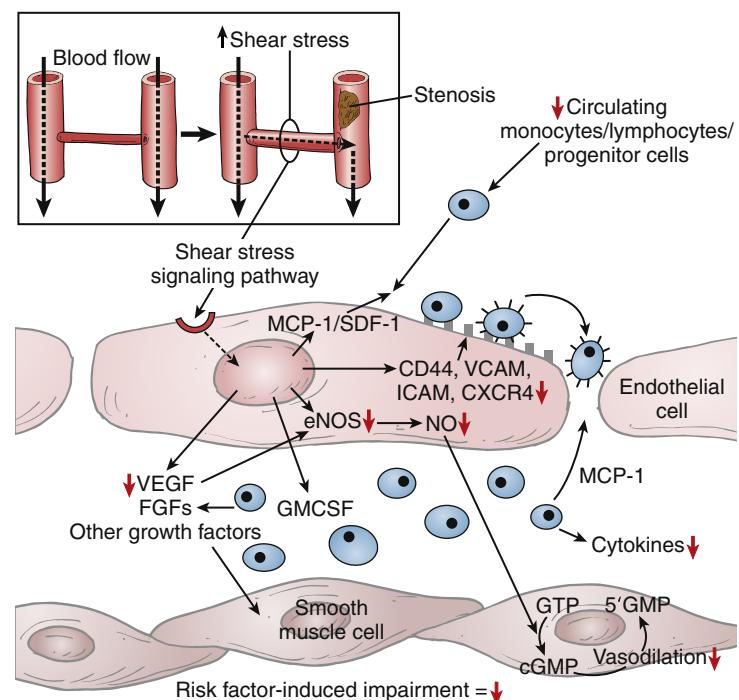


Figure 7.4 Impact of Cardiovascular Risk Factors on Arteriogenesis. With arterial stenosis or occlusion (inset), there is a drop in pressure and subsequent increase in fluid shear stress (FSS). FSS initiates activation of the intracellular signaling cascade, thereby stimulating arteriogenesis. Red arrows indicate steps that may be negatively influenced by cardiovascular risk factors. *cGMP*, cyclic guanosine monophosphate; *CXCR4*, CXC chemokine receptor 4; *eNOS*, endothelial nitric oxide synthase; *FGFs*, fibroblast growth factors; *GMCSF*, granulocyte-macrophage colony-stimulating factor; *GMP*, guanosine monophosphate; *GTP*, guanosine triphosphate; *ICAM*, intercellular adhesion molecule; *MCP-1*, monocyte chemotactic factor-1; *NO*, nitric oxide; *SDF*, stromal cell-derived factor-1; *VCAM*, vascular cell adhesion molecule; *VEGF*, vascular endothelial growth factor. (Redrawn from Kinnaird T, Stabile E, Zbinden S, et al. Cardiovascular risk factors impair native collateral development and may impair efficacy of therapeutic interventions. *Cardiovasc Res*. 2008;78:257–264, with permission from Oxford University Press.)

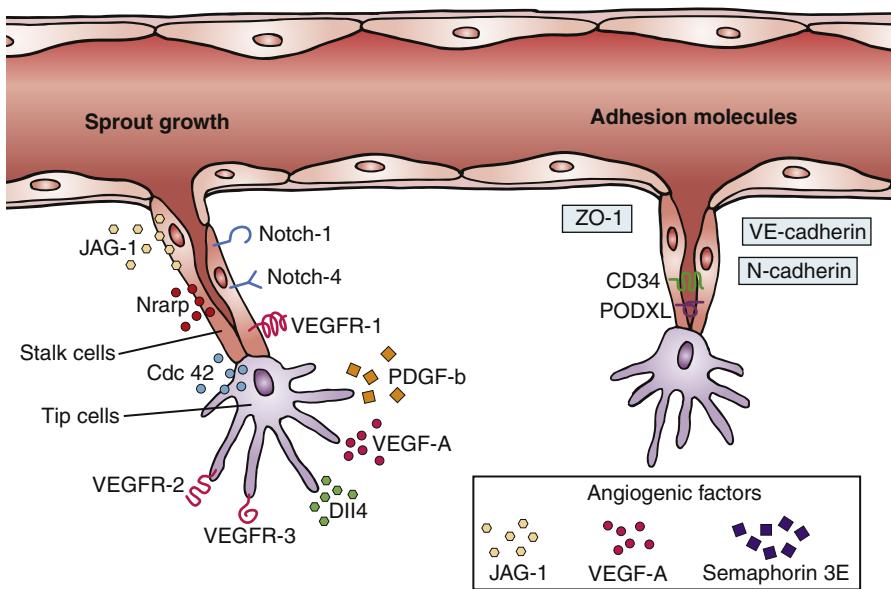


Figure 7.5 Specialization of Cell Phenotypes During Sprouting Angiogenesis. Proangiogenic and antiangiogenic factors regulate phenotypic specialization of cells into “tip,” “stalk,” and “phalanx” cells. Tip cells are responsible for migration of the developing vessel and formation of filopodia. Stalk cells are responsible for proliferation, lumen formation, and providing length to the developing vessel. Phalanx cells are quiescent. *Cdc 42*, Cell division control protein 42 homolog; *JAG-1*, jagged 1 gene; *Nrarp*, notch regulated ankyrin-repeat protein; *PDGF-b*, platelet-derived growth factor; *PODXL*, podocalyxin; *VEGF*, vascular endothelial growth factor; *VEGFR*, VEGF receptor; *ZO-1*, zonula occludens protein-1. (From Ribatti D, Crivellato E. “Sprouting angiogenesis,” a reappraisal. *Dev Biol*. 2012;372:157–165.)

Lumen Formation

Tubulogenesis, or lumen formation, is responsible for transforming endothelial stalks into vessels capable of carrying blood and nutrients to surrounding tissue. This process initially involves establishing endothelial cell apical–basal polarity, mediated by VE-cadherin. Apical borders face apposing cells, whereas the basal border faces the periphery. Beyond this first step, three proposed mechanisms may explain lumen development.

The first process involves development of intracellular pinocytotic vesicles and vacuoles, which progressively fuse within the endothelial cell and then with adjacent cells, leading to lumen formation along the length of the stalk. The second mechanism is similar but involves exocytosis of these vacuoles between endothelial cells along the length of the growing stalk. These vacuoles then coalesce and form a lumen. The third mechanism for lumen formation is by reorganization of intracellular junctions mediated by VE-cadherin. Endothelial cells adhere to each other and become polar cells as VE-cadherin localizes CD34-sialomucins to the apical cell surface.^{32,51–55} The negative charges of sialomucins lead to repulsion of adjacent surfaces of the endothelial cells, inducing lumen slit formation (Fig. 7.6).^{32,51} As the lumen develops, the CD34-sialomucins are rearranged to the lateral surfaces of the cells and F-actin is attracted to the exposed lumen.

VEGF attracts non-muscle myosin II to the cell surface, with formation of an actinomyosin complex along the apical endothelial cell surface. This cytoskeletal interaction encourages cellular morphologic shape changes and further luminal expansion.^{32,51–53} Beyond tubulogenesis, further increases in lumen diameter are primarily related to FSS.^{29,51}

Intussusceptive Angiogenesis

Intussusceptive angiogenesis involves the formation of transcapillary tissue pillars that fuse to form new vessels. This leads

to a rapid increase in the complexity of the capillary plexus and increased surface area for gas and nutrient exchange.^{56–59} Initially cell contact is created between opposing capillary cells, followed by formation of a bilayer with reorganization of endothelial cell junctions. A pillar core then develops as the result of this activity. Pericytes, myofibrils, and interstitial cells are responsible for perforating the endothelial bilayer to define discrete vessels,^{24,59,60} while collagen fibrils are deposited as a foundation for the developing meshwork.⁶⁰ After initial capillary formation by either vasculogenesis or sprouting angiogenesis, intussusception is initiated to allow for rapid capillary expansion and remodeling by three basic mechanisms: intussusceptive microvascular growth (IMG), arborization, and intussusceptive branching remodeling (IBR).

IMG initially involves formation of tissue pillars, which provides an increase in the surface area available for exchange of oxygen, carbon dioxide, and nutrients.^{24,58–60} The next phase, intussusceptive arborization, involves the development of “vertical pillars,” which transform well-perfused capillary segments into arterioles and venules. This process decreases the distance between arteries and veins as the capillary plexus expands. Pillars form from endothelial cell reorganization and merging of developing tissue septa (Fig. 7.7). Horizontal folds develop and lead to pillar detachment from any remaining connections, ultimately separating the feeding vessel from the capillary plexus. Hemodynamic forces therefore have a role in further development of the arterial tree.^{59,60,62}

IBR is the final process by which intussusception alters the vascular network. It involves adaptation of the angulation at branch points to optimize fluid hemodynamics in response to shear stress. Although this process has a role in branch remodeling, it is not involved in the formation of new capillary branches.⁶³ Vascular pruning also occurs with IBR because luminal obstruction leads to regression of the vessel in response to oxygen tension and growth factors, including VEGF, PDGF, and angiopoietin-1 (Ang-1).^{30,59,64,65} Pruning encourages the

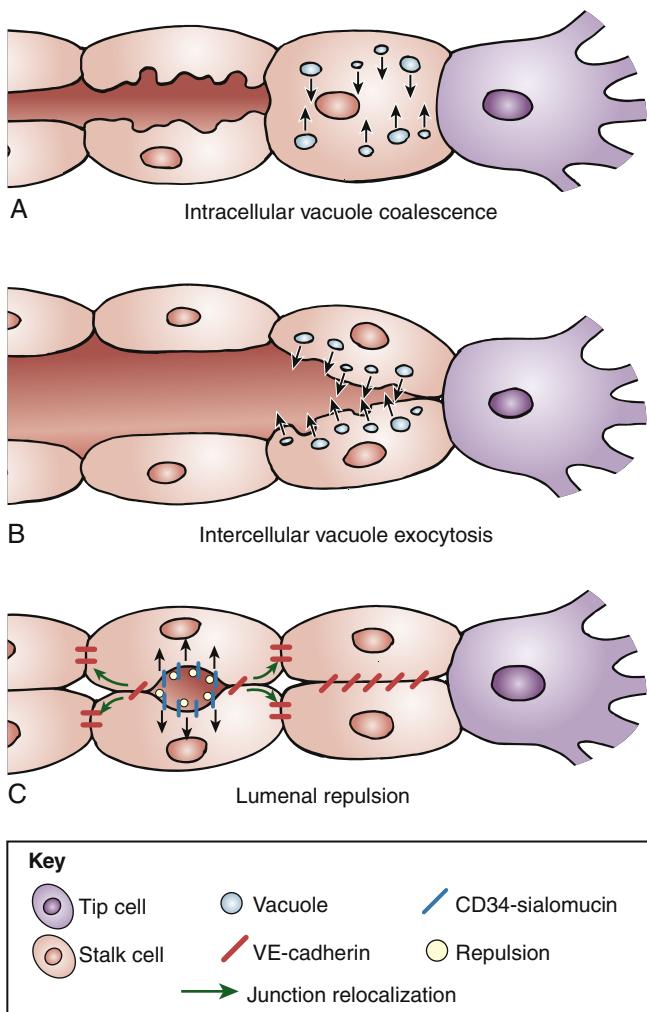


Figure 7.6 Paradigms of Lumen Formation in Angiogenesis. (A) Endothelial cells (ECs) form intracellular pinocytotic vesicles and vacuoles, which fuse within ECs and between adjacent cells, forming a lumen. (B) ECs exocytose vacuoles between cells along the growing stalk. (C) Polarization of ECs by vascular endothelial (VE)-cadherin-mediated localization of CD34-sialomucins to the EC apical surface, leading to apical-basal polarity and subsequent repulsion and lumen formation. (From Geudens I, Gerhardt H. Coordinating cell behavior during blood vessel formation. *Development*. 2011;138:4569–4583.)

predominance of more perfused capillaries with regression of less perfused ones (Fig. 7.8).^{24,59,66}

There are multiple benefits of intussusceptive growth. One is that minimal endothelial proliferation is required given capillary expansion occurs primarily by rearrangement or remodeling of existing endothelial cells. Other advantages include low vascular permeability and minimal tissue disruption leading to less porous capillaries.^{29,59,60}

Remodeling and Pruning

Remodeling of the capillary network occurs in response to the nutritional needs of local tissue. It involves growth of those vessels preferentially receiving flow, with regression of others. Vessels undergo alterations to increase in luminal diameter and mature their walls. Pruning is a dynamic process and involves vessel regression in response to luminal blood flow and possibly

a reduction in VEGF. Areas with low wall shear stress undergo pruning, increasing flow into larger bore vessels.^{29,64,67}

After proliferation and remodeling are complete, blood vessels mature and stabilize largely on the basis of signaling from endothelial cells, pericytes, SMCs, and the ECM.^{29,41} Pericytes invade and surround capillaries; subsequently, and likely in relation to interaction with the pericytes, the endothelial cells wrap around pericytes to stabilize the vessel.^{68–70}

Postnatal angiogenesis is a dynamic process involving capillary growth related to sprouting and remodeling as directed by blood flow and the action of intussusceptive angiogenesis. Functional specialization of the endothelial cells during sprouting, regulation by VEGF and Notch signaling, and functional remodeling related to tissue metabolic needs are all hallmarks of angiogenesis.

ROLE OF NONCODING RNAs IN NEOVASCULARIZATION

In the last two decades, clinical outcomes of growth factor-based therapies in the treatment of CLI have largely been unsuccessful.⁷¹ Multiple studies suggest this is due to impaired downstream signaling, rather than a deficiency of angiogenic growth factors. Patients with CLI demonstrate high levels of proangiogenic factors compared to those with PAD and intermittent claudication.⁷² These studies suggest impaired angiogenic signaling contributes to CLI through mechanisms analogous to a diabetic patient with insulin resistance secondary to impaired insulin signaling.

Noncoding RNAs, including microRNAs (miRNAs) and long noncoding RNAs (lncRNA), are key regulators of signaling pathways implicated in the pathogenesis of PAD and CLI.⁷³ MicroRNAs are a class of short endogenous noncoding RNA molecules that regulate gene expression through inhibition of target gene translation.⁷⁴ These molecules are approximately 22 nucleotides in length and bind to the 3'-untranslated region (UTR) of target messenger RNA (mRNA).^{75,76} The 3'-UTR is a specific section of mRNA following the stop codon of the coding region that contains regulatory regions which can influence post-transcriptional gene expression. Long noncoding RNAs are >200 nucleotides in length and regulate diverse cellular processes in the nucleus including regulation of transcription by sequestering transcription factors, induction of histone modifications, and acting as enhancer RNAs (in comparison, in the cytoplasm lncRNAs control mRNA stabilization, protein phosphorylation, or block miRNA activity).^{73,77,78}

MiRNAs influence both arteriogenesis and angiogenesis through interactions with various transcription factors, cytokines, and CAMs. The same miRNAs that are involved in adaptive vascular remodeling are also key regulators in maladaptive processes, such as atherosclerosis and aneurysm formation.⁷⁹ A few of the miRNAs involved include miR-126, miR-155, and the miRNA families miR-17/92 and miR-23/24/27.⁸⁰ The miR-126 gene is located on chromosome 9 and is one of the most abundantly expressed miRNAs in endothelial cells.⁸¹ VCAM-1 expression is inhibited by

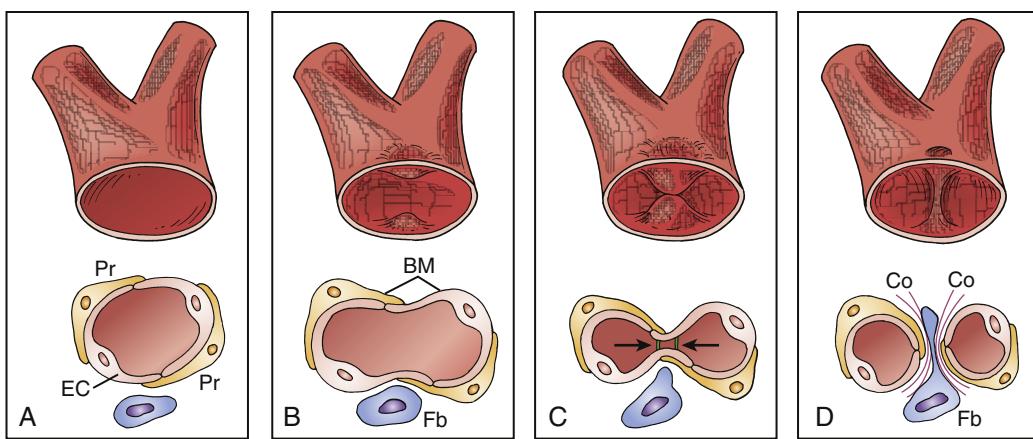


Figure 7.7 Intussusceptive Growth. (A and B) Impingement of endothelial cell wall into lumen, leading to creation of bilayer. This bilayer then develops perforations (C) leading to pillar formation (D). BM, basement membrane; Co, collagen fibrils; EC, endothelial cell; Fb, fibroblasts; Pr, pericytes. (A–D from Kurz H, Burri PH, Djonov VG: Angiogenesis and vascular remodeling by intussusception: from form to function. *News Physiol Sci*. 2003;18:65–70; published by Int Union Physiol Sci/Am Physiol Soc. Entire figure is reproduced from Djonov V, Baum O, Burri PH. Vascular remodeling by intussusceptive angiogenesis. *Cell Tissue Res*. 2003;314:107–117. Published by Springer-Verlag.)

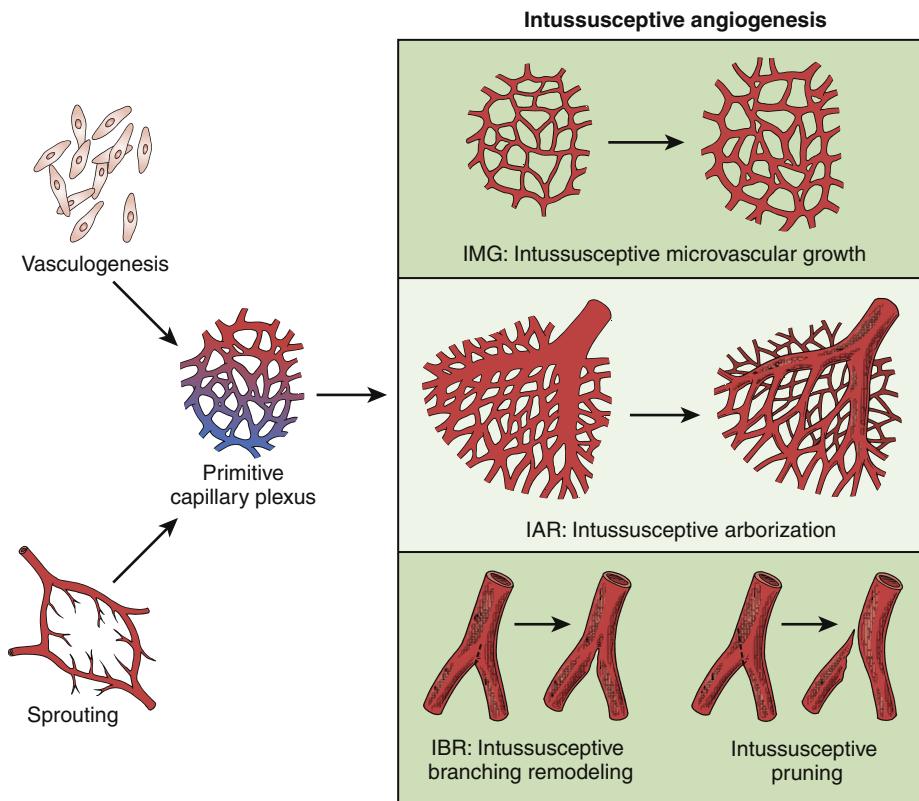


Figure 7.8 Intussusceptive Angiogenesis. The primitive capillary plexus undergoes organization and development into mature vessels via intussusceptive angiogenesis. Pillars develop by apposition of opposing capillary walls. “Vertical” pillars form and fuse, later definitively separating from the capillary plexus and forming new vessels. Local tissue demand promotes further modifications. Small diameter vessel pruning occurs due to low shear stress and possibly a decrease in vascular endothelial growth factor. (From Djonov V, Baum O, Burri PH. Vascular remodeling by intussusceptive angiogenesis. *Cell Tissue Res*. 2003;314:107–117, Springer-Verlag.)

miR-126, which decreases leukocyte adherence to ECs and inhibits angiogenesis.⁸² MiR-126 also promotes angiogenesis through inhibition of SPRED1 and PIK3R2, which are inhibitors of the VEGF signaling pathway.⁸³ The miR-155 gene is located on chromosome 21 and is highly expressed by activated B and T cells, as well as monocytes and macrophages.^{84,85} MiR-155 has antiangiogenic as well as pro-arteriogenic functions through its interactions with AT1R and SOCS1, respectively.⁸⁶ It is co-expressed on SMCs with AT1R, where it inhibits the expression of AT1R and limits

cell responsiveness to angiotensin II.⁸⁷ The pro-arteriogenic effect of miR-155 is thought to be mediated through SOCS1, which is upregulated in miR-155-deficient mice. These mice show significantly reduced levels of pro-arteriogenic cytokines.⁸⁸ These are only a few examples of the influence miRNAs have on neovascularization.

A summary of the ncRNA involved in experimental hind limb ischemia is displayed in Table 7.2.⁷³ As further progress is made in this area of study, the multifactorial role of ncRNAs is certain to be elucidated.

TABLE 7.2 Noncoding RNAs Involved with Angiogenesis/Arteriogenesis in Experimental Hind Limb Ischemia in Mice

	Noncoding RNA	Perfusion	Targets	Delivery
Angiogenesis/ Arteriogenesis	miR-126-3p	Promotes	SPRED1, PIK3R2	IM, IV
	miR-132/212	Promotes	RASA1, SPRY1, SPRED1	TG
	miR-150	Promotes	SRCIN-1	IM
	miR-26b	Promotes	PTEN, PMAIP1	IM
	miR-92a	Promotes	ITGA-5	IV
	miR-146b	Promotes	TRAF-6	IM
	miR-329/487b/494/495	Inhibits	...	IV
	miR-19a/b	Inhibits	FZD-4, LRP-6	TG, IP
	miR-15b	Inhibits	AKT-3	IM
	miR-223	Inhibits	β1 integrin	TG
	miR-100	Inhibits	mTOR	IV
	miR-27a	Inhibits	VE-CAD	IV
	MEG-3	Inhibits	VEGF signaling	IP

FZD4, frizzled class receptor 4; IM, intramuscular; IP, intraperitoneal; ITGA-5, integrin subunit alpha 5; IV, intravenous; LRP6, LDL-receptor related protein 6; mTOR, mammalian target of rapamycin; PIK3R2, phosphoinositide-3-kinase regulatory subunit 2; PMAIP1, phorbol-12-myristate-13-acetate-induced protein 1; PTEN, phosphatase and tensin homolog; RASA1, RAS p21 protein activator 1; SPRED1, sprouty-related EVH1 domain containing 1; SPRY1, sprouty RTK signaling antagonist 1; SRCIN-1, SRC kinase signaling inhibitor 1; TG, transgenic; TRAF, TNF receptor-associated factor; VEGF, vascular endothelial growth factor.⁷³

CLINICAL TRIALS

For a variety of reasons, patients with vascular disease may not be safe candidates for traditional surgical intervention owing to comorbidities or extent of arterial disease. In this challenging patient population, stimulation of neovascularization via gene, protein or cell-based therapies has been proposed. Initial results of gene and protein therapies have been disappointing (Table 7.3); however, more encouraging results have been observed with cell-based therapies (Table 7.4).

Gene and Protein-Based Therapies

Vascular Endothelial Growth Factor

One of the first growth factors to be investigated was VEGF. This growth factor is responsible for promoting endothelial cell proliferation, migration, and lumen formation while inducing monocyte chemotaxis. It has been demonstrated to play an integral part in both arteriogenesis and angiogenesis.^{88–97} VEGF was initially investigated in phase I clinical trials by Isner et al.⁹⁸ and Baumgarten et al.⁹⁹ With intraarterial administration of VEGF, these groups were able to demonstrate an increase in collateral vessels on arteriography, a significant improvement in ankle–brachial index (ABI), improvement in ischemic wounds, and limb salvage in three patients,⁹⁹ with the side effect of peripheral edema of the affected limb.⁹⁸

Since then several phase II clinical studies have further investigated the use of both intraarterial and intramuscular administration of VEGF.^{100–102} Mäkinen et al.¹⁰⁰ in the VEGF peripheral vascular disease (VEGF-PVD) trial, found an increase in collateralization and an improvement in the ABI. Rajagopalan et al.¹⁰¹ in the RAVE (Regional Angiogenesis with Vascular Endothelial Growth Factor) trial were unable to demonstrate any

significant improvement in exercise tolerance or other quality of life indicators and found peripheral edema as a side effect. Kusumanto et al.¹⁰² confirmed an improvement in both ABI and wound healing in a diabetic population, although no significant reduction in amputation rate was identified.

Fibroblast Growth Factor

FGF is a potent stimulator of angiogenesis and arteriogenesis. It induces proliferation, migration, and differentiation of endothelial cells and potentiates VEGF.^{88,90–95,103–105} Several phase II and phase III clinical trials have studied the safety and efficacy of intraarterial and intramuscular injections of FGF. The Therapeutic Angiogenesis with Recombinant Fibroblast Growth Factor-2 for Intermittent Claudication (TRAFFIC) trial noted an improvement in exercise tolerance at 90 days, with more improvement observed in smokers, although this improvement did not persist to 180 days.¹⁰⁶ ABI improvements were also noted and did persist to 180 days.¹⁰⁶ The Therapeutic Angiogenesis Leg Ischemia Study for the Management of Arteriopathy and Non-Healing Ulcer (TALISMAN) trial demonstrated a twofold risk reduction for all amputations and major amputations with FGF therapy, although the therapy achieved no improvement in ulcer healing when compared with placebo.¹⁰⁷ However, a non-significant trend toward a reduced mortality with intramuscular FGF was noted in the treatment population.¹⁰⁷ TAMARIS, the phase III trial to follow TALISMAN, was unable to replicate the reduction in amputations or mortality shown in TALISMAN.¹⁰⁸

Hepatocyte Growth Factor

Hepatocyte growth factor influences angiogenesis via enhancement of endothelial cell function by activation of the Dll4-Notch signaling pathway, inducing proliferation and

TABLE 7.3 Phase II/III Clinical Trials of Gene/Protein-Based Therapy

Trial (Phase)	Therapeutic Intervention	Study Group	Results
VEGF			
Mäkinen (2002) ¹⁰⁰ VEGF in chronic limb ischemia (II)	IA VEGF-165 adenovirus/plasmid, after percutaneous transluminal angioplasty Control: LR	54 patients (18 VEGF-Ad, 17 VEGF-p, 19 LR)	Improved vascularity and ABI Rutherford class increased in both treatment and placebo groups
Rajagopalan (2003) ¹⁰¹ RAVE (Regional Angiogenesis with Vascular Endothelial growth factor trial) (II)	IM VEGF-121 adenovirus Control: placebo	105 patients (40 high dose, 32 low dose, 33 placebo)	No improvement in PWT Increased peripheral edema in treatment group
Kusumanto (2006) ¹⁰² VEGF in diabetes and chronic limb ischemia (II)	IM VEGF-165 adenovirus Control: NSS	54 patients with diabetes mellitus (27 VEGF-Ad, 27 NSS)	Significant improvements in ABI and ulcer healing, no reduction in amputation rates
FGF			
Lederman (2002) ¹⁰⁶ TRAFFIC (Therapeutic Angiogenesis with Recombinant Fibroblast Growth Factor-2 for Intermittent Claudication) (II)	IA FGF-2 Control: placebo	174 patients (63 single dose, 54 double dose, days 1 and 30; 59 placebo)	Improvement in ABI at 90 and 180 days Improvement in PWT at 90 days (more improvement in smokers), not persisting to 180 days
Nikol (2008) ¹⁰⁷ TALISMAN (Therapeutic Angiogenesis Leg Ischemia Study for the Management of Arteriography and Non-Healing Ulcer) (II) ^a	IM NV1FGF (FGF-1) Control: placebo	107 patients (51 NV1FGF, 56 placebo)	Reduction in risk of amputations, no significant improvement in ulcer healing Trend toward decrease mortality (NS)
Belch (2011) ¹⁰⁸ TAMARIS (III) ^a	IM NV1FGF (FGF-1) Control: placebo	525 patients (259 NV1FGF, 266 placebo)	No improvement in death or major amputation
HGF			
Powell (2008) ¹¹³ HGF-STAT	IM plasmid HGF Control: placebo	106 patients (27 low dose, 26 middle dose, 27 high dose, 26 placebo)	tcPO ₂ increased at 6 months in high/mid-dose group No improvement in ABI, TBI, pain relief, wound healing, major amputation
Shigematsu (2010) ¹¹⁴	IM naked plasmid HGF Control: placebo	44 patients (30 HGFP, 14 placebo)	Improvements in ulcer healing and QOL Unable to demonstrate improvement in either ABI or rest pain
Henry (2011) ¹¹⁵ (I)	IM VM202(non-viral DNA plasmid) Control: none	12 patients (dose escalation from 2–16 mg)	Improved ABI/TBI, wound healing and decreased pain
DEL-1			
Grossman (2007) ¹²⁰ DELTA (Del-1 for Therapeutic Angiogenesis) (IIa)	IM VLTS-589/poloxamer 188 plasmid encoding Del-1 Control: poloxamer plasmid	105 patients (52 Del-1, 53 poloxamer 188 only)	Improvements in PWT, onset of claudication, ABI, QOL
HIF			
Creager (2011) ¹³⁰ WALK (effect of HIF-1 gene therapy on walking performance in patients with intermittent claudication) (II)	Induces gene transcription, vasodilation, and endothelial/SMC migration and proliferation to promote angiogenesis	289 patients (3 graded dosing or placebo)	No improvement in PWT, claudication, QOL, ABI

^aTAMARIS is the phase III trial of the same group who performed TALISMAN, a phase II trial; although results in two studies were different.ABI, ankle-brachial index; Ad, adenovirus; Del-1, developmentally regulated endothelial locus-1; eNOS, endothelial nitric oxide synthase; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; HGFP, plasmid HGF; IA, intraarterial; IM, intramuscular; LR, lactated Ringer solution; NS, nonsignificant; NSS, normal saline solution (0.9%); NV1FGF, nonviral 1 FGF; p, plasmid; PVD, peripheral vascular disease; PWT, peak walking time; QOL, quality of life; TBI, toe-brachial index; tcPO₂, transcutaneous oxygen tension; VEGF, vascular endothelial growth factor.(From Idei N, Soga J, Hata T, et al. Autologous bone-marrow mononuclear cell implantation reduces long-term major amputation risk in patients with critical limb ischemia: a comparison of atherosclerotic peripheral arterial disease and Buerger disease. *Circ Cardiovasc Interv*. 2011;4:15–25).

TABLE 7.4 Cell-Based Therapy, Human Trials²²³

Trial (Phase)	Therapeutic Intervention	Study Group	Results
EPCS			
van Royen (2005) ¹³¹ START (STimulation of ARTeriogenesis) trial (II)	SC rhGM-CSF Control: placebo	40 patients (20 rhGM-CSF, 20 placebo)	Increased microcirculatory flow in treatment group No improvement in ABI or exercise tolerance
Kawamoto (2009) ¹³² EPOCH-CLI (I/Ia)	IM EPCs (CD34+) Control: none	17 patients (6 low dose, 8 middle dose, 3 high dose)	Improvements in pain, TBI, pain-free walking distance, ulcer healing
Burt (2010) ¹³⁴ (I)	IM EPCs (CD133+) Control: none	9 patients	Improvements in amputation rate, QOL
Zhang (2016) ¹³⁵ (II)	IV EPCs (CD133+) Control: placebo	53 patients (27 treatment, 26 placebo)	Improvements in Rutherford class, tcPO ₂ , ABI, ulcer healing, and amputation rate
BM-MNC/MSC			
Huang (2005) ²²⁴ (I)	PB-MNCs Control: IV PGE ₁	28 patients (14 PB-MNCs, 14 PGE ₁)	Improvements in limb pain, ulcer healing, ABI, TBI, laser Doppler blood perfusion Angiogenic evidence of new vessel development
Powell (2011) ¹³⁹ RESTORE-CLI (II)	IM BM-MNCs Control: placebo	46 patients (32 BM-MNCs, 14 placebo)	Improvements in time to treatment failure, amputation free survival
Walter (2011) ¹³⁸ PROVASA (II)	IA BM-MNCs Control: placebo	40 patients (19 BM-MNCs, 21 placebo), both groups received BM-MNCs at 3 months	Improvements in ulcer healing and rest pain, associated with repeat administration and BM-MNC number/function
Idei (2011) ²²⁵ (I)	IM BM-MNCs Control: placebo	97 patients (25 w/atherosclerotic PAD, BM-MNCs; 26 w/Buerger disease, BM-MNCs; 30 w/ atherosclerotic PAD, placebo; 16 w/Buerger disease, placebo)	Buerger disease: improvements in ABI and tcPO ₂ at 1 month and 3 years PAD: improvement in ABI at 1 month, gradual return to baseline
Iafrati (2011) ^{143,144} BMAC (Bone Marrow Aspirate Concentrate) (III)	IM BM-MNCs Control: placebo	48 patients (34 BM-MNCs, 14 placebo)	Improvement in amputation, pain, QOL, Rutherford classification, ABI
Lasala (2012) ¹⁴⁰ (II)	IM BM-MNCs/MSCs Control: placebo	26 patients (BM-MNCs/MSCs in more ischemic leg, placebo in contralateral)	Improvements in exercise tolerance, ABI; improved perfusion with Tc-99m tetrofosmin scintigraphy
Losordo (2012) ²²⁶ (I)	IM CD34+ cells—high/low dose, placebo	28 patients	Increased amputation free survival, further increase in high dose group
Fully Differentiated Cells			
Grossman (2016) ¹⁴⁶ (I) MultiGeneAngio(MGA)	IA MGA(fully differentiated venous ECs expressing Ang-1[angiopoietin-1] and SMCs expressing VEGF-165) Control: none	12 patients (dose increases across 3 cohorts)	Improvement in mean PWT, COT ABI did not decrease over study period
Ongoing or Recruiting Phase III Clinical Trials (clinicaltrials.gov)^a			
Maggi SCELTA (III) NCT02454231 Recruiting	Randomized, single center study evaluating IM BM-MNCs vs PB-MCs	(BM-MNCs vs. PB-MCs)	Primary outcome: improvement in perfusion, evaluated by ultrasound Secondary outcomes: ABI, tcPO ₂ , QOL, rest pain, amputation
Dong (III) NCT02089828 Recruiting	Randomized, single blinded study evaluating purified CD34+ cells vs. PB-MNCs	(CD34+ EPCs vs PB-MNCs)	Primary outcome: major amputation free survival Second outcomes: tcPO ₂ , peak pain-free walking time

^aFirst researcher's name, trial acronym, and study number given. Information available online from clinicaltrials.gov.

ABI, ankle–brachial index; BM, bone marrow; BM-MNC, bone marrow–derived mononuclear cell; BM-MNCs/MSCs, bone marrow–derived mononuclear and mesenchymal stem cells; CLI, critical limb ischemia; COT, claudication onset time; EC, endothelial cell; EPCs, endothelial progenitor cells; IA, intraarterial; IM, intramuscular; MGA, MultiGeneAngio; NS, nonsignificant; PAD, peripheral arterial disease; PGE₁, prostaglandin E₁; PWT, peak walking time; QOL, quality of life; rhGM-CSF, recombinant human granulocyte–macrophage colony-stimulating factor; SC, subcutaneous; TBI, toe–brachial index; tcPO₂, transcutaneous oxygen tension. (From Cooke JP, Losordo DW. Modulating the vascular response to limb ischemia: angiogenic and cell therapies. *Circ Res*. 2015;116(9):1561–1578).

migration of endothelial cells.^{109–112} Investigation of intramuscular HGF injection in the HGF-STAT trial demonstrated increased transcutaneous oxygen tension (tcPO₂) when administered in high/moderate doses,¹¹³ although there was no improvement in ABI, wound healing, or amputation rates. In a phase III trial by Shigematsu et al.,¹¹⁴ there was 100% improvement in ulcer healing at 12 weeks and a significant improvement in quality of life, although no improvement in rest pain. Henry et al.¹¹⁵ and Gu et al.¹¹⁶ were both able to show an increase in median ABI and decrease in pain among patients with significant PAD using a novel form of intramuscular HGF. VM202 is a nonviral DNA plasmid that expresses two isoforms of HGF.^{115,116} A more recent phase II trial involving VM202 showed significant reduction in ulcer size and an increase in tcPO₂ but failed to demonstrate an improvement in ABI.¹¹⁷

Developmentally Regulated Endothelial Locus-1

Developmentally regulated endothelial locus-1 (Del-1) is an angiogenesis protein capable of producing a highly angiogenic response by initiating α_vβ₃-dependent endothelial cell attachment and migration, upregulating an angiogenic phenotype.^{111,118–120} In the DELTA (Del-1 for Therapeutic Angiogenesis) trial, intramuscular injection of Del-1 led to improvement in exercise tolerance, delayed the onset of claudication, and improved both quality of life and ABI, as demonstrated by Grossman et al.¹²⁰

Hypoxia-Inducible Factor

HIF-1 is a transcriptional factor that regulates neovascularization in response to hypoxia. It induces gene transcription of VEGF and eNOS, promoting angiogenesis in ischemic environments. It induces vasodilatation, as well as cell proliferation and migration for sprouting angiogenesis. VEGF also recruits pericytes and SMCs and initiates ECM digestion via activation of MMP.^{121–129} Creager et al.¹³⁰ have investigated HIF in the WALK (effect of HIF-1 gene therapy on walking performance in patients with intermittent claudication) trial to evaluate the effects in patients with claudication. These investigators were unable to demonstrate an improvement in exercise tolerance, claudication, quality of life, or ABI.

Cell-Based Therapies

As highlighted by the studies summarized in Table 7.4 and in this section, the benefits of cell therapy for CLI include improved ABI, rest pain, and ulcer healing. Despite these benefits, however, individual trials have not demonstrated improved limb salvage over the last decade.

Endothelial Progenitor Cells

EPCs are derived from bone marrow or peripheral blood and are responsible for initiating postnatal vasculogenesis, given their ability to proliferate and provide both cytokines and growth factors necessary for vessel development.^{111,131} Investigation has focused on using recombinant human GM-CSF (rhGM-CSF) to stimulate release of these cells from bone

marrow into the peripheral blood to stimulate angiogenesis in patients with PAD.

The START (STimulation of ARTeriogenesis using subcutaneous application of GM-CSF as a new treatment for peripheral vascular disease) trial, a phase II clinical trial conducted by van Royen et al.¹³¹ described efficacy of the use of rhGM-CSF in 40 patients with moderate to severe claudication. There was a tendency toward higher peak flow and peak-minus rest flow in the group treated with rhGM-CSF, thought to be related to enhanced endothelial function at the level of the microcirculation. These researchers were able to demonstrate only a temporary increase in monocyte and CD34 stem cell counts over the study period. A large placebo effect was observed as both the treatment and placebo groups experienced an increase in walking distance over a two-week period.¹³¹

Another phase I/IIa clinical trial, conducted by Kawamoto et al.,¹³² evaluated the use of GM-CSF-mobilized CD34 EPCs in CLI patients with no options for vascularization or secondary to Buerger disease. GM-CSF apheresis was used to collect the EPCs for use as intramuscular injections in 17 patients. The findings indicated that use of CD34 EPCs was safe and effective as patients experienced improvements in pain, pain-free walking distance, and wound healing. In addition, toe-brachial index and tcPO₂ were improved.¹³²

A prospective phase II clinical trial using autologous granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood-derived CD34+ cell transplantation to treat hemodialysis patients with CLI was done by Ohtake et al. in 2018. The study enrolled six patients with CLI (two with Rutherford category 4 and four with Rutherford category 5). No major adverse events were observed at 52 weeks following treatment. Amputation-free survival was noted in all six patients at 1 year. Ulcer size significantly improved as early as four weeks after therapy compared with baseline ($P < 0.01$), and three out of five ulcers completely healed within 12 weeks after cell transplantation.¹³³

Burt et al.¹³⁴ performed a phase I trial using GM-CSF-mobilized CD133+ cells from patients with CLI and no other options for revascularization. Intramuscular injection of CD133+ EPCs was found to be safe and prevented amputation in seven of nine patients. In addition, quality of life improved at 3 and 6 months, but effects did not persist at 1 year. A trend toward improvement in symptom-free ambulation and increased exercise capacity was noted at 1 year, although there was no improvement in ABI.¹³⁴

Zhang et al.¹³⁵ used intraarterial CD133+ cells to study microvascular circulation and angiogenesis in patients with CLI. They first observed improved flow on a macroscopic level by performing angioplasty on infra-aortic and infra-popliteal lesions. In patients with restored flow through the tibial arteries, significant improvements in ABI, tcPO₂, and Rutherford classification were seen at 18 months.¹³⁵

Bone Marrow-Derived Cells

BMCs include endothelial stem and progenitor cells, hemangioblasts, angioblasts, and mesenchymal and hematopoietic stem cells. Some of these cell lines are able to differentiate into

endothelial cells for angiogenesis,^{111,136,137} whereas others, including mesenchymal and hematopoietic stem cells, are not. Most BMCs, regardless of ability to transform into endothelial cells, are proangiogenic and participate in angiogenesis via paracrine signaling and/or the ability to take on endothelial cell-like characteristics.¹³⁶

Phase II clinical trials have been conducted focusing on the safety and efficacy of intraarterial or intramuscular injection of BM-MNCs with or without bone marrow-derived mesenchymal stem cells (BM-MSCs). Initial results have been promising in the treatment of patients with CLI. In the PROVASA trial, a multicenter, double-blinded, randomized phase II trial, Walter et al.¹³⁸ examined 40 patients with severe CLI who received intraarterial BM-MNCs or placebo. At 3 months, all patients (treatment and placebo groups) received intra-arterial BM-MNCs. In the treatment group a significant improvement was seen in ulcer healing and rest pain within 3 months. Both increased number and functionality of the BM-MNCs and repeated administration were associated with greater improvements in wound healing, which then correlated with limb salvage. There was no difference in amputation-free survival or improvement in ABI between the groups.¹³⁸

Two trials have been conducted using a combination product of BMCs. In the RESTORE-CLI trial (a randomized, double-blind multicenter phase II trial comparing expanded autologous bone marrow-derived tissue repair cells [TRCs] and placebo), Powell et al.¹³⁹ evaluated patients with CLI and no options for revascularization. Bone marrow aspiration was performed in 46 patients, and the aspirate was processed to obtain a population of TRCs. This cell population represents a collection of nucleated cells, including endothelial, mesenchymal, and hematopoietic stem and progenitor cells.^{137,139} With intramuscular injection of TRCs, improvement was seen in time to treatment failure and in amputation-free survival. There was also a nonsignificant trend toward wound healing in the treatment group.

Lasala et al.¹⁴⁰ conducted a single-center, prospective, non-randomized, placebo-controlled phase II clinical trial of the intramuscular injection of a similar combination bone marrow product. Their findings demonstrated improvement in walking time and ABI as well as increased perfusion when evaluated with technetium (Tc-99m) tetrofosmin scintigraphy. The purpose of using a BMC combination product, as in these two trials, is to provide growth factors, ECM molecules, and pericytes that are provided by MSC to interplay with EPCs for enhanced vascular growth and repair.^{139–141}

A meta-analysis of 37 clinical trials using G-CSF-mobilized peripheral blood cells (PBCs) or autologous BMCs in patients with PAD¹⁴² revealed superiority of the intramuscular route of administration (versus intraarterial) and BMCs (over PBCs). BMCs were found to improve subjective symptoms, including pain and pain-free walking distance. There was also a significant improvement in ulcer healing. TcPO₂ and ABIs were greater in the treatment group. Comparatively, a slight and nonsignificant improvement in pain scale was seen with PBCs mobilized by G-CSF.¹⁴²

Although early clinical trials using PBCs mobilized by G-CSF did not yield promising results, clinical trials using BMCs continue to demonstrate promising results in the treatment of CLI. Iafrati et al.^{143,144} conducted a randomized, placebo-controlled, phase III trial using BM-MNCs in a group of 48 patients with Rutherford classification 4 and 5. Follow-up at three months showed an improvement in pain, quality of life, ABI, and Rutherford classification, as well as decreased rates of amputation in the study group. Further follow-up at 6 months demonstrated a decrease in amputation rates among study patients.¹⁴⁴

A 2018 Cochrane review by Abdul Wahid et al. compared the efficacy and safety of autologous cells derived from bone marrow–mononuclear cells (BM-MNCs) versus mobilized peripheral blood stem cells (mPBSCs) as treatment for no-option CLI patients. The review included 359 patients including seven RCTs. Main findings demonstrated no clear differences in almost all comparisons between different cell sources and implantation regimens (intramuscular vs intraarterial) for the major clinical outcomes of all-cause mortality, amputation rate, improvement in rest pain, number of participants with healing ulcer, and improvement in ankle–brachial index (ABI).¹⁴⁵ The studies included in this review were underpowered; thus, the long-term safety profile for BM-MNCs and mPBSCs is lacking.

Fully Differentiated Cells

There have been few studies evaluating the role of fully differentiated cells and their use in PAD. Grossman et al.¹⁴⁶ used a combination of fully differentiated autologous venous SMCs expressing VEGF-165 and endothelial cells expressing Ang-1 in a phase I trial. These cells were isolated from a short superficial vein segment, either basilic or cephalic, from the individual's arm. The endothelial and SMCs were transduced ex vivo using pseudo-typed retroviral vectors encoding Ang-1 and VEGF-165, respectively. Following cell processing, the therapeutic combination was administered intraarterially in the subject's most symptomatic leg, and they were monitored for 1 year. This trial demonstrated an increase in mean peak walking time and claudication onset time, as well as no decrease in ABI over the study period. Phase Ib trial using this unique combination, termed MultiGeneAngio (MGA), randomized two concentrations of the product to 18 patients with rest pain or nonhealing ulcers. One-year amputation-free survival rate was 72% (13/18) with no difference in outcomes between the two doses used.¹⁴⁷

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Arterial Hemodynamics

C. ALBERTO FIGUEROA

INTRODUCTION: THE PHYSICS OF ARTERIAL FLOW 88

Pulsatile Pressure and Flow Waves 88

Important Physical Concepts in Blood Flow 88

UNDERSTANDING PULSATILE PRESSURE AND FLOW WAVES IN THE ARTERIAL SYSTEM 93

The Windkessel Model 93

Vascular Impedance 93

Pulse Wave Velocity 94

Pressure Amplification, Aortic Stiffness, and Hypertension 95

SHORT-TERM REGULATIONS OF HEMODYNAMICS 95

LONG-TERM REGULATIONS OF HEMODYNAMICS 96

IMAGE-BASED COMPUTATIONAL ANALYSIS OF ARTERIAL HEMODYNAMICS 98

INTRODUCTION: THE PHYSICS OF ARTERIAL FLOW

Haemo: from ancient Greek *aíouo*-(haimo-). Pertaining to blood.

Dynamics: the branch of mechanics concerned with the motion of bodies under the action of forces. It differs from “kinematics”, which is concerned with the motion of bodies without regard to the forces that cause it.

This chapter assumes knowledge on anatomy and composition of blood and blood vessels. It is concerned with providing a description of the physical principles ruling the hemodynamics of the arterial system, to serve as a reference for vascular surgeons, cardiac surgeons, cardiologists, radiologists, and other clinicians with an interest in the topic. It is far too common that the understanding of concepts used in physics and engineering is loose and inaccurate, leading to frictions in communication between engineers and clinicians.

The arterial system is an engineering wonder of transport efficiency. With clockwork precision, 35 million pulses travel through the system each year, delivering oxygen, nutrients, enzymes, hormones, and heat to every point in the body. The pump (heart), distribution system (arteries), exchange system (capillaries) and fluid (blood) work following the laws of fluid dynamics. In this chapter, we provide an overview of basic engineering concepts used to describe the behavior of flow and pressure waveforms through the arterial system. We also describe bio-physical processes grouped in three different categories: (1) pulsatile pressure and flow waves down the arterial

system; (2) short-term intrinsic and extrinsic mechanisms of autoregulation via vascular smooth muscle tone; and (3) long-term growth and remodeling of arterial tissue in response to chronic alterations in biomechanical stresses.

Pulsatile Pressure and Flow Waves

Figure 8.1 illustrates pressure waveforms down the arterial system, from the aorta to the capillaries, and then on to the venous side of the systemic circulation.¹ The depicted behavior corresponds to healthy conditions: arterial occlusive disease may significantly alter the patterns of pressure and flow.

Mean arterial pressure (MAP) is relatively constant in the aorta and elastic and muscular arteries but experiences a sharp decline at the level of arterioles. Pulsatile behavior is apparent in the aorta, elastic arteries and muscular arteries. Pulsatility also decays greatly in the arterioles and is absent thereafter. Next, an overview of several physical parameters key to understanding pulsatile flow in elastic arteries is provided.

Important Physical Concepts in Blood Flow

Resistance: Resistance refers to the ratio of drop in pressure to flow in a vascular territory:

$$\text{Resistance (R)} = \text{change in pressure / flow} = \frac{\Delta P}{Q} \quad (8.1)$$

It is apparent that the arterioles constitute the vascular territory with the largest resistance, as they experience the largest

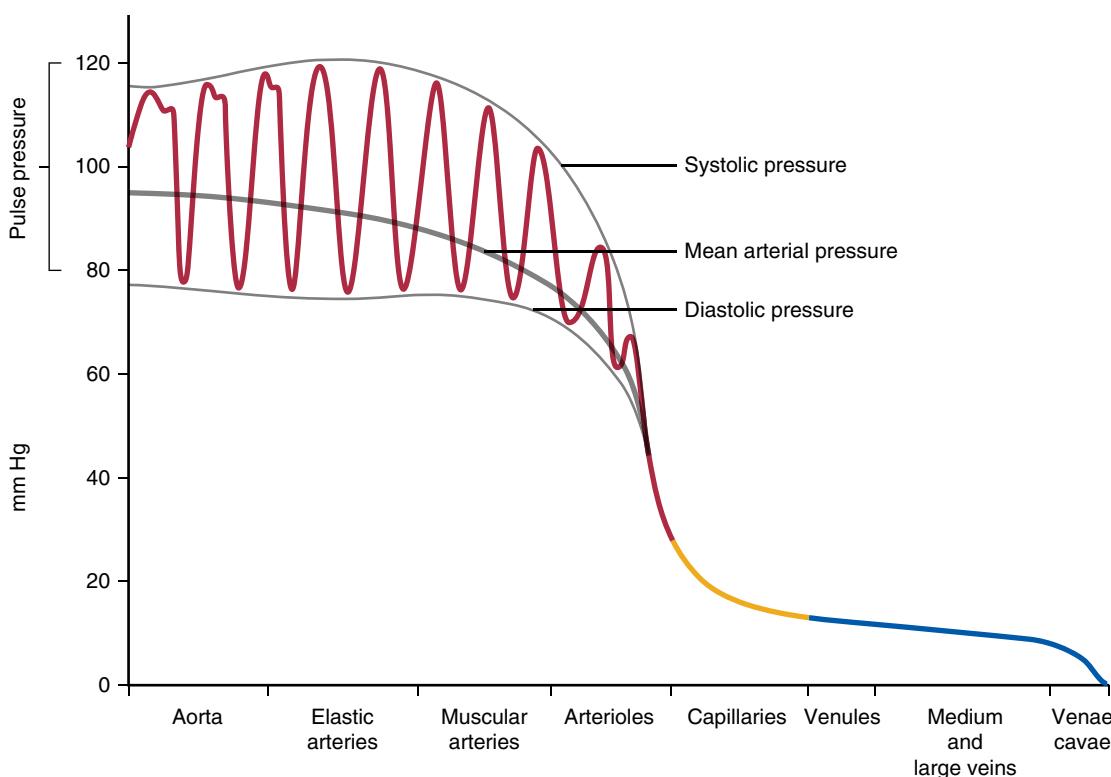


Figure 8.1 Schematic representation of blood pressure down the arterial system, from the aorta, all the way to the capillaries and then on to the venous side. (From: 20.2 Blood Flow, Blood Pressure, and Resistance – Anatomy and Physiology. OpenStax; 2013. <https://openstax.org/books/anatomy-and-physiology/pages/20-2-blood-flow-blood-pressure-and-resistance>.)

drop in pressure. These vessels are known as *resistive* arteries. The resistance is determined by several factors, including vessel length and diameter. *Poiseuille* flow is a useful concept from fluid mechanics to understand the relationship between flow, pressure, and resistance (see Fig. 8.2). Here, a pressure gradient (ΔP) drives flow through the vessel, which moves from a point of higher pressure (Pressure_{proximal}) to a point of lower pressure (Pressure_{distal}). The flow has a parabolic shape, with maximum velocity (Velocity_{max}) at the center of the lumen, and zero velocity at the interface with the endothelial surface.

In Poiseuille's flow, the relationship between flow (Q), pressure drop ΔP , and Resistance are:

$$Q = \frac{\pi R^4}{8\mu L} \Delta P \quad (8.2)$$

$$\text{Resistance} = \frac{8\mu L}{\pi R^4} \quad (8.3)$$

where μ is the blood **viscosity**, L is the vessel length over which the given pressure drop ΔP takes place, and R is the vessel radius. It is thus apparent that the vessel radius plays a much larger role on vascular resistance than the vessel length, due to its power of 4 exponent. This explains why relatively small changes in vascular tone significantly alter vascular resistance (e.g., a 10% vasoconstriction results in an increase of over 50% in vascular resistance).

Another important biomechanical concept that can be easily illustrated with Poiseuille's flow is **wall shear stress** (τ), the tangential stress of the flowing blood on the endothelial surface of the blood vessel² (Fig. 8.2). Stress is force per unit area. Therefore, wall shear stress and pressure have both the same units. Their orientation and magnitude are drastically different though: while typical values of wall shear stress in the arterial system are around 10–100 dynes/cm² acting tangentially to the endothelial surface, pressure is over a thousand times larger (100 mm Hg = 133,322 dynes/cm²) and acts perpendicularly to the vessel wall.^{3,4} Figure 8.3 shows a computational fluid dynamics (CFD) analysis of wall shear stress and pressure values acting on a thoracic aortic endograft, highlighting the difference in magnitude and orientation between these quantities.

Using Poiseuille's solution, the wall shear stress (τ) can be estimated by:

$$\text{Wall shear stress } (\tau) = \mu \frac{\text{Velocity}_{\text{max}}}{R} \quad (8.4)$$

For a given vessel radius, larger velocities will lead to larger wall shear stress. Larger viscosity also results in larger wall shear stress. Both pressure and wall shear stress are important drivers of vascular mechanobiology, as discussed later.

Hoop wall stress: the hoop wall stress (σ_{hoop}) refers to the stress induced by the blood pressure inside the vessel wall. This stress acts circumferentially within the wall and is a key

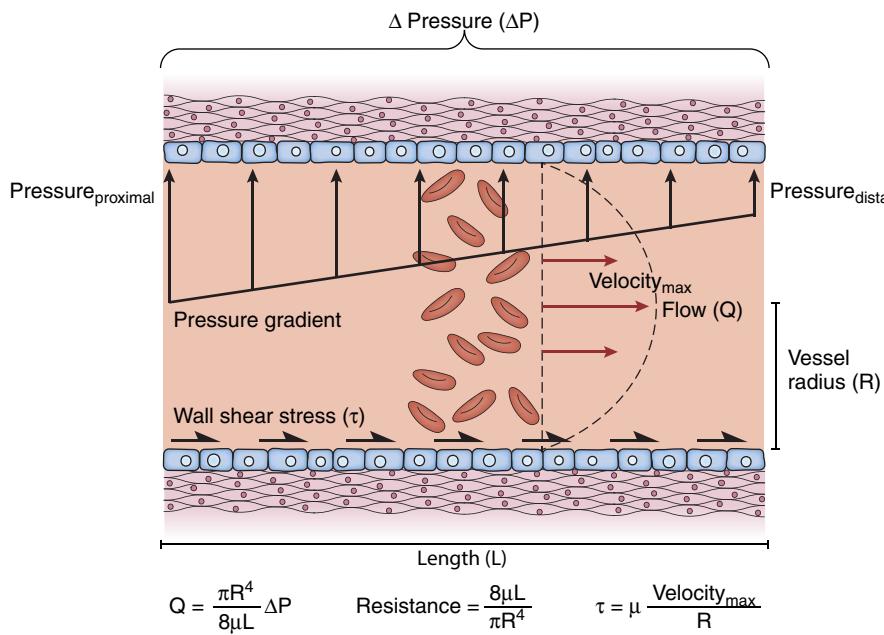


Figure 8.2 Poiseuille's flow has a parabolic velocity profile with maximum velocity in the center of the vessel and is driven by a pressure gradient.

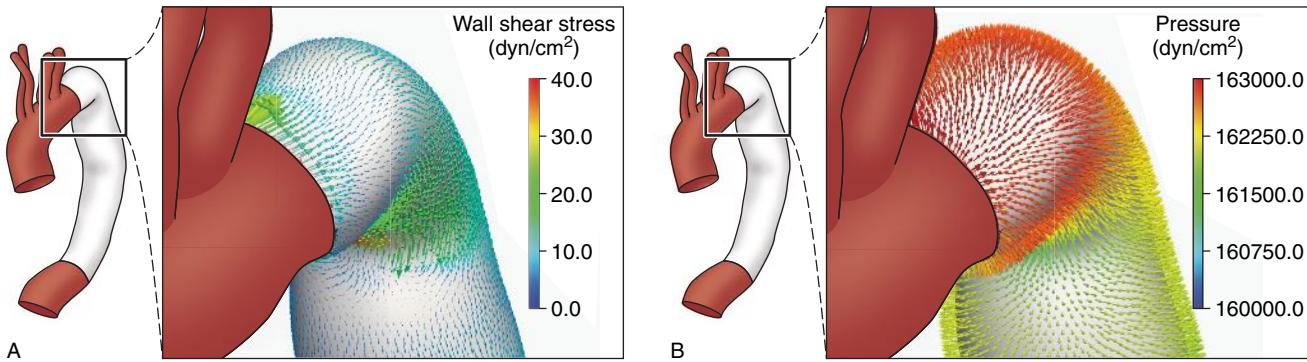


Figure 8.3 Maps of wall shear stress (A) and pressure (B) on the surface of a thoracic aortic endograft at peak systole.³ The wall shear stress acts tangentially to the surface of the endograft, whereas the pressure acts perpendicularly to the surface of the endograft. The pressure is approximately 8000 times larger than the wall shear stress. The vectors of pressure and wall shear stress are drawn using different scales for visualization purposes. (From Figueroa CA, Taylor CA, Chiou AJ, et al. Magnitude and direction of pulsatile displacement forces acting on thoracic aortic endografts. *J Endovasc Ther*. 2009;16(3):350–358.)

biomechanical driver of vessel function.^{5–7} The stress can be understood by another fundamental equilibrium equation in biomechanics, *Laplace's law* (Fig. 8.4, left). Laplace's law states that the hoop stress σ_{hoop} is directly proportional to the product of the pressure P and the vessel radius R and inversely proportional to the wall thickness h :

$$(\sigma_{hoop}) = \frac{P \cdot R}{h} \quad (8.5)$$

It is important not to confuse the hoop stress with the wall shear stress. The hoop stress acts *within* the vessel wall. Assuming a ratio $R/h = 10$, and a mean pressure to 100 mm Hg, typical magnitudes of the hoop stress are 1,333,333 dynes/ cm^2 , which is approximately 30,000 times larger than the magnitude of the wall shear stress.

Axial wall stress: the axial wall stress (σ_{axial}) is due to the tethering of the blood vessel in the axial direction, which imposes a force F_{axial} on the vessel (Fig. 8.4, right). The axial stress is the result of dividing the axial force by the cross-sectional area of the vessel:

$$\sigma_{axial} = \frac{F_{axial}}{\pi h (h + 2R)} \quad (8.6)$$

Vascular compliance and stiffness: the pulsatility of the pressure waveforms is greatly influenced by the *stiffness* of the arterial wall.^{4,8,9} Vascular stiffness (E) is determined by the ratio of elastin to collagen fibers (the more collagen, the stiffer) and by the thickness (h) of the vessel wall (the thicker the wall, the stiffer). The intrinsic stiffness of the vessel wall is known

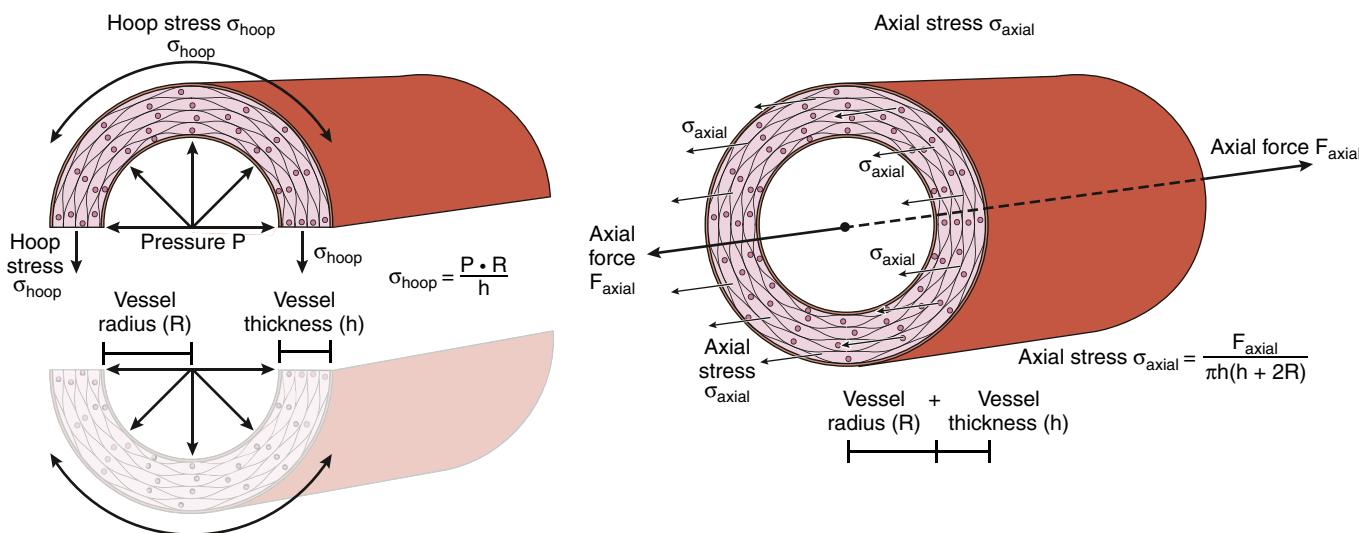


Figure 8.4 **Left:** Laplace's law: the tensile/circumferential stress σ_{hoop} is directly proportional to the pressure load on the blood vessel (P), the vessel radius (R), and inversely proportional to the vessel thickness (h). **Right:** The axial stress σ_{axial} is due to the tethering of the blood vessel in the axial direction, which imposes a force F_{axial} . The axial stress σ_{axial} is the axial force F_{axial} divided by cross-sectional area of the vessel, $\pi h(h + 2R)$.

as *material stiffness*. The product of the thickness and material stiffness is known as *structural stiffness* $E_{\text{structural}} = E \cdot h$. Vascular stiffness is widely accepted as a metric of vascular health, as it plays a critical role in dampening the pulse of pressure as it travels down the arterial system.^{4,9–11} Often, terms such as compliance, distensibility, and stiffness are loosely used to refer to the elastic properties of arteries. For the reader's clarity, simple definitions are given below:

Compliance: Compliance (C) is the change in volume (ΔV) imposed on the vessel by a given change in pressure (ΔP) such as the pulse pressure (PP) between systole and diastole, i.e.:

$$C = \frac{\Delta V}{\Delta P} = \frac{\Delta V}{\Delta PP} \quad (8.7)$$

The compliance of an artery depends on the pressure that it is subjected to. The larger the pressure, the smaller the compliance (see left panel in Fig. 8.5).¹²

Distensibility: distensibility (D) refers to the ratio of changes in luminal area between systole and diastole, divided by the pressure pulse (PP) between systole and diastole, i.e.:

$$D = \frac{(\text{Area}_{\text{systole}} - \text{Area}_{\text{diastole}})}{\text{Area}_{\text{diastole}} \cdot \text{PP}} = \frac{\Delta \text{Area}}{\text{Area}_{\text{diastole}} \cdot \text{PP}} \quad (8.8)$$

Distensibility is therefore a similar metric to compliance, obtained via changes in luminal area of the vessel rather than via changes in volume.

Stiffness: Stiffness (E) is the ratio between increments in stress (σ) and strain (ϵ):

$$E = \frac{\Delta \sigma}{\Delta \epsilon} \quad (8.9)$$

In general, both stress and strain are multi-axial quantities with components in the circumferential and axial directions of the vessels. Therefore, they are more general than the simple pressure, area, and volume quantities used in the definitions of compliance and distensibility. This is the reason engineering analysis often relies on complex, multi-axial stiffness characterization of blood vessels.¹³

Blood vessels exhibit a nonlinear relationship between stress and strain. At low strains and pressures, the burden of bearing the stress is carried by the elastin matrix, which has low values of stiffness.¹⁴ At higher stress, the collagen fibers become engaged and confer the vessel a much stiffer behavior. Note that the shape of the compliance and stiffness curves for vascular tissue is different, due to the axes of stress/pressure and strain/volume being switched (Fig. 8.5, right).

Blood viscosity: viscosity (μ) is another important biophysical concept in blood flow. It refers to the friction between layers of fluid as they slide relative to each other. Figure 8.6 (left) shows a classic experiment used to determine viscosity known as *Couette* flow. Here, a fluid is contained between two parallel plates. The bottom plate is fixed, and the top plate is subject to a force, F , parallel to the bottom plate, which results in the plate moving at a constant velocity v . Therefore, the fluid has a velocity ranging from zero at the interface with the bottom plate to v at the interface with the top plate. Here, the shear rate ($\dot{\gamma}$) is the ratio of change in velocity over a certain distance, $\dot{\gamma} = v/h$, where h is the gap between the plates. In Poiseuille's flow, the shear rate can be approximated by the maximum centerline velocity of blood divided by the vessel radius, see Fig. 8.2, Eq. 8.4.

The bottom plate experiences a wall shear stress (τ), given by the product of the fluid viscosity and the shear rate. Given that in this experiment the shear stress (τ) can be obtained by dividing the force exerted on the top plate F by its area A , the

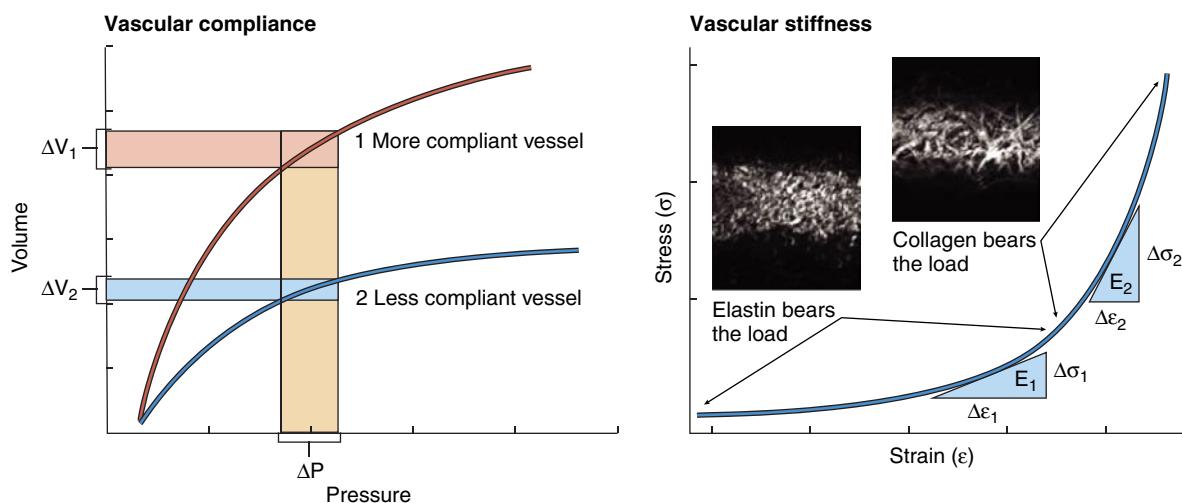


Figure 8.5 **Left:** Vascular compliance curves of two different blood vessels. Vessel 1 is more compliant since for a given change in pressure (ΔP) it accommodates a larger change in volume ΔV . Both vessels become less compliant for larger values of pressure. **Right:** Vascular stiffness (E) is the relationship between increments in stress ($\Delta\sigma$) and strain ($\Delta\epsilon$). At low strains and pressures, the burden of bearing the stress is carried by the elastin fibers, which offer low stiffness. At higher stress, the collagen fibers become engaged and confer the vessel a much stiffer behavior. Insets show nonlinear optical microscopy images of fibrillar collagen from second harmonic generation in a carotid artery. The images reveal a marked undulation of collagen in an unloaded configuration (0 mm Hg), but recruitment and straightening of the fibers when loaded at *in vivo* conditions (80 mm Hg). (From Ferruzzi J, Collins MJ, Yeh AT, et al. Mechanical assessment of elastin integrity in fibrillin-1-deficient carotid arteries: implications for Marfan syndrome. *Cardiovasc Res*. 2011;92(2):287.)

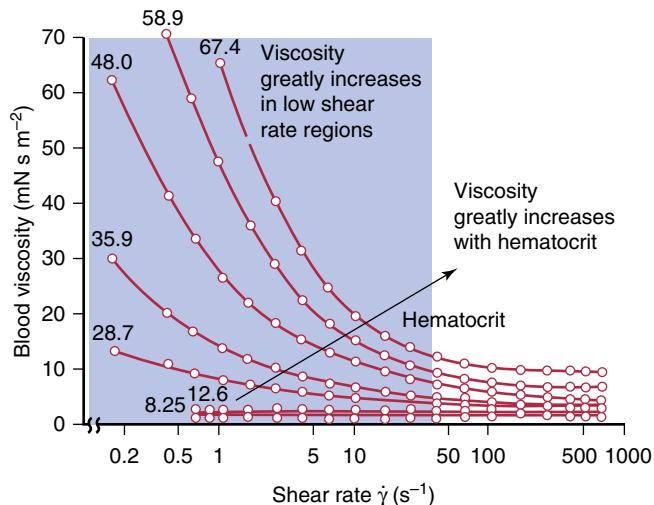
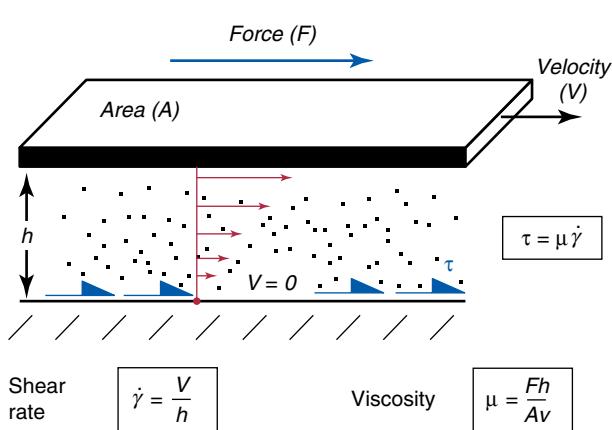


Figure 8.6 **Left:** Couette flow experiment used to determine the viscosity (μ) of a fluid, which is given by the ratio of shear stress (τ) to the shear rate ($\dot{\gamma}$). **Right:** Relationships between viscosity and shear rate for human plasma (straight lines) and increasing levels of hematocrit (12.6–67.4).¹⁵ Results show that viscosity greatly increases for lower values of shear rate. Furthermore, higher hematocrit levels greatly increase the viscosity for all shear rates, with more pronounced effects for lower shear rates.

experiment provides a formula to measure the viscosity of the fluid given by:

$$\mu = \frac{Fh}{Av} \quad (8.10)$$

As we saw earlier, viscosity is an important contributor to resistance to flow (Eq. 8.3). In simple fluids like plasma, the viscosity is constant property of the fluid. However, whole blood has a complex viscosity behavior, due to being a mixture of plasma, cells, and

proteins.^{15,16} This results in viscosity being higher at lower values of shear rate, typically occurring on the venous circulation and on regions of slow re-circulating flow (Fig. 8.6, right). Furthermore, hematocrit levels greatly affect viscosity as well. The right panel of Figure 8.6 shows experimental data for the relationship between viscosity and shear rate for human plasma (straight lines) and increasing levels of hematocrit (from 12.6 to 67.4).¹⁵ Higher hematocrit levels greatly increase the viscosity for all shear rates, with more pronounced effects in the lower shear rate range.

UNDERSTANDING PULSATILE PRESSURE AND FLOW WAVES IN THE ARTERIAL SYSTEM

The Windkessel Model

Some of the most commonly used simplified models of the circulation are the so-called “lumped-parameter models,” which use analogues between fluid flow and current in electric circuits. There is a direct analogue between pressure gradient and voltage, blood flow and current, and resistance to flow and current. The Windkessel model, first described by Otto Frank in the late 19th century,¹⁷ is a widely used lumped-parameter model for arterial hemodynamics.^{4,18,19} The Windkessel effect describes the shape of the arterial pressure waveform, given an input flow waveform (Q) and the interaction between the stroke volume and the compliance of the aorta and large elastic arteries (C) and the resistance of the smaller arteries and arterioles (R). The total arterial resistance can be evaluated by the ratio of mean arterial pressure by the cardiac output (see Eq. 8.1).

$$\text{Total Arterial Resistance } (R) = \frac{\Delta P}{\text{Flow}} = \frac{\text{Mean arterial pressure}}{\text{Cardiac output}} \quad (8.11)$$

The total arterial compliance (C) may be calculated using Eq. 8.7 or similar formulas, to estimate the change in total blood volume associated with a total change in pulse pressure.²⁰ In the three-element Windkessel model, the total arterial resistance, R , is divided between proximal and distal resistances (R_{proximal}) and (R_{distal}), which account for 5% and 95% of the total resistance R , respectively (Fig. 8.7, top panel). The Windkessel model explains the decay in diastolic aortic pressure following aortic valve closure, and can estimate the workload on the heart in terms of peripheral resistance and total arterial compliance.²¹ Windkessel models have also been widely used to characterize parameters such as arterial compliance, peripheral resistance and as a means to derive aortic flow or arterial pressure from image data.²² The bottom panel illustrates how the three-element Windkessel model works. Given values of proximal and distal resistance (R_{proximal} and R_{distal}) and total compliance (C), the Windkessel model takes an inflow waveform (Q), given by the blue curve, and predicts a pressure waveform (P), given by the red curve. The pressure waveform shows a constant diastolic decay. Furthermore, **the pressure waveform clearly lags the flow waveform**. This is a fundamental feature of arterial hemodynamics, and it is present in any mathematical model of pulsatile flow.

Vascular Impedance

The Windkessel model is also useful to understand **vascular impedance**, another important concept in arterial hemodynamics. The impedance, Z , is defined as the ratio of the pulsatile components of the pressure and flow:

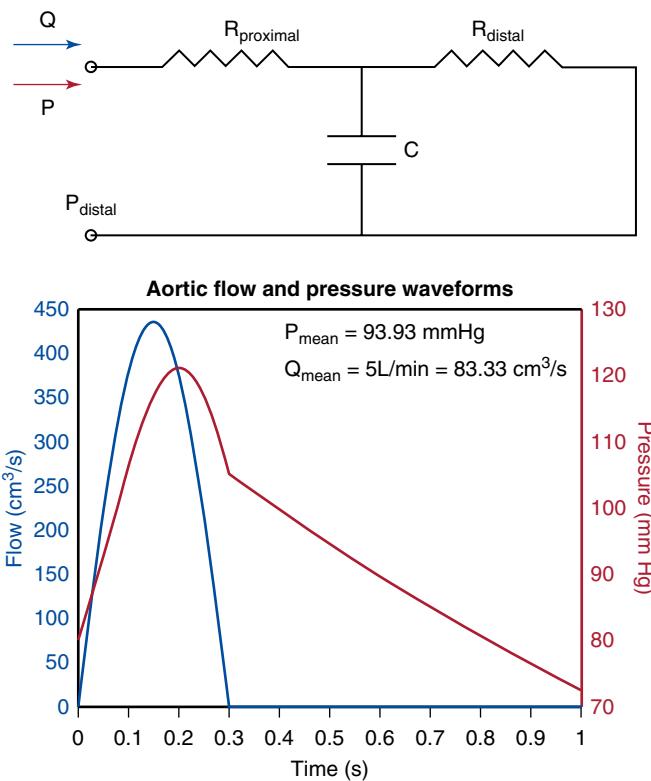


Figure 8.7 Top: Schematic of the most commonly used lumped-parameter model of the arterial circulation, the three-element Windkessel. Bottom: Predicted pressure wave (red), given known parameters and an inflow wave (blue curve).

$$Z(n\omega) = \frac{P(n\omega)}{Q(n\omega)}, \quad n = 0, 1, \dots, N \quad (8.12)$$

where ω is the angular frequency of the pressure and flow waveforms ($\omega = 2\pi / T$), and T is the cardiac cycle. The pressure and flow waveforms can be divided into fundamental components, also known as *harmonics*, using Fourier decomposition of the signals: $P(0)$, $P(\omega)$, $P(2\omega)$, etc. up to N harmonics. Therefore, it follows that the first component of the impedance (when $n = 0$), is equivalent to the resistance:

$$Z(n\omega) |_{n=0} = \frac{P(n\omega)}{Q(n\omega)} |_{n=0} = \frac{P(0)}{Q(0)} = R \quad (8.13)$$

Therefore, the impedance can be understood as a generalization of the resistance in pulsatile, cycle-to-cycle periodic signals, instead of the ratio of mean values utilized by the resistance.

Each vascular bed is characterized by its own input impedance, defined by the ratio of harmonics of pressure and flow waveforms in that territory. This is apparent in the differences in the flow waveforms reported in Figure 8.8. The figure shows a computer model of the aorta and its main branches, together with flow data acquired at several locations down the aorta (sections A, B, C) and at the common carotid artery (D), innominate artery (E), left carotid (F), left subclavian (G), and left renal (H) arteries.¹⁸ The 3D anatomical data was obtained using magnetic resonance imaging (MRI) in a 28-year-old male healthy

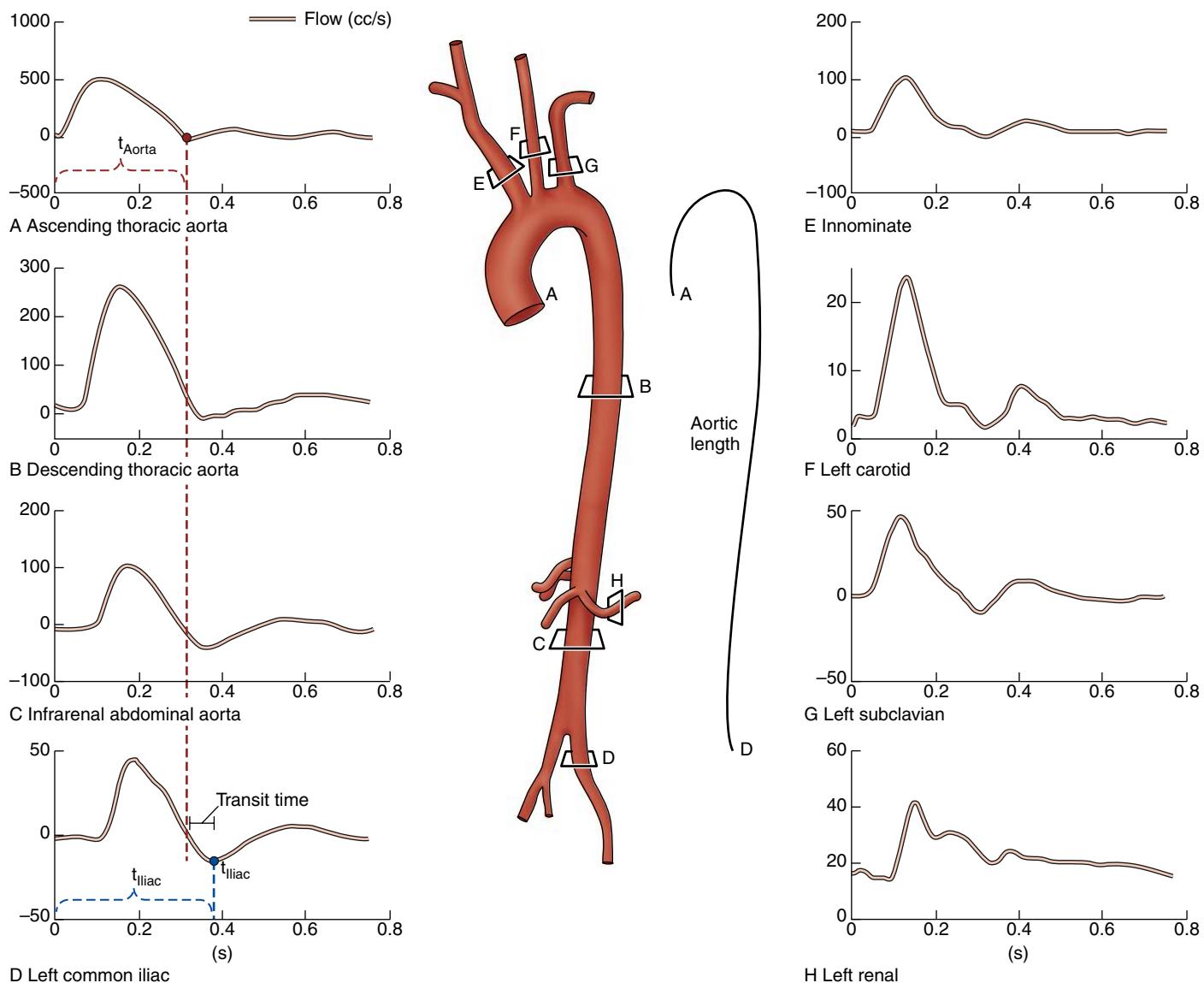


Figure 8.8 A computer model of the full aorta and main branches, reconstructed from MRI data from a 28-year-old healthy volunteer. Planes show flow waveforms acquired using PC-MRI at several locations down the aorta and different branches. (From Arthurs CJ, Xiao N, Moireau P, et al. A flexible framework for sequential estimation of model parameters in computational hemodynamics. *Adv Model Simul Eng Sci.* 2020;7(1):48.)

volunteer. Through-plane velocity data was acquired using 2D phase-contrast MRI (PC-MRI), to produce flow waveforms at several locations down the aorta and different branches.

Pulse Wave Velocity

In addition to the distinct shapes and magnitudes of the different aortic waveforms, this data also reveals another important feature of arterial hemodynamics: **pulse wave velocity (PWV)**.

This is the velocity at which the pressure and flow waves propagate through the circulatory system, not to be confused with the blood velocity. The pulse wave velocity is the speed at which a wave travels down an elastic vessel, with typical values in the 5–10 m/s range.^{4,8} The stiffer the vessel, the faster the speed.

In Figure 8.8, the transit time required by the flow waveforms to travel from the aortic inflow (section A) to the left

common iliac artery (section D) is indicated by identifying a common feature of the waveforms such as the foot of the wave at the start of diastole (labelled t_{aorta} and t_{iliac} , respectively). Knowing the distance between these two locations (aortic length) in the figure, the PWV can be determined as:

$$\text{PWV (m/s)} = \frac{\text{Aortic length (m)}}{\text{transit time (s)}} \quad (8.14)$$

An important analytical equation to understand PWV is the **Moens-Korteweg formula**, which relates PWV with the vessel wall stiffness (E), thickness (h), radius (R) and density (ρ):

$$\text{PWV (m/s)} = \sqrt{\frac{E \cdot h}{2R\rho}} \quad (8.15)$$

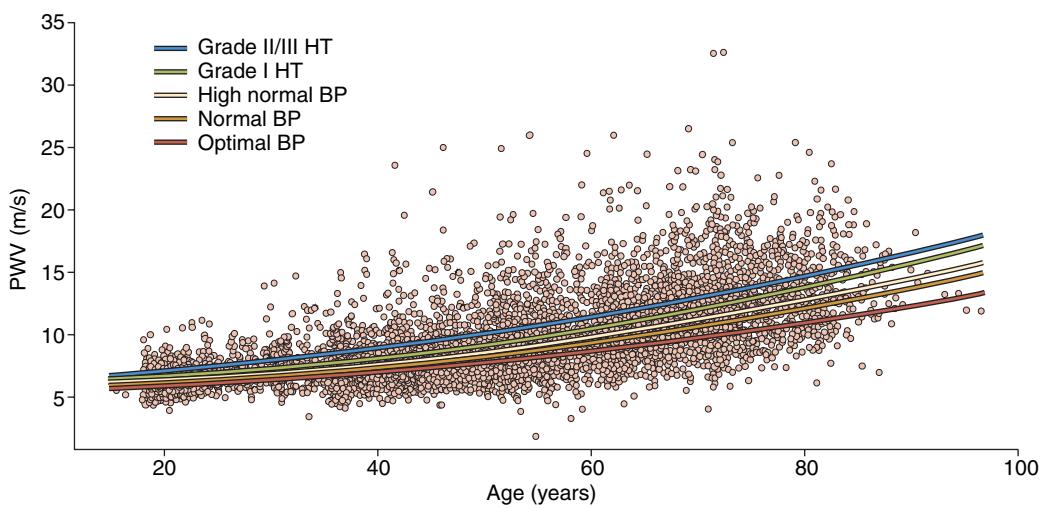


Figure 8.9 Pulse wave velocity (PWV) vs. age in a study involving 11,092 subjects. Regression lines denote the results of regression on age for different blood pressure (BP) categories. (From Mattace-Raso FUS, Hofman A, Verwoert GC, et al. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values.' *Eur Heart J*. 2010;31(19):2338–2350.)

PWV is therefore a biomarker of arterial stiffness (E), which is a well-known independent predictor of all-cause and cardiovascular mortality in hypertensive patients.^{9,11} Figure 8.9 shows data on PWV versus age for different blood pressure categories (from optimal to grade II/III hypertensive) from a study involving over 11,000 subjects free from overt cardiovascular disease, nondiabetic, and untreated by antihypertensive or lipid-lowering drugs. Data shows that, for a given age, the more severe the blood pressure category, the larger the PWV. The data also shows that PWV increases with age for every blood pressure category, indicating an overall stiffening of the aorta with age.

Pressure Amplification, Aortic Stiffness, and Hypertension

The impact of aging on aortic stiffening and pressure wave propagation is further illustrated in Figure 8.10. The left panel shows pressure waveforms from the ascending aorta down to the femoral artery, for three different healthy individuals, ages 24, 54, and 68.^{10,12} In the 24-year-old, a marked increase in pulse pressure (amplitude of the waveform) can be observed from the ascending aorta to the femoral artery. This pulse pressure increase, known as *pulse amplification*, is due to the tapering of the aorta and to the changes in vessel wall composition: at the ascending aorta, the ratio of elastin to collagen fibers is highest, making it the most compliant (least stiff) segment of the aorta. This ratio becomes smaller and smaller for the descending and iliac and femoral arteries, making them less compliant.¹³ The pulse pressure amplification down the aorta for the young subject is also apparent in the schematic of Figure 8.1.

The top right panel of Figure 8.10 illustrates the increase in aortic stiffness in the human aorta for different age groups. The differences between central and peripheral

stiffness are more pronounced for the younger age group.^{8,13} Higher stiffness (or smaller compliance) in the distal aorta results in an increased pulse pressure (see Eq. 8.7). For the oldest subject, the amplitude of the ascending aortic waveform is nearly identical to that in the peripheral locations. This is due to larger stiffening of the ascending aorta with age, more pronounced than in other locations.^{8,13} The physics of increased pulse pressure in the ascending aorta with age are illustrated in the bottom right panel of Figure 8.10. Using *wave intensity analysis*,^{23,24} the pressure waveform can be separated into its main forward traveling wave (purple curve) and its main backwards traveling wave (green curve). The superposition of the two waves gives the total pressure wave. In an older subject with a stiffer aorta, the backwards traveling wave reflects faster in the cardiac cycle, and its peak is more closely aligned with that of the forward traveling wave, resulting in an amplification of the total pressure wave in systole.⁴

SHORT-TERM REGULATIONS OF HEMODYNAMICS

The cardiovascular system is equipped with well-tuned **control mechanisms** that play a key role in regulating hemodynamics via changes in smooth muscle tone to meet the demands of the organism in healthy and diseased states. These control mechanisms can be broadly categorized in two groups:

1. Local (intrinsic) mechanisms: these are the myogenic, endothelial (wall shear stress dependent), and metabolic.^{25,26}
2. Global (extrinsic) mechanisms: these included the neural systems such as the baroreflex (with the sympathetic and parasympathetic pathways)^{27–29} and humoral mechanisms, including circulating catecholamines (epinephrine and norepinephrine),³⁰ the renin–angiotensin–aldosterone

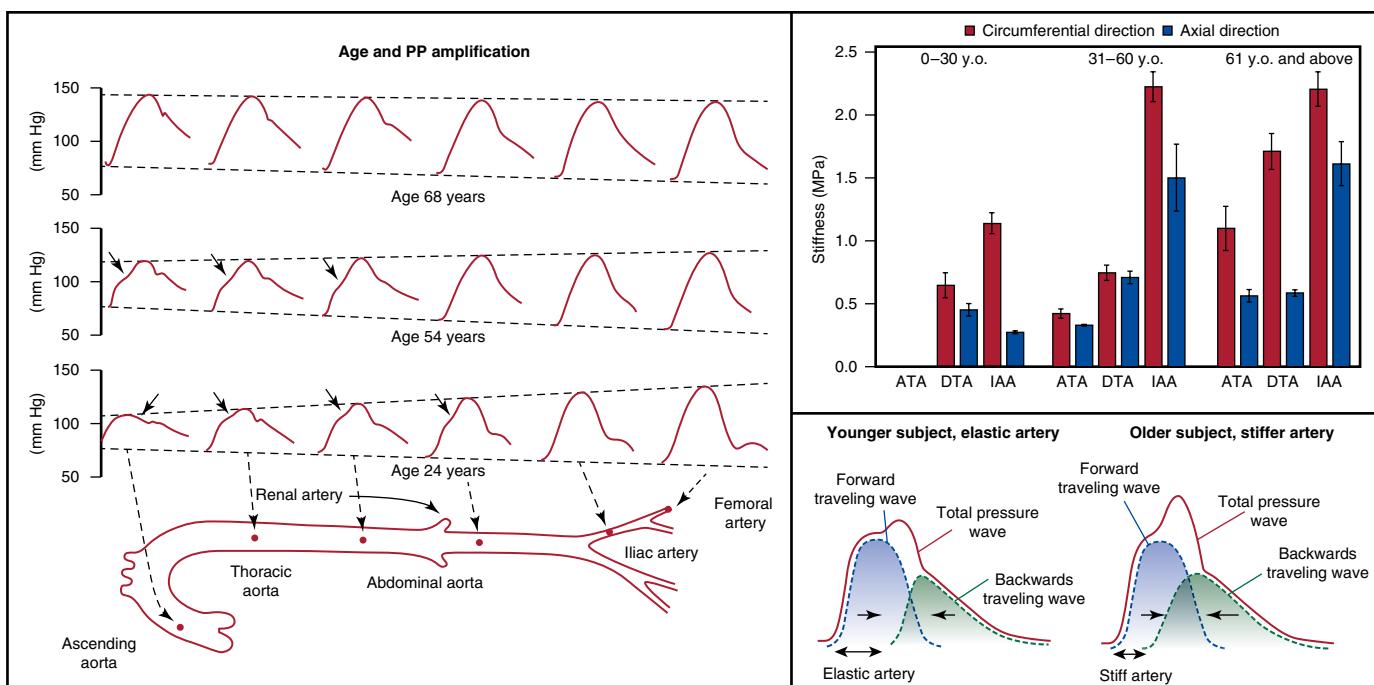


Figure 8.10 Left: Propagation of the pulse pressure wave from central to peripheral arteries at different ages in humans. In younger subjects (age 24), peak systolic blood pressure (SBP) increases markedly from central to peripheral arteries, while end-diastolic blood pressure (DBP) tends to be reduced and mean arterial pressure (MAP) remains unchanged. In older subjects (age 68) with stiffer aortas, because of the more rapid propagation of pressure wave with faster wave reflections, the amplification of the pulse pressure disappears, equalizing the central and peripheral waveforms. **Top Right:** Black and dark gray bars represent values of circumferential and axial stiffness (in MPa), respectively, in the human ascending thoracic aorta (ATA), descending thoracic aorta (DTA), and infrarenal abdominal aorta (IAA) for different age groups. **Bottom Right:** Schematic representation of the faster wave reflections in older individuals with stiffer aortas. (From Safar ME, Laurent P. Pulse pressure and arterial stiffness in rats: comparison with humans. *Am J Physiol Circ Physiol*. 2003;285(4):H1363–H1369. <https://doi.org/10.1152/ajpheart.00513.2003>. Rocabianca S, Figueiro CA, Tellides G, et al. Quantification of regional differences in aortic stiffness in the aging human. *J Mech Behav Biomed Mater*. 2014;29:618–634.)

system,³¹ vasopressin (antidiuretic hormone),³² atrial natriuretic peptide,³³ and endothelin.

Hemodynamic stresses such as hoop stress (σ_{hoop}) and wall shear stress (τ) provide the stimuli for many acute and chronic biologic adaptations. In the myogenic response, increases in pressure increase the hoop stress, σ_{hoop} , which increase the stretch in smooth muscle cells, resulting in increased intracellular Ca^{2+} , which ultimately leads to vasoconstriction despite the increase in pressure. During exercise, increases in blood flow driven by increased metabolic activity result in larger wall shear stress (τ) on the endothelial cells, which release nitric oxide (NO), leading to a decrease in free Ca^{2+} , an ultimately smooth muscle relaxation, increased vessel diameter, and smaller vascular resistance which accommodates larger flows without increasing the driving pressure.³⁴

In the baroreflex system, stretch-sensing cells in the aorta and the carotid bifurcation send signals through the afferent nervous system to the vasomotor center, which in turn sends signals through the efferent nervous system to the heart (to modulate contractility and heart rate) and the blood vessels, which via smooth muscle relaxation or contraction decrease or increase vessel resistance, compliance and unstressed venous volume^{27,29} to modulate blood pressure.

LONG-TERM REGULATIONS OF HEMODYNAMICS

In addition to the short-term adaptations described above, the cardiovascular system also adapts to long-term anatomic and physiologic changes that occur during growth, aging, injury, increased or decreased physical activity levels, and disease. Hemodynamic stresses again play important roles in long-term vascular adaptations. Changes in blood velocity and pressure, sensed at a cellular level, initiate a cascade of biochemical signals leading to hierarchical reorganization across molecular, cellular, tissue, and system scales.⁶ This field of study is known as vascular mechano-transduction.^{35,36} Figure 8.11 shows a schematic of the main biomechanical stresses and their role in vascular mechano-transduction: hoop stress σ_{hoop} (Eq. 8.5), axial stress σ_{axial} (Eq. 8.6), and wall shear stress τ (Eq. 8.4). Increased hoop stress σ_{hoop} triggers thickening in the vessel wall that restores previous homeostatic values; increased wall shear stress τ induced by chronic changes in flow trigger vessel enlargement that restores previous homeostatic values; lastly axial stress σ_{axial} is associated with increased collagen synthesis that may result in vessel lengthening.

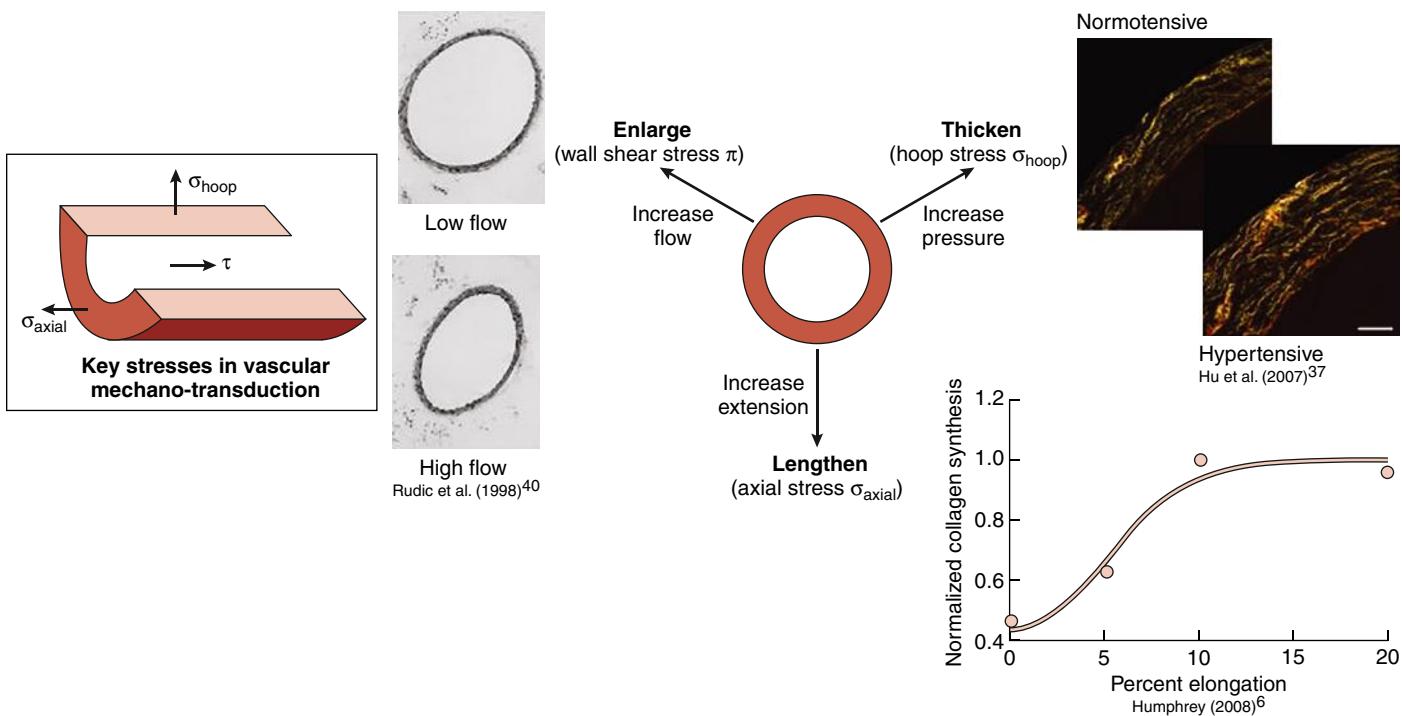


Figure 8.11 Key biomechanical stresses in vascular mechano-transduction and their main associated responses: hoop stress (σ_{hoop}) triggers thickening in the vessel wall that restores previous homeostatic values; wall shear stress (τ) induced by chronic changes in flow trigger vessel enlargement that restores previous homeostatic values of wall shear stress; axial stress σ_{axial} is associated with increased collagen synthesis that may result in vessel lengthening.

Increases in blood pressure increase the hoop stress in the vessel, which alters basic cellular functions including the production and degradation of extracellular matrix as well as proliferation, migration, and apoptosis. This results in increased fibrillar collagen content in the media and adventitia as well as increased smooth muscle in the media, which leads to stiffer vessels and thickening of the wall, which increases the structural stiffness.³⁷ The thickening following the increase in pressure restores the previous (homeostatic) value of hoop stress (see Laplace's formula, Eq. 8.5). Recent work has highlighted a positive feedback loop between central artery stiffening, characterized by the hoop stress induced thickening in response to elevations in blood pressure, and global changes in hemodynamics due to increased PWV, which lead to accumulation of reflected waves in systole and further increases in blood pressure³⁸ (Fig. 8.12). This feedback mechanism stresses the link between local mechanics affecting cell mechanobiology and global hemodynamics controlling systemic physiology.

Blood vessel enlargement in response to chronic increases in blood flow through increases in endothelial NOS due to increased wall shear stress, τ , has long been documented.^{39,40} In contrast to vascular enlargement in response to increased flow, blood vessels reduce in caliber in response to reductions in blood flow.⁴¹ This response is critical in defining the success of arteriovenous fistulas, in which simultaneous changes in hoop stress (due to changes in pressure) and wall shear stress (due to changes in flow) occur.^{2,42}

Endothelial cells are sensitive to magnitude and orientation of wall shear stress signals. The observation that

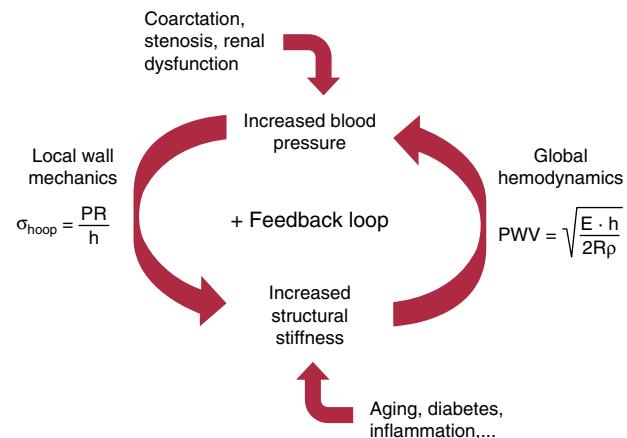


Figure 8.12 Possible positive feedback loop in central arteries that links local wall mechanics (thickening due to increases in hoop stress), which lead to increases in structural stiffness and global hemodynamics (increases in PWV), which leads to accumulation of reflected waves in systole and further increase in pressure. This stresses the link between local mechanics affecting cell mechanobiology and global hemodynamics controlling systemic physiology. (From Humphrey JD, Harrison DG, Figueroa CA, et al. Central Artery stiffness in hypertension and aging a problem with cause and consequence. *Circ Res*. 2016;118(3):379–381.)

atherosclerosis occurs preferentially in localized regions of the vasculature with disturbed flow (carotid bifurcation, infrarenal aorta) has led to the hypothesis that hemodynamic factors play a critical role in its development.^{43–45} This has motivated the application of experimental and computational methods to quantify hemodynamics and vessel wall biomechanics in

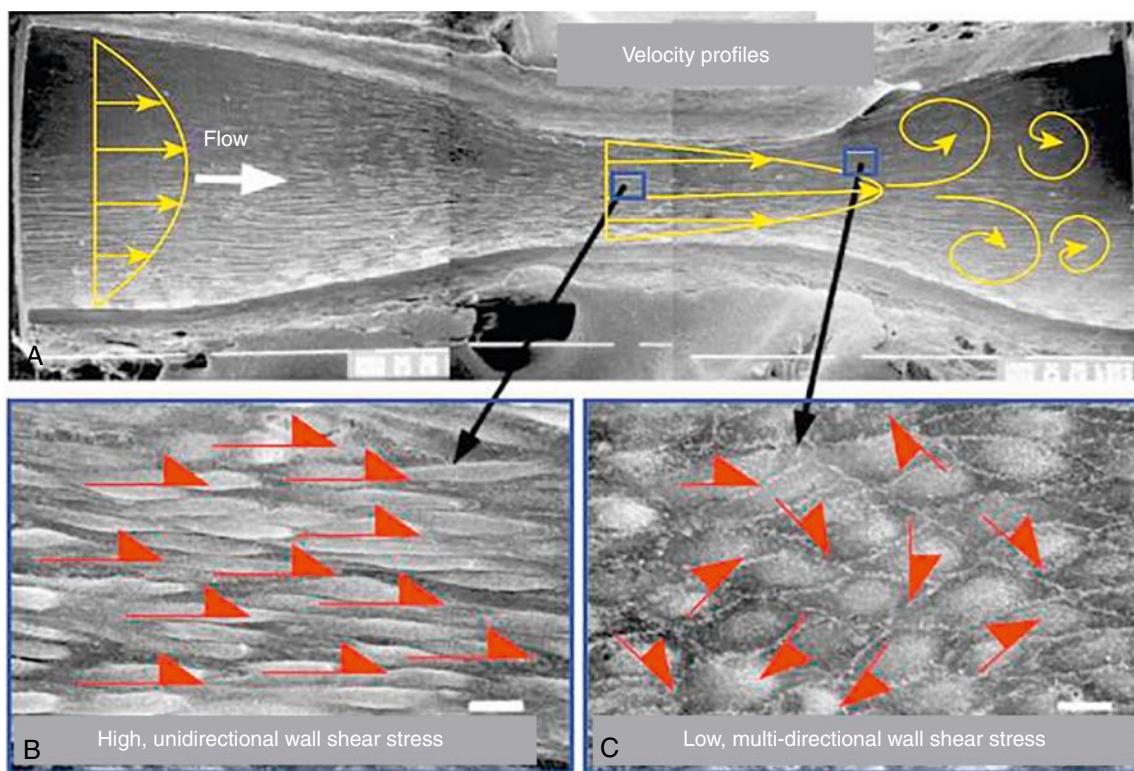


Figure 8.13 Scanning electron microscopy micrographs of the lumen surface of canine common carotid artery coarctation after 2 weeks. A coarctation was made using a silver clip (lumen diameter 1.0 mm, lumen length 3.0 mm) (A). Flow is from left to right (white arrow). In the coarctation channel, endothelial cells are narrow, elongated, and protruded (B). In the post-stenotic area, endothelial cells are short and wide with many fine projections (C). (From Masuda H, Kawamura K, Nanjo H, et al. Ultrastructure of endothelial cells under flow alteration. *Microsc Res Tech*. 2003;60(1):2–12.)

human arteries. Figure 8.13 shows the structural changes in endothelial cells after 2 weeks due to flow-induced alterations in wall shear stress in a common carotid artery banding experiment performed in a dog.⁴⁶ Panel A shows schematic representations of flow patterns (in yellow) proximal, in the banding region, and distal to the banding. Flow is unidirectional prior to the banding and in the banding region, with larger velocities developing in the banding due to the smaller radius. Then, flow becomes disorganized and develops recirculation patterns in the post-stenotic region. Panels B and C show the structure of endothelial cells and schematic representations of wall shear stress (τ) (red vectors) in the banding (B) and post-stenotic (C) regions.

In the banding section (panel B), wall shear stress is high due to the increased shear rates resulting from the larger centerline velocities (Eq. 8.4). The orientation of the vectors is also uniform. The endothelial cells are narrow, elongated in the direction of the flow, and protruded. In contrast, in the post-stenotic section (panel C), the wall shear stress vectors are smaller in magnitude and lack a preferential orientation, due to the disturbed, recirculating flow. Here, endothelial cells are short and wide, with numerous projections. This drastic difference in cellular organization may have implications in endothelial permeability and vascular health.⁴⁷

IMAGE-BASED COMPUTATIONAL ANALYSIS OF ARTERIAL HEMODYNAMICS

Development of image-based modeling technologies for simulating blood flow began in the late 1990s. Since that time, many groups have developed and utilized these techniques to investigate cardiovascular disease, develop and optimize medical devices, optimized surgical planning, and, more recently, aid with noninvasive diagnosis of arterial disease.^{48,49}

Patient-specific modeling of cardiovascular mechanics requires methods to: (a) construct geometric models from 3D magnetic resonance imaging (MRI), computed tomography (CT), or ultrasound (US) anatomical data; (b) extract preoperative physiologic data from cine phase contrast MRI, US, or catheterization data to inform boundary conditions for the computer model; and (c) advanced computational methods to solve the governing equations of blood flow and pressure in the patient-specific models.

Currently, there are highly sophisticated open-source software packages that have been developed specifically for simulation of blood flow in patient-specific anatomical models of arteries, such as SimVascular⁵⁰ and CRIMSON.¹⁹

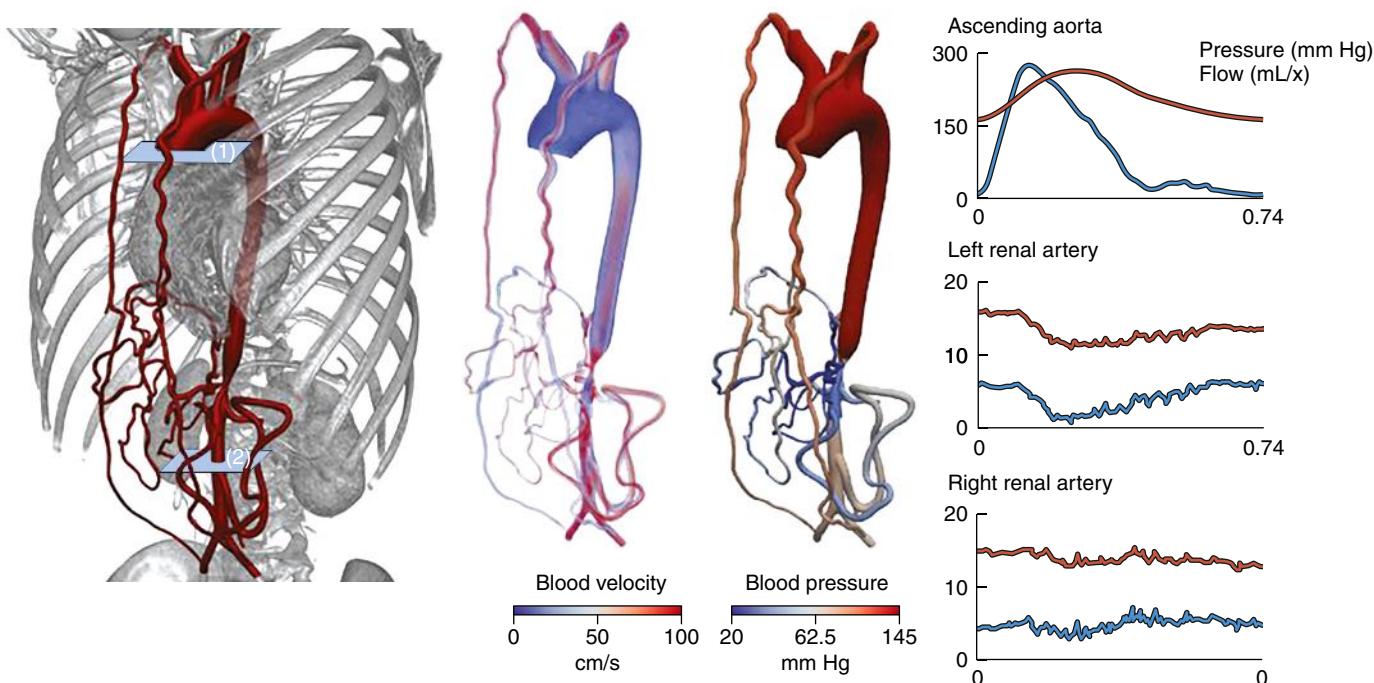


Figure 8.14 **Left:** Medical image data on anatomy (CT, MRI) is used to generate patient-specific models of the vessel of interest. Flow data is available at planes (1) and (2). **Center:** Maps of blood velocity and pressure at peak systole. **Right:** Computed ascending aortic and renal artery pressure and flow waveforms. Renal waveforms show greatly disturbed patterns with diastolically dominated flows and high-frequency components due to the disturbances in blood flow induced by the stenosis and pressure at peak systole. (From Tossas-Betancourt C, van Bakel TMJ, Arthurs CJ, et al. Computational analysis of renal artery flow characteristics by modeling aortoplasty and aortic bypass interventions for abdominal aortic coarctation. *J Vasc Surg.* 2020;71:505–516.e4. Mosby Inc.)

Figure 8.14 shows a schematic representation of the image-based hemodynamic simulation paradigm using CRIMSON. The left panel shows CT medical image data used to generate a patient-specific model of the aorta and main branches of a 9-year-old female subject with mid-aortic syndrome (67% diameter reduction is shown). PC-MRI data on flow is available at planes (1) and (2). This data is used to inform the boundary conditions of the computational analysis. Then, computational analysis methods are used to solve the equations describing the physics of flow and pressure in the anatomical model, using the available boundary conditions. These simulations usually require powerful hardware to solve for velocity, pressure, wall shear stress and other quantities of interest in millions of nodes distributed along the vascular anatomy. This approach provides spatial and temporal resolution in the hemodynamic quantities which are not possible to achieve through imaging. The center panel of Figure 8.14 shows maps of blood velocity.

Lastly, the right panel shows pressure and flow waveforms which can be extracted anywhere in the anatomical model. Here, ascending aortic and renal artery flow and pressure waveforms are shown. The renal waveforms show greatly disturbed patterns with diastolically dominated flows and high-frequency components due to the disturbances in blood flow induced by the stenosis.⁵¹

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Venous Pathophysiology

ANDREA OBI and PETER K. HENKE

INTRODUCTION	100
BASIC CONSIDERATIONS	100
Endothelium and Hemostasis	100
Venous Biomechanics	101
DEEP VENOUS THROMBOSIS	101
Venous Thrombosis Pathways	101
Coagulation Cascade	101
Platelets	101
Natural Anticoagulants	102
Thrombolysis	103

Plasminogen Inhibitors and Thrombosis	103
Inflammation and Thrombosis	103
Thrombus Resolution and Vein Wall Remodeling	104
CHRONIC VENOUS INSUFFICIENCY	105
Historical Perspective and General Background	106
Varicose Veins	106
Pathophysiology of Stasis Dermatitis and Dermal Fibrosis	106
THROMBOPHLEBITIS	107
VENOUS ANEURYSMS	107
CONCLUSION	107

INTRODUCTION

The veins are complex “organs,” and much like arteries, are well suited to their physiologic purpose. Venous diseases represent a major concern in the general population and are influenced by genetics, environment, and acquired conditions. Understanding the basic physiologic and molecular responses to venous injury is essential for designing effective and safe therapies. Deep venous thrombosis (DVT) refers to the formation of one or more thrombi within the deep veins, most commonly in the lower limbs. The thrombus may cause partial or complete blockage of the circulation, which may lead to characteristic symptoms such as pain, swelling, tenderness, discoloration, or redness of the affected area, and skin ulcers. In 2008, the Surgeon General’s call to action to prevent DVT and pulmonary embolism (PE) was published.¹ Recently, a multidisciplinary group convened to sketch out some of the major research priorities over the next decade in venous thrombotic disease.²

BASIC CONSIDERATIONS

Endothelium and Hemostasis

The endothelium forms the inner cell lining of all blood vessels in the body and is a spatially distributed tissue. In an average individual, the endothelium weighs approximately 1 kg and covers a total surface area of 4000 to 7000 square meters.³ The endothelium has been described as a primary determinant of

pathophysiology or as a target for collateral damage in most, if not all, disease processes.^{3,4} Endothelial cells play a critical role in the balance between procoagulant and anticoagulant mechanisms in healthy individuals. Most of the thrombosis–thrombolysis processes occur in juxtaposition to the endothelium, and hence the endothelium is one of the pivotal regulators of homeostasis.⁵

Under normal conditions, endothelial cells maintain a vasodilatory and local fibrinolytic state in which coagulation, platelet adhesion, and activation are suppressed. A non-thrombogenic endothelial surface is maintained by a number of mechanisms, including: (1) endothelial production of thrombomodulin and subsequent activation of protein C; (2) endothelial expression of heparan sulfate and dermatan sulfate, which accelerate anti-thrombin and heparin cofactor II activity; (3) constitutive expression of tissue factor pathway inhibitor (TFPI); and (4) local production of tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). In addition, the production of nitric oxide (NO) and prostacyclin by the endothelium inhibits the adhesion and activation of leukocytes and produces vasodilation.⁶ Tissue factor (TF) production is also inhibited by NO.⁷

Veins and arteries may differentially express homeostatic mediators. For example, von Willebrand factor (vWF) is expressed to a greater extent on the endothelium of veins compared to arterial endothelium, and tPA is less commonly expressed in venous endothelium.⁸ Systemic inflammatory insults such as conferred by tumor necrosis factor- α (TNF- α) may cause