Energy Balance and Cancer 7

Andrew J. Dannenberg
Nathan A. Berger *Editors*

Obesity, Inflammation and Cancer

Springer

Energy Balance and Cancer

Series Editor:
Nathan A. Berger,
Case Western Reserve University,
Cleveland, OH, USA

For further volumes:
http://www.springer.com/series/8282

Andrew J. Dannenberg • Nathan A. Berger
Editors

Obesity, Inflammation and Cancer

Springer

Editors
Andrew J. Dannenberg
Department of Medicine
Weill Cornell Medical College
New York, NY, USA

Nathan A. Berger
Center for Science, Health & Society
Case Western Reserve University,
School of Medicine
Cleveland, OH, USA

ISBN 978-1-4614-6818-9 ISBN 978-1-4614-6819-6 (eBook)
DOI 10.1007/978-1-4614-6819-6
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013937294

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface: What's Old Is New Again and Now It's Red Hot

As the worldwide obesity pandemic expands, obesity has been associated with an increased risk of more and more cancer types. The original malignancies shown to be associated with obesity included esophageal adenocarcinoma, colon cancer, renal cell cancer, postmenopausal breast cancer, endometrial cancer, and advanced prostate cancer. More recently, obesity has been identified as a risk factor for cancers of the pancreas, gall bladder, and ovary and several hematologic malignancies including leukemia, lymphomas, and myeloma, and the list continues to grow.

From a historical viewpoint, while early studies considered the possibility that inflammation initiated the process of carcinogenesis, this was generally considered to be a local effect associated with tissue injury or chronic infection. With elucidation of DNA structure and function and development of the concept of chemical carcinogens as mutagens, attention turned to identification of activated oncogenes and deactivated tumor suppressor genes in the carcinogenic process. Separate studies demonstrated that inflammation extended beyond the local site, mediated by cellular and humoral components. As noted above, independent epidemiologic studies confirmed an association of obesity with cancer incidence, morbidity, and mortality. Studies to identify the mediators of these processes focused on the effects of obesity on growth factors and hormones and the mechanisms of carcinogenesis they commonly affect. More recently, it has become apparent that adipose tissue, in addition to serving as a fat storage depot, is an intensely active metabolic organ. In obesity, low-grade chronic adipose tissue inflammation occurs, resulting in multiple cellular and humoral inflammatory factors. Seminal studies showing that systemic metabolic disorders, such as insulin resistance, could be mediated, in part, by inflammatory cytokines, synthesized and secreted by adipose tissue, resulted in a whole new approach to understanding and attempting to control obesity-associated comorbidities. Moreover, elucidation of the prostaglandin pathway and its role in inflammation, as well as the observations that anti-inflammatory agents, especially the nonsteroidal anti-inflammatory drugs (NSAIDs), could prevent the development and progression of several forms of neoplasia, provided a major stimulus to the field. A major goal of ongoing research is to inhibit inflammation as an approach to cancer prevention and control.

The above brief description traces the complex transdisciplinary evolution of this area of research endeavor. Not only does it illustrate the impact of sometimes divergent disciplines on the evolution of a concept, but it also indicates the potential value of moving forward in this field with a transdisciplinary approach. Accordingly, the goal of this volume of Energy Balance and Cancer, volume 7 in the series, is to highlight the cutting-edge transdisciplinary science linking obesity, inflammation, and cancer. We are grateful to all the authors listed below for their contributions to this volume and look forward to their collective impact in further advancing this rapidly developing field.

This volume first provides information on inflammation as an important link between obesity and insulin resistance, which is in itself linked to promotion of cancer through hyperinsulinemia. The volume then covers some of the most important mechanisms by which obesity leads to inflammation, including the novel inflammasome concept, alterations in chromatin structure, circulating inflammatory factors, unique cellular interactions between adipocytes and macrophages, and the direct link of dietary fat to inflammation and cancer. Subsequently addressed in this volume are a number of target organs and interventional strategies for disrupting inflammation and their effects on cancer prevention and control.

In Chap. 1, Lesley G. Ellies, Andrew Johnson, and Jerrold M. Olefsky (University of California, San Diego) describe the mechanisms by which obesity stimulates low-grade inflammation leading to insulin resistance. Chapter 2, written by Tuo Deng, Christopher J. Lyon, Nan Zhang, Helen Y. Wang, Rong-fu Wang, and Willa A. Hsueh (Weill Cornell Medical College) and Jun Cui (Sun Yat-sen University), reviews the basis for understanding the emerging concept of the inflammasome and its mechanisms of activation and role in obesity. Gerald V. Denis and Deborah J. Bowen (Boston University School of Public Health) describe in Chap. 3 chromatin-based, transcription co-regulatory mechanisms that may link obesity, inflammation, and cancer. Carey Nien-Kai Lumeng (University of Michigan Medical School), in Chap. 4, describes the important role that adipose tissue macrophages play in breast and ovarian cancer. In Chap. 5, Stephanie K. Doerner and Nathan A. Berger (Case Western Reserve University School of Medicine) discuss the impact of different dietary fatty acids on promoting or suppressing colorectal cancer. In Chap. 6, Anamay Sharma, Ahmed Elebiary, Sonia Chowdhury, and Navtej Buttar (Mayo Clinic) describe the contribution of gastric reflux to inflammation in Barrett's esophagus and esophageal adenocarcinoma and potential interventions. In Chap. 7, Stephanie K. Doerner (Case Western Reserve University School of Medicine) and Jason D. Heaney (Baylor College of Medicine) describe the role of obesity-induced intestinal inflammation on colorectal cancer incidence. In Chap. 8, Neil M. Iyengar, Patrick G. Morris and Clifford A. Hudis (Memorial Sloan-Kettering Cancer Center) and Andrew J. Dannenberg (Weill Cornell Medical College) review the emerging evidence supporting the contribution of adipose tissue and chronic breast inflammation to the development of breast cancer. In Chap. 9, the relation of obesity, inflammation, and hepatocellular cancer is discussed by Naim Alkhouri and Arthur McCullough (Cleveland Clinic Lerner College of Medicine at Case Western Reserve University), and in Chap. 10, Jorge Blando, Achinto Saha, Kaoru Kiguchi, and John

DiGiovanni (University of Texas at Austin) describes the role of obesity and inflammation in prostate cancer. Louise R. Howe (Weill Cornell Medical College), in Chap. 11, describes the central role of cyclooxygenase-derived prostaglandins as potential mediators of obesity-related cancer and outlines how targeting this pathway may be protective against obesity-associated carcinogenesis. In Chap. 12, Harmony F. Turk, Jennifer M. Monk, Tim Y. Hou, and Robert S. Chapkin (Texas A&M University) discuss mechanisms through which n-3 polyunsaturated fatty acids interfere with the inflammatory process to suppress carcinogenesis, and in Chap. 13, Gary Stoner and Li-Shu Wang (Medical College of Wisconsin) describe key mechanisms by which naturally occurring dietary compounds reduce the harmful effects of inflammation and the risk for cancer development. In Chap. 14, Stephen D. Hursting, Nikki A. Ford, Sarah M. Dunlap, and Laura M. Lashinger (University of Texas at Austin) and Marcie J. Hursting (Clinical Science Consulting) describe the modification of inflammatory pathways and their impact on cancer by diet and caloric restriction. Ahmad Salameh and Mikhail G. Kolonin, in Chap. 15, describe an innovative approach to adipose tissue control by vascular targeting. In Chap. 16, Michael Gleeson (Loughborough University) describes the anti-inflammatory effects of exercise.

Overall, this volume on Obesity, Inflammation, and Cancer provides an up-to-date status report on the latest developments and state-of-the-art understanding of the role of inflammation in mediating the effects of obesity on cancer and describes possible strategies for targeting inflammation as an approach to cancer prevention and control. The book should be useful for students, researchers, and clinicians, especially those interested in the role of inflammation and its impact on cancer. It is our expectation that this volume will both stimulate research on the role of inflammation in cancer etiology and progression and lead to new approaches and clinical trials for cancer prevention and control by targeting obesity-related inflammation.

New York, NY, USA — Andrew J. Dannenberg
Cleveland, OH, USA — Nathan A. Berger

Contents

1 Obesity, Inflammation, and Insulin Resistance 1
Lesley G. Ellies, Andrew Johnson, and Jerrold M. Olefsky

2 Inflammasomes and Obesity 25
Tuo Deng, Jun Cui, Christopher J. Lyon, Nan Zhang, Helen Y. Wang, Rong-fu Wang, and Willa A. Hsueh

3 Uncoupling Obesity from Cancer: Bromodomain Co-regulators That Control Inflammatory Networks 61
Gerald V. Denis and Deborah J. Bowen

4 Adipose Tissue Macrophages in Obesity, Inflammation, and Cancer 83
Carey Nien-Kai Lumeng

5 Dietary Fats as Mediators of Obesity, Inflammation, and Colon Cancer 99
Stephanie K. Doerner and Nathan A. Berger

6 Inflammation, Obesity, Barrett's Esophagus, and Esophageal Adenocarcinoma 133
Anamay Sharma, Ahmed Elebiary, Sonia Chowdhury, and Navtej Buttar

7 Inflammation, Obesity, and Colon Cancer 147
Stephanie K. Doerner and Jason D. Heaney

8 Obesity, Inflammation, and Breast Cancer 181
Neil M. Iyengar, Patrick G. Morris, Clifford A. Hudis, and Andrew J. Dannenberg

9 Obesity, Inflammation, Nonalcoholic Fatty Liver Disease, and Hepatocellular Carcinoma 219
Naim Alkhouri and Arthur McCullough

10 Obesity, Inflammation, and Prostate Cancer 235
Jorge Blando, Achinto Saha, Kaoru Kiguchi, and John DiGiovanni

11 Pharmacologic Interventions with NSAIDs 257
Louise R. Howe

12 Omega-3 Fatty Acids in Cancer Prevention and Control: A Membrane Perspective 305
Harmony F. Turk, Jennifer M. Monk, Tim Y. Hou, and Robert S. Chapkin

13 Natural Products as Anti-inflammatory Agents 341
Gary Stoner and Li-Shu Wang

14 Calorie Restriction and Cancer Prevention: Established and Emerging Mechanisms 363
Stephen D. Hursting, Nikki A. Ford, Sarah M. Dunlap, Marcie J. Hursting, and Laura M. Lashinger

15 Vascular Targeting of Adipose Tissue 381
Ahmad Salameh and Mikhail G. Kolonin

16 Anti-inflammatory Effects of Exercise 401
Michael Gleeson

Index 425

Contributors

Naim Alkhouri Digestive Disease Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

Nathan A. Berger Center for Science, Health and Society, Case Western Reserve University, School of Medicine, Cleveland, OH, USA

Jorge Blando Division of Pharmacology and Toxicology, University of Texas at Austin, Austin, TX, USA

Deborah J. Bowen Department of Community Health Sciences, Boston University School of Public Health, Boston, MA, USA

Navtej Buttar Mayo Clinic, Rochester, MN, USA

Robert S. Chapkin Kleberg Center, Texas A&M University, College Station, TX, USA

Sonia Chowdhury Mayo Clinic, Rochester, MN, USA

Jun Cui School of Life Sciences, Sun Yat-sen University, Guangzhou, China

Andrew J. Dannenberg Department of Medicine, Weill Cornell Medical College, New York, NY, USA

Tuo Deng Methodist Hospital Research Institute, Weill Cornell Medical College, Houston, TX, USA

Gerald V. Denis Section of Hematology Oncology, Department of Pharmacology and Experimental Therapeutics and Cancer Research Center, Boston University School of Medicine, Boston, MA, USA

John DiGiovanni Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX, USA

Department of Nutritional Sciences, College of Natural Sciences, The University of Texas at Austin, Austin, TX, USA

Dell Pediatric Research Institute, The University of Texas at Austin, Austin, TX, USA

Stephanie K. Doerner Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH, USA

Sarah M. Dunlap Department of Nutritional Sciences, The University of Texas at Austin, Austin, TX, USA

Ahmed Elebiary Mayo Clinic, Rochester, MN, USA

Lesley G. Ellies Department of Pathology, University of California, San Diego, La Jolla, CA, USA

Nikki A. Ford Department of Nutritional Sciences, The University of Texas at Austin, Austin, TX, USA

Michael Gleeson School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, Leicestershire, UK

Jason D. Heaney Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Tim Y. Hou Program in Integrative Nutrition and Complex Diseases and the Center for Environmental and Rural Health Texas A&M University, College Station, TX, USA

Louise R. Howe Department of Cell and Developmental Biology, Weill Cornell Medical College, New York, NY, USA

Willa A. Hsueh Methodist Hospital Research Institute, Weill Cornell Medical College, Houston, TX, USA

Clifford A. Hudis Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Marcie J. Hursting Clinical Science Consulting, Austin, TX, USA

Stephen D. Hursting Division of Nutritional Sciences, The University of Texas at Austin, Austin, USA

Department of Molecular Carcinogenesis, The University of Texas-MD Anderson Cancer Center, Smithville, TX, USA

Neil M. Iyengar Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Andrew Johnson Department of Medicine, Division of Endocrinology and Metabolism, University of California, San Diego, La Jolla, CA, USA

Kaoru Kiguchi Division of Pharmacology and Toxicology, University of Texas at Austin, Austin, TX, USA

Mikhail G. Kolonin The University of Texas Health Science Center at Houston, Houston, TX, USA

Laura M. Lashinger Department of Nutritional Sciences, The University of Texas at Austin, Austin, TX, USA

Carey Nien-Kai Lumeng Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, MI, USA

Christopher J. Lyon Methodist Hospital Research Institute, Weill Cornell Medical College, Houston, TX, USA

Arthur McCullough Digestive Disease Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

Jennifer M. Monk Program in Integrative Nutrition and Complex Diseases and the Center for Environmental and Rural Health Texas A&M University, College Station, TX, USA

Patrick G. Morris Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Jerrold M. Olefsky Department of Medicine, Division of Endocrinology and Metabolism, University of California, San Diego, La Jolla, CA, USA

Achinto Saha Division of Pharmacology and Toxicology, University of Texas at Austin, Austin, TX, USA

Ahmad Salameh The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

Anamay Sharma Mayo Clinic, Rochester, MN, USA

Gary Stoner Division of Hematology and Oncology, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI, USA

Harmony F. Turk Program in Integrative Nutrition and Complex Diseases and the Center for Environmental and Rural Health Texas A&M University, College Station, TX, USA

Helen Y. Wang Methodist Hospital Research Institute, Weill Cornell Medical College, Houston, TX, USA

Li-Shu Wang Division of Hematology and Oncology, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI, USA

Rong-fu Wang Methodist Hospital Research Institute, Weill Cornell Medical College, Houston, TX, USA

Nan Zhang Methodist Hospital Research Institute, Weill Cornell Medical College, Houston, TX, USA

Chapter 1
Obesity, Inflammation, and Insulin Resistance

Lesley G. Ellies, Andrew Johnson, and Jerrold M. Olefsky

Abstract Obesity is a pressing public health concern as it leads to a collection of abnormalities often termed the metabolic syndrome. Molecular studies are revealing novel pathways by which obesity-associated hormonal, nutrient, and tissue factors can stimulate the chronic low-grade inflammation that leads to insulin resistance. Signaling interactions between proinflammatory immune cells, particularly macrophages and lymphocytes, and insulin target cells in the liver and adipose tissue are key to this process and provide potential opportunities for the development of targeted therapies to improve insulin sensitivity and correct energy imbalance.

Abbreviations

ATM	Adipose tissue macrophage
DIO	Diet-induced obesity
FFA	Free fatty acid
GPCR	G protein-coupled receptor
HFD	High-fat diet
IL	Interleukin
SAT	Subcutaneous adipose tissue
SFA	Saturated fatty acid

L.G. Ellies, Ph.D.
Department of Pathology, University of California, San Diego, La Jolla, CA 92093, USA
e-mail: lellies@ucsd.edu

A. Johnson • J.M. Olefsky, M.D. (✉)
Department of Medicine, Division of Endocrinology and Metabolism, University of California, San Diego, La Jolla, CA 92093, USA
e-mail: anj004@ucsd.edu; jolefsky@ucsd.edu

A.J. Dannenberg and N.A. Berger (eds.), *Obesity, Inflammation and Cancer*, Energy Balance and Cancer 7, DOI 10.1007/978-1-4614-6819-6_1,

TNF	Tumor necrosis factor
VAT	Visceral adipose tissue
WAT	White adipose tissue

1.1 Introduction

Overnutrition leads to energy imbalance and obesity, a precursor to the metabolic syndrome and type 2 diabetes, with increased risks of cardiovascular disease and certain types of cancer, resulting in a serious global health issue [1–3]. Traits of efficient nutrient storage and responsive immune cell activation that were advantageous during human evolution have become detrimental to human health in times of food excess [4]. In combination with the reduced physical activity and increasingly sedentary lifestyles associated with improved technology, the sequelae associated with obesity are rapidly increasing. More than one third of adults and almost 17 % of youths in the United States are obese as defined by having a body mass index (BMI, kg/m^2) of at least 30 [5]. Moreover, the most recent data from the National Health and Nutrition Examination Survey indicate that 68.3 % of individuals studied were overweight, having a BMI of at least 25 [4]. Thus, there is a pressing need to understand the molecular mechanisms underpinning obesity so that novel therapies can be developed.

Excess nutrients lead to the expansion of adipose tissues throughout the body and it is not surprising that adipocytes play a major role in obesity-induced insulin resistance [6, 7]. The discovery that low-grade, chronic inflammation in adipose tissue activated proinflammatory pathways critical for the development of insulin resistance has had a major impact on our understanding of the pathophysiology of obesity and type 2 diabetes mellitus. Hotamisligil et al. [8] provided the first evidence that proinflammatory tumor necrosis factor-α (TNFα) produced by adipose tissue could impair insulin signaling and that blocking TNFα activity ameliorated insulin resistance. Another milestone in this field was the work done by Xu et al. and Weisberg et al. [9, 10], who showed that in obesity large numbers of proinflammatory adipose tissue macrophages (ATMs) accumulate in various fat depots. The location of adipose tissue is also important in the development of insulin resistance. Adipose tissue that is deposited centrally, visceral adipose tissue (VAT), is more metabolically detrimental than subcutaneous adipose tissue (SAT) [11], and central obesity is more strongly associated with an increased risk of insulin resistance, the metabolic syndrome, and cardiovascular disease than BMI alone [12, 13]. Gender differences in fat distribution can also affect the incidence of obesity-associated diseases as women have relatively more SAT than VAT compared with men [14]. Men have approximately twice as much VAT as women and this correlates with a higher prevalence of the metabolic syndrome [15]. Estrogen may be a key regulator in mediating these effects as postmenopausal women undergo a redistribution of adipose tissue with increased amounts of VAT and an increased risk of obesity-related metabolic disorders [14]. Interestingly, epidemiologic studies suggest that

obesity in premenopausal women is protective against the development of breast cancer, while obesity in postmenopausal women increases the risk for the disease [16]. Regardless of menopausal status, both obesity and type 2 diabetes are associated with breast cancers that are more aggressive at the time of diagnosis and have a poorer prognosis [17, 18].

1.2 Insulin Signaling

Insulin regulates the metabolism of glucose and lipids and insulin signaling is a complex cascade of events downstream of the insulin receptor (IR). There are two main pathways: the phosphatidylinositol 3-kinase (PI3K)-AKT pathway, which mediates glucose uptake and suppresses gluconeogenesis, and the Ras-extracellular signal-related kinase (ERK) pathway, which mediates gene expression and also interacts with the PI3K-AKT pathway to control cell growth and proliferation [19]. Insulin receptor substrates (IRSs) are key mediators of insulin signaling and include four distinct family members, IRS-1–4. IRS-1 and IRS-2 are widely expressed in mammalian tissues, while IRS-3 and IRS-4 have a more restricted distribution. Tyrosine phosphorylation of IRS by the IR generates binding sites for Src homology 2 (SH2) domain proteins, including the p85 regulatory unit of PI3K. The IRS-dependent activation of PI3K is critical for insulin-mediated regulation of metabolism as it leads to the activation of the serine/threonine kinase AKT. AKT phosphorylates key regulatory proteins in multiple tissues, leading to increased glucose transport in muscle and adipocytes, decreased gluconeogenesis and glycogenolysis in hepatocytes, and anti-lipolysis in adipocytes. Shc is another IR substrate, which subsequently engages the Grb21-Sos1-Ras pathway. Although IRS and Shc proteins are the major substrates of the IR and IGFR tyrosine kinases, other substrates such as Grb2-associated binder (GAB) and downstream of kinases (DOKs) can act as tissue- or pathway-specific alternatives to IRS [19].

Insulin resistance can arise when insulin signaling is restricted at any point in this signaling cascade. In inflammation, immune cells release increased levels of obesity-associated inflammatory cytokines which activate serine kinases such as c-Jun amino-terminal kinase (JNK) [20], inhibitor of nuclear factor kappa-B kinase subunit β (IKKβ) [21], and protein kinase Cθ (PKCθ) [22], resulting in serine phosphorylation of IRS-1. This impairs tyrosine phosphorylation and activation of IRS-1, reducing insulin receptor-mediated signaling and leading to insulin resistance.

The importance of immune cells in the etiology of metabolic disease has led to the emergence of a new field termed immunometabolism [23]. While the precise sequence of physiological events initiating inflammation in obesity remains poorly understood, as with other chronic inflammatory conditions, there is a failure in the control mechanisms that rein in overactive immune responses. This review will focus on the immune cells regulating early events in obesity-mediated inflammation and how nutrients and inflammation-related proteins may impact insulin sensitivity.

1.3 Immune Cells

The inflammatory response that occurs during obesity involves multiple immune cell types. Macrophages, neutrophils, $CD4^+$ and $CD8^+$ T cells, B cells, natural killer T (NKT) cells, eosinophils, and mast cells can all be targeted in ways which either positively or negatively regulate inflammation [24]. Thus, the immune response should be considered as a multifaceted, interactive process whereby many cell populations are inter-reliant (Fig. 1.1).

1.3.1 Macrophages

Tissue macrophage populations encompass a heterogeneous group of cells with diverse phenotypes and functions. Canonically, "classically activated" M1 macrophages, expressing the integrin CD11c, are proinflammatory, whereas "alternatively activated" M2 macrophages, which do not express CD11c, are anti-inflammatory [25]. However, such clear distinction into M1 and M2 phenotypes is not always apparent in vivo, and it is likely that a continuum exists between pro- and anti-inflammatory states [25]. Obesity is characterized by a substantial accumulation of $CD11c^+$ macrophages in the VAT and liver and an overall imbalance towards a more proinflammatory phenotype [9, 10, 26, 27].

The central role of macrophages in mediating obesity-associated insulin resistance is best demonstrated by the numerous genetic studies targeting macrophages and macrophage signaling which ameliorate or exacerbate the insulin-resistant state [28]. For example, the depletion of proinflammatory $CD11c^+$ macrophages [29] or the macrophage-intrinsic deletion of the inflammatory mediators JNK1 [30] or IKK-β [31] improves insulin sensitivity, whereas increasing inflammatory macrophage polarization via cell-intrinsic deletion of PPARγ [32] renders mice more insulin resistant [32].

In obesity, adipocytes and endothelial cells secrete chemokines, such as MCP-I and leukotriene B4 (LTB_4), that attract monocytes into the adipose tissue and liver where they differentiate into macrophages [33–36]. Once inflammation is established, the production of chemokines and cytokines by infiltrating immune cells including ATMs provides a feed-forward mechanism exacerbating macrophage recruitment (Fig. 1.1). MCP-1 mediates recruitment by binding to its cognate receptor CCR2 [37, 38]. In diet-induced obesity (DIO) mice, *Ccr2* deficiency reduced ATM accumulation, the inflammatory profile of adipose tissue, and ultimately improved systemic glucose homeostasis and insulin sensitivity [33]. In the same study, short-term treatment of obese mice with a Ccr2 antagonist also reduced ATM content and improved insulin sensitivity. However, these results have not been consistently observed in MCP-I or *Ccr2* knockout (KO) mice [39, 40] suggesting that there is a degree of redundancy in chemoattractant pathways in vivo.

Leukotriene B4 (LTB4) is a proinflammatory lipid mediator generated from arachidonic acid that promotes chemotaxis [41, 42]. Its potent biological actions are

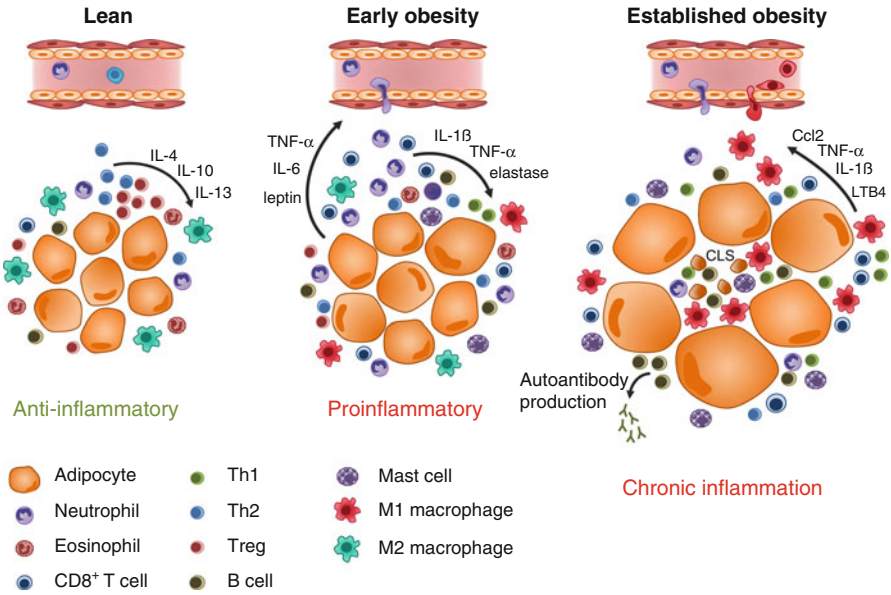

Fig. 1.1 Initiation of adipose tissue inflammation in obesity. In the lean state Th2 T cells, Tregs and eosinophils secrete cytokines such as IL-4, IL-10 and IL-13 that maintain resident macrophages in the M2 state. Early in obesity, there is an influx of neutrophils responding to chemotactic factors released by stressed adipocytes. Neutrophils release neutrophil elastase that promotes proinflammatory cytokine release from $CD8^+$ T cells and Th1 cells that begin to accumulate. This initiates the recruitment of proinflammatory macrophages that are polarized towards the M1 state. In established obesity, M1 macrophages release cyokines that act in a paracrine fashion to maintain the abnormal level of inflammation. Hypoxia and adipocyte cell death fuels the cycle of cytokine and chemokine production that results in a loss of Th2 and Treg cells, while increasing $CD8^+$ T cells, B cells and mast cells. Immune cells congregate in regions of adipocyte lysis forming crown-like structures (CLSs). Cytokines can act locally to cause insulin resistance and can be released into the circulation to promote inflammation in distant tissues

mediated primarily through binding to its G protein-coupled receptor (GPCR), BLT1 [43]. Spite et al. [35] showed that DIO increases the circulating levels of $BLT1^+$ monocytes and genetic ablation of BLT1 reduces ATM accumulation, the expression of proinflammatory cytokines and chemokines and improves insulin sensitivity [35]. Thus, the LTB_4-BLT1 pathway represents a second chemotactic axis promoting macrophage infiltration to adipose tissue and insulin resistance.

Recently, three GPCRs, GPR120, GPR105, and GPR21, have also been implicated in the regulation of macrophage chemotaxis and insulin sensitivity [44–47]. The genetic deletion of GPR105 and GPR21 reduces macrophage chemotaxis to liver or adipose tissue and thus improves insulin sensitivity in DIO mice [44, 46, 47]. The ligand for GPR105 is UDP-glucose, released from injured hepatocytes, and plasma levels of UDP-glucose are elevated in obese mice [44]. Therefore, this is likely to represent a chemotactic pathway whereby macrophages are recruited to

sites of liver damage. In contrast, the ligand for GPR21 at present remains unknown. GPR21 KO macrophages are broadly compromised in their cytoskeletal response to inflammatory stimuli, including MCP-1, suggesting a more general defect in their capacity to migrate [46]. GPR120 is a receptor for anti-inflammatory, omega-3 fatty acids. Omega-3 fatty acids inhibit macrophage chemotaxis, ameliorate adipose tissue inflammation, and enhance insulin sensitivity in a GPR120-dependent manner indicating that this is a potent anti-inflammatory pathway in vivo [45, 48]. Interestingly, a loss of function polymorphism in the human GPR120 gene has recently been associated with obesity emphasizing that this pathway has translational and possibly therapeutic significance [48].

1.3.2 Neutrophils

Among the first cells to arrive at sites of inflammation are neutrophils (Fig. 1.1). Neutrophil deployment is normally tightly regulated since they carry potent cargoes of proteases used to dispose of harmful bacteria. Within 3–7 days of initiating a high-fat diet (HFD), an increase in neutrophil recruitment to adipose tissue occurs [49], suggesting that neutrophils could play a role in initiating the inflammatory cascade in response to excess nutrients. Examination of adipose tissue neutrophils (ATNs) over a longer period of HFD showed that ATN numbers remained elevated for up to 90 days [50]. Expression of neutrophil elastase (NE), a potent serine protease known to have proinflammatory effects [51], was also higher in the obese mice. Pharmacologic or genetic inhibition of the NE improved glucose tolerance of obese mice, while administration of recombinant mouse NE to normal chow-fed mice caused glucose intolerance [50]. In concordance with previous studies demonstrating degradation of IRS by NE in tumor cells [52, 53], NE led to decreased IRS1 levels in mouse and human hepatocytes with subsequent impaired insulin signaling, increased hepatic glucose production, and insulin resistance, indicating that neutrophils may play a previously unsuspected role in the initiation of obesity-induced insulin resistance [50].

1.3.3 CD4⁺ T Cells: T-Helper and T-Regulatory Cell Subsets

$CD4^+$ T cells can be separated into distinct subsets with diverse phenotypes and functions referred to as T-helper (Th1, Th2, and Th17) cells and T-regulatory (Treg) cells, and each of these subsets is present in VAT [24]. Treg and Th2 cells predominate in lean VAT where they play a role in preventing the onset of inflammation [54, 55]. Interestingly, the proportion of Treg cells within the VAT (~40 %) is higher than in any other tissue in the body other than the colon indicating a strong requirement for anti-inflammatory strategies in this tissue [54]. The preferential accumulation of Treg cells in lean VAT is mediated by expression of the transcription factor

PPARγ [56]. Treg cell-specific PPARγ KO mice have reduced Treg cell numbers in VAT and subsequently display enhanced insulin resistance on an HFD [56]. Conversely, treatment of mice with the PPARγ agonist, pioglitazone, enhances Treg cell numbers in obese adipose tissue, and this is part of the mechanism by which pioglitazone increases insulin sensitivity [56]. The precise mechanisms by which Treg and Th2 cells act to limit inflammation in VAT are unknown; however, high expression of the anti-inflammatory cytokine IL-10, especially by Treg cells [54], together with the canonical Th2 cytokines, IL-4 and IL-13, could polarize macrophages towards a less inflammatory state. In contrast, obese adipose tissue is characterized by a specific accumulation of Th1 cells defined by their production of proinflammatory cytokines, such as IFN-γ and unchanged or declining numbers of Th2, Th17, and Treg cells [54, 55, 57]. The accumulation of Th1 cells is subsequent to macrophage and neutrophil infiltration (occurring between 4 and 20 weeks after HFD feeding in mice), and the relatively narrow T cell receptor repertoire of these cells suggests a requirement for antigen presentation [55]. A reversal of this Th1 cell dominance either by anti-CD3 antibody treatment [55] or by pioglitazone-mediated expansion of Treg cells [56] increases insulin sensitivity, and so Th1 cells are thought to contribute to the insulin-resistant state.

1.3.4 CD8⁺ T Cells

$CD8^+$ T cells also accumulate in obese adipose tissue and this begins within 2 weeks of HFD feeding and peaks approximately 9 weeks later [58]. Depletion of $CD8^+$ T cells either prophylactically or after the onset of inflammation improves insulin sensitivity indicating that $CD8^+$ T cells contribute to the insulin-resistant state [58]. $CD8^+$ T cells act to enhance inflammatory macrophage recruitment and differentiation emphasizing the integration of different immune cell pathways during obesity.

1.3.5 B Cells

B cells are recruited to adipose tissues shortly after initiation of an HFD [59], and an absence of B cells protects mice from the development of insulin resistance [60]. Transfer of IgG antibodies from obese WT mice to B cell-deficient mice enhances TNFα production, proinflammatory macrophage polarization and decreases glucose tolerance [60]. Therefore, antibody production is one mechanism by which B cells promote insulin resistance in DIO mice. In human studies, islet cell autoantibodies have been identified in ~10 % of type 2 diabetes patients, and the levels of these antibodies correlate with the need for insulin therapy [61]. Furthermore, autoantibodies against glial fibrillary acid protein (GFAP), one of the antigens most strongly associated with insulin resistance, occur in approximately 30 % of people with type 2 diabetes [62]. Interestingly, B cells deficient in the antigen presentation, major histocompatibility

molecules (MHC-I and MHC-II), do not promote insulin resistance, indicating that an interaction with T cells is a feature of B cell-mediated insulin resistance [60].

1.3.6 Natural Killer T Cells

NKT cells recognize glycolipid antigens and are present in significant proportions in lean VAT and the liver, although their numbers are depleted after the onset of obesity [63–66]. A similar depletion is also observed in obese humans [65]. Studies in mice have shown little or no effect of NKT cell deficiency on the development of insulin resistance on an HFD [64–66]. However, a recent study found that NKT cell-deficient mice display reduced glucose tolerance in lean settings suggesting that NKT cells might be protective during homeostasis [66]. Furthermore, activation of NKT cells with the model ligand, α-Galactosylceramide, can increase glucose tolerance in obese mice by promoting anti-inflammatory macrophage polarization [65]. However, previous studies in younger NKT cell-deficient mice did not observe the same protective effect [63], and therefore, the role for NKT cells in regulating insulin sensitivity remains to be clarified.

1.3.7 Eosinophils

Eosinophils migrate into adipose tissue by an integrin-dependent process and promote M2 macrophage polarization by secretion of IL-4- or IL-13. Eosinophil-deficient mice fed with an HFD develop increased body fat, increased inflammation, impaired glucose tolerance, and insulin resistance, suggesting a role for eosinophils in protecting from DIO [67]. Interestingly, infection by parasitic helminth worms induces an adipose eosinophilia that enhances glucose tolerance, suggesting that targeting eosinophils to increase their numbers or enhance their function could be a useful therapeutic strategy to increase insulin sensitivity [67].

1.3.8 Mast Cells

Mast cells are typically associated with allergic hyperresponsiveness [68]. Increased numbers of mast cells are found in obese adipose tissues of mice and humans compared with their lean counterparts [69–71], and this is accompanied by increased circulating levels of the mast cell protease tryptase [69]. Genetic depletion of mast cells or pharmacologic inhibition of mast cell function reduces body weight gain; reduces the levels of inflammatory cytokines, chemokines, and proteases in serum and adipose tissue; increases energy expenditure; and improves glucose homeostasis [69]. Therefore, mast cells function to promote obesity-associated metabolic changes.

1.4 Signaling Pathways Linking Inflammation and Insulin Resistance

1.4.1 Cytokine Signaling

The inflammatory state of obese adipose tissue leads to increased local cytokine secretion, which directly causes decreased insulin sensitivity. While a number of immune cell types can produce these factors, the macrophage is the major cell type behind the release of proinflammatory cytokines. TNFα is the most well studied and stimulates serine kinases including IKK [72], JNK [20], S6 kinase (S6K) [73, 74], mammalian target of rapamycin (mTOR) [74], and double-stranded RNA-dependent protein kinase (PKR) [75] that can phosphorylate IRS1 on serine residues attenuating downstream insulin signaling (Fig. 1.2).

A variety of interleukins are released during inflammatory responses, and the two most prominent proinflammatory interleukins upregulated in obesity are IL-1β and IL-6 [76, 77]. IL-1β protein levels are increased in mice on HFD and *Il1r1* KO mice are protected from adipose tissue inflammation [78].

1.4.2 Lipid Signaling

The association between increased circulating free fatty acid (FFA) levels and insulin resistance is well known [79]. In the context of obesity, ATMs and other immune cells are exposed to high local concentrations of FFAs released by adipocyte lipolysis. The effects of saturated fatty acids (SFAs) are mediated in part through activation of the pattern recognition receptors (PRRs) Toll-like receptor 4 (Tlr4) and/or Tlr2 [26, 80, 81]. Tlrs normally recognize pathogen-associated molecular patterns (PAMPs) such as bacterial lipopolysaccharides (LPS). SFA-mediated proinflammatory signaling is attenuated in adipocytes deficient in Tlr4 [82] and *Tlr2* or *Tlr4* KO mice are partially protected against HFD-induced insulin resistance [82–85].

Early binding studies indicated that SFAs do not interact directly with Tlr4 [86]. Recent evidence suggests that fetuin A (FetA), a liver-derived circulating glycoprotein, serves as an adaptor molecule presenting FFAs to Tlr4 and activating the Tlr4 inflammatory pathway [87] (Fig. 1.2). *FetA* knockdown in insulin-resistant DIO mice resulted in reduced Tlr4-mediated inflammatory signaling in adipose tissue, whereas administration of FetA restored inflammatory signaling and induced insulin resistance. In addition, fetuin-deficient mice are protected against aging-associated obesity and insulin resistance [88]. FetA levels are elevated in type 2 diabetes and could therefore serve as a biomarker for the inflammatory state, providing an attractive target to improve glucose homeostasis without affecting immune function [89].

SFAs can also activate the Tlr4 pathway by inducing dimerization and recruitment of Tlr4 into lipid rafts, thereby enhancing its association with adaptor

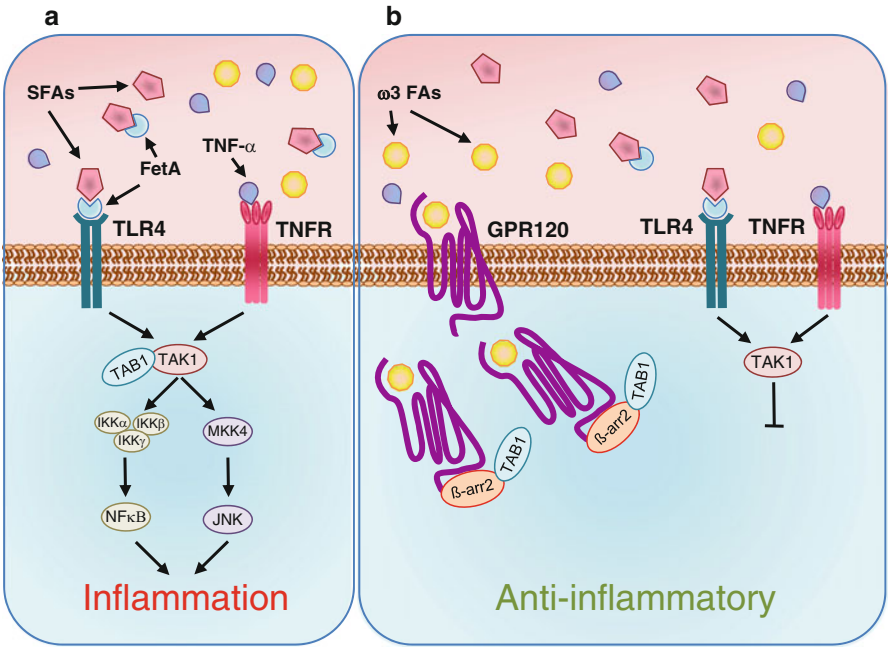

Fig. 1.2 Mechanism for proinflammatory saturated fatty acid (SFA) and anti-inflammatory omega-3 fatty acid (ω3 FA) activity. (**a**) Circulating FetA produced by the liver, functions as an adaptor between SFAs and toll-like receptor 4 (TLR4) signaling. TLR4 activated by SFAs and tumor necrosis factor receptor (TNFR) activated by TNF-α interact with transforming growth factor β (TGF-β) activated kinase 1 (TAK1) with TAK1 binding protein 1(TAB1), initiating a proinflammatory signaling cascade by activating nuclear factor kappa B kinase (NF-κB) and c-Jun N-terminal kinase (JNK). (**b**) Binding of ω3 FAs to GPR120 activates and internalizes the receptor which then binds to β-arrestin 2 (β-arr2) and sequesters TAB1, inhibiting inflammation

molecules TRIF and MyD88 [90]. This process is dependent on the production of reactive oxygen species (ROS) and leads to activation of downstream signaling and increased target gene expression. Other studies have shown that SFAs can alter the membrane distribution of c-Src, partitioning it into lipid rafts where it becomes activated [91]. This leads to signaling via JNK and the transcription of proinflammatory genes. SFAs such as palmitate activate a specialized inflammasome activation pathway in which SFAs inhibit AMP-activated protein kinase (AMPK) leading to defective autophagy, which then activates the inflammasome, resulting in IL-1β cleavage and release [92]. IL-1β stimulates TNFα production and the lack of NLRP3 and its adaptor protein, apoptotic speck protein containing a caspase recruitment domain (ASC), prevents HFD-induced inflammation.

In addition to stimulating inflammatory pathways, metabolites of SFAs, such as ceramide, can directly inhibit insulin signaling by inhibiting Akt. Virtually all stress stimuli increase rates of ceramide synthesis, and numerous studies have

demonstrated a strong association between intracellular ceramide levels and the development of insulin resistance [93–96]. Recent studies have revealed an IKKβ-dependent pathway whereby Tpl2 or Tnf2 induce expression of the genes driving ceramide biosynthesis [93], indicating an additional mechanism by which inflammatory cytokines can act to inhibit insulin signaling [93, 97]. Thus, in addition to acting as ceramide precursors, SFAs stimulate ceramide biosynthesis, amplifying the effects of ceramide on insulin signaling [98].

1.4.3 ER Stress

ER stress activates the unfolded protein response (UPR) to restore ER homeostasis by inhibiting protein synthesis, increasing the degradation of proteins from the ER, and increasing the level of chaperone proteins to assist in protein folding [99]. If these adaptive mechanisms are insufficient to restore ER homeostasis, the cell undergoes programmed cell death [100]. Downstream signaling through regulators of the UPR, PKR-like eukaryotic initiation factor 2α kinase (PERK), inositol-requiring enzyme 1 (IRE-1), and activating transcription factor 6 (ATF-6) can activate both JNK and IKK, leading to the expression of inflammatory cytokines [101, 102]. In addition to protein folding, ER stress response genes play an important role in lipid metabolism as indicated by abnormal lipid processing in the absence of these genes [103–105]. Numerous studies have shown increased ER stress in fatty liver tissues from obese mice.

ER chaperones and folding enzymes such as glucose-regulated protein (GRP) 78, GRP94, protein disulphide isomerase (PDI), calnexin (CNX), and calreticulin (CRT) assist in protein folding and prevent aggregation of unfolded or misfolded proteins [106]. Treating obese or diabetic mice with chemical chaperones [107] or overexpressing GRP78 [108] restores systemic insulin sensitivity and improves hepatic steatosis. Mice deficient in X-box-binding protein-1 (XBP-1), a transcription factor, have an aggravated UPR response, develop insulin resistance and glucose intolerance [109], suggesting that ER stress could be a factor in initiating metabolic inflammation.

1.4.4 Hypoxia

In vivo measurements demonstrate that fat depots in obese animal and human subjects contain distinct regions of microvascularity and exist in a hypoxic state [110, 111]. Hypoxic adipose tissue shows a substantial induction of hypoxia-inducible factor (HIF). Evidence suggests that HIF-1α can activate inflammatory pathways and Krishnan et al. [112] have shown in mice that Hif-1α in VAT is critical for development of glucose intolerance and insulin resistance in HFD mice.