Vascular inflammation is a missing link for diabetes-enhanced atherosclerotic cardiovascular diseases

Najeeb A Shirwany[1], Ming-Hui Zou[1]

[1]*Section of Molecular Medicine, Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA*

TABLE OF CONTENTS

1. ABSTRACT

Diabetes is associated with major life-threatening complications such as a markedly increased risk of cardiovascular disease, even in the presence of rigid glycemic control. Indeed, nearly 75% of diabetic patients eventually die of cardiovascular disease or cardiovascular complications. A striking feature of the diabetic cardiovascular phenotype is the appearance of accelerated atherosclerosis, which resembles atherosclerosis that may be encountered in the non-diabetic individual, except that it is more extensive, aggressive, and occurs at an earlier age. Atherosclerosis (or atherosclerotic vascular disease; ASVD), is a pathological syndrome affecting arterial vessels characterized by narrowing of the vascular lumen secondary to intravascular buildup of fatty material such as cholesterol, aggregated cellular debris, and inflammatory change in the vascular endothelium. Seemingly distinct, these two well-defined disorders are nevertheless, intimately and intricately linked. In fact, these two pathologies appear to be linked by common signaling pathways and shared regulatory systems that appear to go awry in an as yet poorly understood manner. In recent years, a body of evidence has been growing that suggests that inflammation peculiar to the vascular system, occurs in the diabetic patient. This review aims to present the empirical underpinning of the hypothesis that inflammatory change in the vasculature might be the integrated mechanism that connects a diabetic phenotype with its attendant vascular complications.

2. INTRODUCTION

Diabetes is a metabolic disorder characterized by hyperglycemia and glucose intolerance due to insulin deficiency or insulin insensitivity, or both. The two clinical subtypes of diabetes are type I diabetes mellitus (T1DM), and type II diabetes mellitus (T2DM), which are distinct from one another in terms of clinical presentation, epidemiology, prognosis and outcomes. T1DM is causally linked to autoimmune destruction of pancreatic beta-cells which leads to loss of insulin production. This variant is somewhat less prevalent in the general population than T2DM, with the latter accounting for nearly 95% of all diabetics, nearly half of whom are obese. The hallmark of T2DM is peripheral insulin resistance or insensitivity.

3. VASCULAR INFLAMMATION AND ATHEROSCLEROSIS

Classical systemic inflammation was recognized by the ancient Greeks and described as having five classical features: Dolor, Calor, Rubor, Tumor and Functio laesa (pain, heat, redness, swelling, and loss of function). In the context of the cardiovascular system, and in particular the vasculature, inflammatory change is qualitatively distinct from this classical description of systemic inflammation and refers to a cellular and humoral response to specific pathophysiological triggers, that provoke well-defined alterations in vascular function. In the vasculature, risk factors such as diabetes, obesity, and smoking, provoke a

coordinated alteration in the cellular milieu of the vessel intima and wall that is akin to systemic inflammation. For example, the fatty streak (the earliest identifiable change in atherogenesis), is clearly an inflammatory reaction typified by the accumulation of lipids, monocytes, and T-lymphocytes in response to vascular injury or by the presence of high levels of blood glucose (as in diabetes). Not normally resident in the vessel wall, monocytes and lymphocytes transmigrate into the subendothelial space through the actions of locally expressed chemotactic cytokines and adhesion molecules by the injured or disturbed endothelial surface. Ongoing recruitment of additional circulating immune competent cells into the injured vessel wall along with low-density lipoprotein (LDL) oxidation and reactive proliferation of smooth muscle cells ultimately manifests as a fully evolved atherosclerotic lesion. At the site of the lesion, over time, extracellular secretion of metalloproteinases (MMPs) and cathepsins by resident macrophages destabilizes the fibrous cap, leads to plaque rupture, vascular occlusion, and thromboembolic events that prove catastrophic and even fatal, as is the case with subsequent cardiac or cerebral ischemia (1) (Progression of the atheromatous lesion is summarized in Figure 1).

In recent years, it has been widely recognized based on clinical and laboratory evidence that inflammation not only contributes to cardiovascular events in general, but is also a key player in the initiation and progression of atherosclerosis (2). Identifying people at high risk of cardiovascular events has been the cornerstone of cardiovascular medicine and the primary aim of prevention of cardiovascular disease for many years. In the context of inflammation in the cardiovascular system, the value of inflammatory and oxidative markers for the prediction of cardiovascular mortality, is an area of intense interest at the present time. Historically, large studies like the Framingham trial that resulted in the creation of a variety of risk scores, have been available for their discriminative power in predicting cardiovascular events using traditional risk factors, such as sex, age, diabetes, blood pressure, smoking, and LDL-and high-density lipoprotein (HDL)-cholesterol profile (3). Despite the fact that more recently acquired understanding of inflammation and oxidative stress in the pathogenesis of atherosclerosis and other cardiovascular diseases, has been validated in many studies, this information has not been translated into routine clinical practice, where the mainstay of treatment is still modification of the lipid profile. Nevertheless, risk prediction using biomarkers is rapidly evolving and numerous recent reports have established a relationship between various inflammatory and oxidative biomarkers and cardiovascular risk, in both healthy individuals as well as those with established cardiovascular disease (3). There is a wide range of biomarkers that have been proposed for diagnostic and predictive use and these include oxidized LDL, myeloperoxidase (MPO), lipoprotein-associated phospholipase A2, pentraxin-3, cytokines, such as IL-6, proteases such as MMP-9, and C-reactive protein (4-7). C-reactive protein (CRP) is an early response biomarker that has been studies for several decades. In clinical practice, it has become the gold standard to detect and to monitor infectious diseases. It has a half-life of about 19 hours and has a well-established range of increase following an inflammatory stimulus, of up to 100-fold from baseline levels. Thus, CRP is the prototypic acute phase response protein and is mainly produced by hepatocytes, as well as in small amounts by monocytes and macrophages, and possibly by smooth muscle cells, in response to pro-inflammatory cytokines like IL-1 and IL-6 (8). More than 20 different prospective studies have reported a significant and independent association between increased concentrations of CRP and future cardiovascular events in healthy subjects.

Myeloperoxidase (MPO) is a cationic protein, which is found predominantly in granules of neutrophils as well as in some subsets of monocytes/macrophages (8). MPO is secreted after leukocytes are activated and induces the formation of potent oxidants that contributes to innate host defenses. It has been implicated in several inflammatory conditions, including atherosclerosis (8) and related reactive oxidant species have been detected in atherosclerotic lesions. MPO derived oxidative products such as oxidized-LDL and HDL are known to be involved in all stages of atherogenesis. In a recent study called CAPTURE, at increased risk was found for subsequent cardiac events in both the short and medium term, were found to be correlated with MPO levels (9).

In addition to MPO and CRP, protein tyrosine nitration is also emerging as a marker of pathophysiological events implicated in cardiovascular disease. In particular, the modulation of nitric oxide (NO) has been reported to occur in a number of disease states (10). This process is characterized by a series of oxidation processes which modify the tyrosine residues on specific molecules mediated by reactive nitrogen species (RNS). RNS are generated through the reaction of excess or deregulated NO with reactive oxygen species (ROS) (10). As a consequence, the nitration of tyrosine residues to form 3-nitrotyrosine (3-NT), has generally been considered a marker for the formation of peroxynitrite radical ($ONOO^-$). Increased production of $ONOO^-$ has been observed under hyper oxygenation states such as those that may occur in myocardial ischemia-reperfusion injury (10). Furthermore, the extent of protein tyrosine nitration and chlorination of ApoA-I, in both the plasma as well as the arterial wall, has been reported to be significantly higher in coronary artery disease (11). In addition, for example, the levels of nitrated proteins in aortic tissue are increased more than 6-fold in mice lacking the low-density lipoprotein receptor and apobec (LA) and LA mice with genetic deletion of apoA-I (LA-apoA-I-/-). In addition to these well studied biomarkers, ongoing research is also attempting to define the role of many other inflammatory biomarkers relevant to cardiovascular disease and atherosclerosis. These include a variety of cytokines [IL-6, IL-18, IL-10, monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor alpha (TNFalpha)], a variety of cell adhesion molecules (ICAMs, VCAMs, selectins), and several markers of plaque in destabilization and rupture [MPO, MMPs, placental growth factor (PIGF), pregnancy associated plasma protein-A (PAPP-A), CD40L]. Despite these well-

established correlations, it is not clearly understood how inflammation, cardiovascular diseases, and diabetes are interrelated.

The classical description of atherosclerosis is a deposition of lipids within the vessel wall of medium-sized and large arteries. In atherosclerosis, very early change in the blood vessel is an increase in the number of intimal macrophages and the presence of a few foam cells and these are common in infancy and childhood (12). However, the majority of these lesions regress and only a few progress, over many years or decades, to form frank atheromata. The determinants of these contrasting fates of early intravascular events are not well understood. It is becoming more evident however, that failure to regress is likely associated with persistent inflammation in the vascular wall (13).

In the early phases of the atherogenic process, plasma proteins may enter the vessel wall to a higher degree than usual (14). In addition to this, monocytes which are rich in the chemokine receptor 1 (CX3CR1), also infiltrate the vessel wall and initiate an inflammatory response. In the late 1990s, Ross proposed that "endothelial activation" (a term referring to a specific change in the endothelial phenotype characterized by an increase in endothelial–leukocyte interactions and permeability) may be initiated by several different pathways such as an altered response to flow, oxidized or otherwise modified LDL, bacterial antigens, membrane components of bacteria, and endogenous inflammatory signals like cytokines (2). Such early phase activation of the endothelium, results in infiltration of the intima by leukocytes, migrating and proliferating smooth muscle cells, and the subsequent formation of the "fatty streak" (the first grossly visible lesion in the development of atherosclerosis composed of leukocyte macrophages and appearing as an irregular off-white to yellow-white discoloration near the luminal surface of the artery) (15).

In later phases of the formation of an atherosclerotic plaque, other cell types also accumulate in the vessel wall. For example, during the establishment of the plaque , many monocytes invade the vessel wall (15). In the wall, monocytes eventually become lipid-laden macrophages (the so-called "foam cells"), and these form the "core region" of the plaque. In addition, T-cells as well as others have been detected in plaques (16) and include mast cells as well as B-cells. As outlined in an excellent review by Libby, several major steps pertaining to the evolving inflammatory reaction in the artery, can be recognized in the formation of atheromatous plaque (17):

1. Activation of adhesion molecules such as vascular cell adhesion molecule (VCAM) in the endothelium.
2. Adhesion to the endothelium of monocytes and penetration of this barrier by these cells.
3. Increased synthesis of chemokines by the monocytes.
4. Transformation of monocytes to foam cells in response to cytokines such as macrophage colony stimulating factor (MCSF).

5. Alteration of plaque stability by cytokines via modification of the "fibrous cap" with a potential for rupture and thrombosis of the plaque resulting in stroke or myocardial infarction.

Cumulatively, these changes lead to an evolving inflammatory reaction, which is instrumental in the initiation of atherosclerotic plaques, the progression of these lesions and ultimately, their destabilization and rupture (18,19). Because inflammation or inflammatory signaling affects the function of several cell types, including vascular smooth muscle cells (VSMC), platelets, leukocytes, renal mesangial cells, retinal pericytes, and macrophages, it is not surprising that inflammatory processes underpin many if not most aspects of endothelial dysfunction (ED; broadly defined as an imbalance between vasodilating and vasoconstricting substances produced by or acting on the endothelium) and atherosclerosis.

4. VASCULAR INFLAMMATION AND DIABETES-ENHANCED CARDIOVASCULAR DISEASES

Cardiovascular disease is the most common cause of morbidity and mortality in patients with diabetes (20). Individuals with either T1DM or T2DM, have cardiovascular disease rates 4 to 10 times higher than those observed in non-diabetic subjects (20). In addition, patients with diabetes also show more advanced atherosclerosis, for example, as measured by the thickness of the intima and media in the carotid arteries or as a measure of coronary artery calcium scores. Type 2 diabetics also manifest a number of other systemic abnormalities that include hypertension, disturbed lipoprotein metabolism, anomalies in the inflammatory responses as well as abnormal coagulation. These disturbances are all thought to exacerbate atherogenicity and increase cardiovascular disease risk based on a large number of observational as well as mechanistic studies conducted both in human populations, and experimental models of diabetes.

Although diabetes-enhanced CVD might be multifactorial, inflammation might be a common pathophysiological pathway for enhanced CVD morbidity and mortality in diabetes (Figure 1). Direct evidence for inflammation as a causal mechanism in diabetes comes from clinical studies where either small molecules with anti-inflammatory properties, or biological agents that specifically target pro-inflammatory cytokine pathways, have been used for blood glucose control (21). The most promising approaches include selective blockade of IL–1R1 using an antibody-based approach, and inhibition of the NFkappaB pathway with derivatives of salicylate, such as salsalate. It has been reported that both approaches have achieved blood glucose control as well as improved beta-cell function and insulin sensitivity, along with attenuation of systemic inflammation (22,23). These early studies have validated the appropriateness of targeting inflammatory mediators as a treatment for T2DM and also support a cause-and-effect role of inflammation in diabetes. Furthermore, these seminal studies could pave the way for the development of new therapeutic approaches that could effectively modify diabetes progression to the extent that
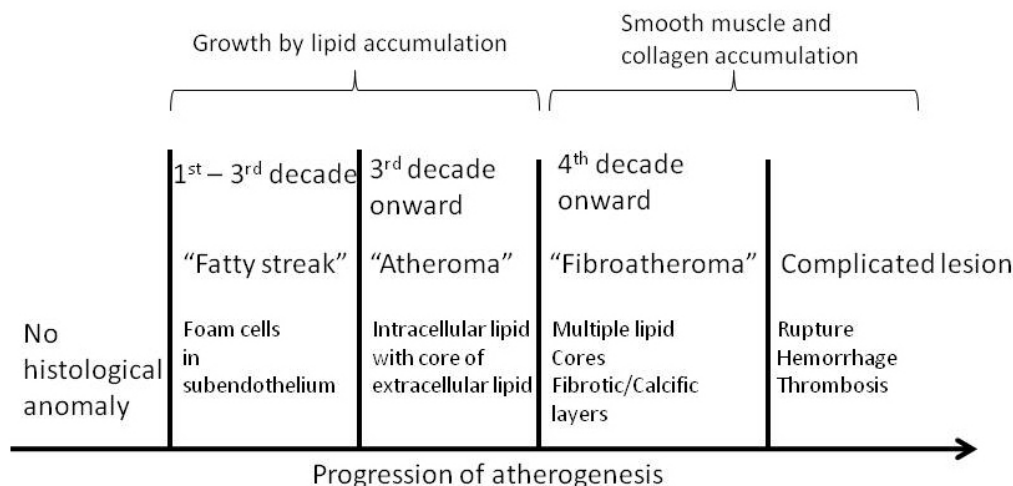
Figure 1. Summary of changes in atherogenesis as a function of age. A timeline is depicted as decades of age, key features of the lesion and its constituents as they change and evolve with age.

simple palliation would become redundant. Thus, in several preclinical studies, three anti-inflammatory approaches have been tested overall: (1) blockade of TNF, (2) antagonism of IL-1beta, and (3), treatment with salsalate (21). Of these, antagonism of TNF has not proven successful (24,25). Interestingly however, in patients who were being treated for rheumatoid arthritis with TNF blockers who had diabetes co-morbidity, benefits in terms of blood glucose levels were clearly identified (21). The most recent interest in a variety of trials has focused on the use of salsalate and IL-1beta antagonsism. The advantage of the former is that it can be administered orally while IL-1β antagonists are only available as injections. Salsalate, however, has a very short half life which requires multiple daily doses to maintain therapeutic blood levels. The trials that have reported positive effects of these anti-inflammatory therapeutic strategies, largely from phase 2 trials with IL-1 blockade or inhibition of the NFkappaB system, include reductions in glycated hemoglobin, attenuation of CRP levels, increased insulin synthesis by the pancreas, improved insulin sensitivity, improved lipid profile, and reduced fasting blood glucose levels (21).

Therefore, it is clear both from mechanistic studies as well early and intermediate phase trials, is that inflammation in the cardiovascular system, is likely a causal phenomena in diabetes-associated vascular disease. These trials with anti-inflammatory compounds have shown great promise but a final verdict has to await completion of trials, regulatory approval, and long-term testing in the clinical realm.

5. ETIOLOGY OF ABERRANT VASCULAR INFLAMMATION AND ATHEROSCLEROSIS IN DIABETES

5.1.Hyperglycemia

The primary clinical manifestation of diabetes, both in T1DM and T2DM, is hyperglycemia. Whether hyperglycemia is caused by insufficient or absent insulin synthesis by the pancreas, or by peripheral insulin resistance, the end result is elevated blood glucose levels [defined characteristically as being higher than 10 mM/L (or 180 mg/dL)]. Tissue that is damaged by hyperglycemia is generally composed of cell types that are unable to effectively control their intracellular glucose concentration. These include neurons, endothelial cells and renal mesangial cells (26). Raised level of glucose inside the cell results in an increased flux through the catalytic pathway and the Krebs cycle. In turn, this results in increased production of reducing equivalents, nicotinamide adenine dinucleotide (NADH), and succinate. Reducing equivalents donate electrons to the mitochondrial respiratory chain and the passage of electrons along the chain enables hydrogen pumping across the inner mitochondrial membrane with the generation of a pH gradient. This gradient is then used by ATP synthase to produce ATP and provide for the energy needs of the cell. The magnitude of this gradient is positively correlated with the production of superoxide (O_2^-) (27). Under the influence of hyperglycemia, the increased production of NADH and succinate results in amplified production of O_2^-. O_2^-, which has the ability to damage DNA, creates cascades of aberrant signaling resulting in further compromise of endothelial cellular function, including cell death (Figure 2).

Raised blood glucose levels are also becoming recognized as being pro-inflammatory as well as pro-oxidant (26). For example, mononuclear cells isolated from healthy volunteers exhibited increased nuclear factor kappa B (NFkappaB) binding, with raised ROS levels as well as increased mRNA for tumor necrosis factor-alpha (TNFalpha), following exposure to hyperglycemia (28,29). In addition, glucose fluctuations in patients with T2DM were found to be associated with increased urinary levels of 8-iso prostaglandin F2 (a marker of oxidative stress) (30). These types of findings have also been validated in animal diabetic models and as an example, in diabetic rats, plasma levels of interleukin-6 and -10 (IL-6 and -10) have been

Intracellular consequences of hyperglycemia
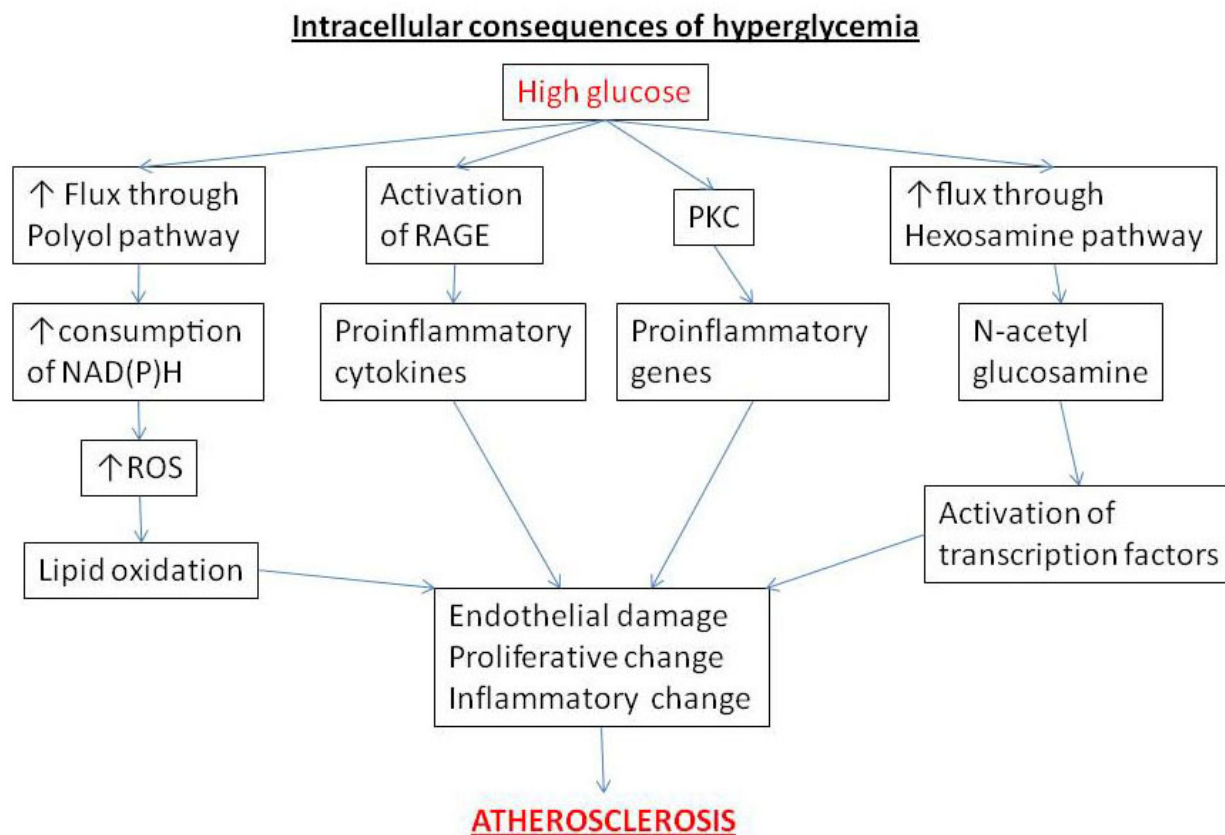
Figure 2. Intracellular consequences of hyperglycemia.

reported to be significantly higher compared to non-diabetic animals (31). Hyperglycemia also tends to produce a hypercoagulable state through an increased expression of tissue factor (TF), which activates factor VII in the coagulation cascade and ultimately results in the generation of thrombin, which converts fibrinogen to fibrin.

Exposure to glucose alone can cause monocytes and macrophages to activate *in vitro*. For example, monocytes grown in high glucose conditions show evidence of increased expression of the cytokines IL-1beta and IL-6 (32). These inflammatory changes are also associated with induction of protein kinase C (PKC), activation of NFkappaB and increased synthesis of ROS, such as O_2^-, and these further participate in glucose mediated oxidative stress.

5.2. Free fatty acids (FFA)

FFA have been shown to disrupt insulin stimulated glucose transport (33). Plasma FFA enter cells and are either oxidized to generate energy in the form of ATP or are re-esterified for storage as triglycerides (TG). Therefore, *in situ*ations where plasma levels of FFAs are high (as in the case of diabetes) intracellular (intramyocellular or intrahepatocellular) accumulation of TGs occurs (34). Increased FFA levels also results in accumulation of several metabolites involved in FFA re-esterification including long chain acyl-CoA and

diacylglycerol (DAG) (35). DAG is a potent activator of PKC isoforms . In addition, other serine/threonine kinases, such as IkappaB kinase complex (IkappaK-β) and the c-Jun NH2-terminal kinase (JNK), are also activated by raised plasma FFA levels (36). Once activated, these serine/threonine kinases can interrupt insulin signaling by attenuating tyrosine phosphorylation of the insulin receptor substrate 1 or 2 (IRS 1/2) (37). This inhibits the IRS/PI3 kinase/Akt pathway which regulates critical insulin actions including glucose uptake, glycogen synthesis, glycogenolysis, and lipolysis (38). The IRS/PI3 kinase/Akt pathway is also critical for the activation of endothelial nitric oxide synthase (eNOS) and the production of NO. In addition, FFA can reduce NO production through another mechanism which acts via stimulation of nicotinamide adenine dinucleotide phosphate oxidase [NAD(P)H oxidase]. This has been shown to occur in a PKC-dependent manner, and leads to increased production of ROS and a decrease in NO (39).

5.3. Adipokines

Recent advances in adipocyte biology has revealed that these cell types are actually an active endocrine organ which secretes several important bioactive compounds called "adipokines" (40). These include several types of cytokines (e.g. TNFalpha and IL-6), chemokines (like IL-8 and MCP-1), and hormones (such as leptin, resistin, and adiponectin) (41). These adipokines have been

the subject of intense scrutiny by biologists and physicians in the context of metabolic disorders given that obesity has reached pandemic proportions, virtually on a global scale (42). Obesity appears to be an independent risk factor for the development of coronary artery atherosclerosis (43) which has led to a frantic attempt on the part of public health agencies, physicians, and policymakers in the western hemisphere, to address the challenge of combating dramatic increases in obesity in western populations. Abdominal obesity or adiposity (also called central or visceral obesity) in particular, is a major factor associated with accelerated progression of atherosclerosis. It is therefore not surprising to find that obesity is associated with a chronic low-grade inflammatory condition in adipose tissue (44). In obese individuals, adipose tissue is infiltrated by macrophages which secrete a number of pro-inflammatory cytokines (generally referred to as adipokines). Recently, T-cells have been detected in adipose tissue found infiltrating sites of high-fat accumulation in obese individuals (45). Adipokines have been shown to mediate the recruitment of immune cells, supporting the view that a feedback regulation mechanism exists, which perpetuates the chronic inflammatory state observed in obese individuals and animal models (46). In mouse models of atherosclerosis, an increased inflammatory response in peri-adventitial and visceral adipose tissue has been demonstrated (47). In these animals, fat depots revealed chronically increased infiltration by macrophages. When these fat depots were transplanted into atherosclerosis-prone ApoE-knockout animals, the plasma levels of leptin, resistin and monocyte chemotactic protein-1 (MCP-1), were all increased. Mice transplanted with visceral fat also developed significantly more atherosclerosis compared with sham-transplanted animals. All of this data has prompted research into defining and identifying the hormones or chemicals released by adipose tissue. Some adipokines are pro-atherogenic while others have protective properties against the formation of atherosclerotic lesions. Adiponectin exerts a protective effect and in *in vitro* studies for example, it has been shown to inhibit TNF-alpha-induced increase in the endothelial expression of inter-cellular adhesion molecule 1 (ICAM-1), VCAM-1, and E-selectin (48). Adiponectin also suppresses VSMC proliferation, as well as the transformation of macrophages to foam cells (49,50). In addition, Adiponectin reduces lipid accumulation in foam cells and attenuates the formation of atherosclerotic lesions by enhancing the uptake of cholesterol into macrophages (51,52). *In vivo* studies have shown that ApoE/Adiponectin-double knockout mice have increased levels in the plasma of Interferon gamma (IFN-gamma)-induced expression of interferon gamma-induced protein-10 (IP-10) as well as increased atheromatous plaques compared with controls. IP-10 also inhibits the production of chemokine ligands in macrophages and reduces T-lymphocyte recruitment during the process of atherogenesis (53). Adiponectin also has been shown to prevent fibroblasts in the adventitia of blood vessels, from proliferating and transforming into myofibroblasts, migrating to the intima, thereby worsening atherosclerosis (54). Mice that were transgenic for macrophage derived Adiponectin, exhibited reduced macrophage foam cell

formation in the arterial wall when these animals were crossbred with LDL-deficient mice (55). In ApoE knockout mice, when plasma Adiponectin levels were artificially boosted by using an adenovirus transfection system, there was a significant suppression in the progression of atherosclerotic lesions in the aortic sinus (56). In contrast to Adiponectin, another important adipokine called Leptin, exhibits some discrepancies in data regarding its role in atherogenesis. As an example, a study has suggested that Leptin treatment of ApoE knockout mice did not alter the size of atherosclerotic lesions or the surface area but did increase the calcification of these lesions and the expression of markers specific for osteoblasts, namely osteocalcin and osteopontin (57). In another study using ApoE knockout mice, treatment with recombinant Leptin resulted in an increase in atherosclerosis in terms of the size of the surface of the lesion as well as a shortening of the time required for these lesions to completely occlude the vessel lumen (58). The deficiency of Leptin in LDL receptor (LDLr) deficient mice has also been shown to induce a several fold reduction in the size of atherosclerotic lesions (59). Although these studies appear to identify a critical role for Leptin and Leptin receptors in the modulation of atherogenesis, further studies are clearly needed before definitive conclusions can be reached. Another interesting example of an adipocyte derived chemokine, is Resistin. This protein was originally discovered to be secreted by adipocytes in mice. In contrast to rodents, where adipocytes are the major source of Resistin, humans appear to express this protein only in macrophages (60). However, Resistin is present in both murine and human atherosclerotic lesions. ApoE knockout mice, for example, have significantly higher Resistin mRNA and protein levels in the aorta as well as having increased levels in the circulation. Overall, from these studies the conclusion has been drawn that complex, interactive and reciprocal regulation may occur among adipokines. Quite recently, another adipokine, Visfatin, has been discovered to play a specific newly defined role in insulin resistance (61). In this instance, Visfatin treatment has been shown to increase the level of circulating IL-6 without affecting the levels of TNF (62).

5.4. Advanced glycation endproducts (AGE)

AGEs are products of non-enzymatic glycation and oxidation of proteins and lipids. These products accumulate in the vessel wall, especially in diabetes, and are thought to be driven by oxidant stress (63). Although there are a wide range of AGE-related chemical structures known to be present in the vasculature, specific AGEs commonly found in diabetic tissue include carboxymethyllysine (CML)-protein adducts, carboxyetyl-lysine (CEL)-adducts, pentosidine-adducts, pyrallines, imidazoles, metylglyoxal and crosslines (64-67). AGEs may directly impact the integrity of the vessel wall as well as the underlying basement membrane. In particular, increased cross-linking of matrix molecules such as collagen, may disrupt matrix-matrix and matrix-cell interactions (68). In addition, AGEs have also been shown to quench NO, thereby impacting vascular relaxation and function (69). A number of cell surface interaction sites for AGEs have been discovered over the years. These include,

aside from the canonical RAGE (receptor for advanced glycation endproducts), molecules such as macrophage scavenger receptor (MSR), oligosaccharyltransferase 48kD (OST-48), 80K-H (a substrate of PKC), Galectin-3 and CD36 (70). RAGE has been identified as the main signal transduction receptor for AGEs. It mediates the effects of CML-adducts and physiologically relevant concentrations of these adducts have been reported, both *in vivo* and *in vivo*, to activate endothelial cells, VSMCs, and mononuclear phagocytes. In addition, the activity of RAGE, causes expression of a variety of pro-inflammatory molecules as well as the activation of NFkappaB (71). It has been proposed, that mitochondrial-derived ROS, generate the pathogenic phenotype of diabetic vascular complications, partly via the interaction of AGE with RAGE (72).

5.5. Dyslipidemia

It has proven challenging to develop and use animal models of T1DM or T2DM, because, as is the case in humans, it is difficult to separate the effects of hyperglycemia from those of other atherogenic factors, primarily dyslipidemias which frequently accompany insulin resistance and the metabolic syndrome. Bearing this limitation in mind, data from animal models of diabetes can be better appreciated in a critical light. Early studies in rabbits, which had been made diabetic by the administration of Alloxan was seen to cause marked hypertriglyceridemia. Clearly this created a marked confounding problem for atherosclerosis studies (73), because a change in triglycerides in the circulation is known to have a significant impact on atherogenesis. In research done in pigs, the presence of diabetes has been shown to increase atherosclerosis in the presence of hyperlipidemia (74). These and other similar results, demonstrate that glucose and lipids might act synergistically to accelerate the formation of atherosclerotic lesions. Similar to higher mammals, studies in the mouse have also demonstrated confounding problems between the atherosclerotic propensity in diabetes and hyperlipidemia. For example, increased atherosclerosis observed in the diabetic ApoE-deficient mouse was associated with an increase in plasma cholesterol levels (75). Diabetes was also associated with an increased formation of atherosclerotic lesions in severely hyperlipidemic LDLr deficient mice (76). In this mouse model, diabetes has been shown to accelerate the formation of the atherosclerotic lesion, as evidenced by an increase in the accumulation of macrophages and the formation of fatty streaks as well as increased intra-plaque hemorrhage in the brachiocephalic artery (77). In these studies, the effects of diabetes on the initiation of atherosclerotic lesions, appear to be independent of differences in plasma lipid levels and therefore appeared to be most likely due to hyperglycemia or the consequences of hyperglycemia. In addition, insulin therapy in these and other studies appear to normalize the initiation and progression of lesions, which further provide proof that the likely mechanism was hyperglycemia in the pathogenesis of atherosclerosis. It is also fascinating to note that mice are relatively deficient in the aldose reductase enzyme, which is capable of generating toxic products of glucose (73). In fact, LDLr deficient mice with a human

aldose reductase transgene developed more atherosclerosis than controls (78).

Overall, it appears that although hyperglycemia plays a central role in the initiation of the atherosclerotic lesion, it is likely that lipids are required for this effect. In humans, for example, the onset of high blood glucose levels often occurs many years after the onset of lipid abnormalities, such as those associated with the development of the metabolic syndrome (79).

5.6. Insulin resistance

The most important mechanism by which the body maintains glucose homeostasis is the rapidity with which insulin acts to stimulate glucose uptake and metabolism in peripheral tissues. Resistance to the actions of insulin in skeletal muscle is a major pathogenic factor in both T1DM and T2DM. In the latter, insulin resistance is thought to be the fundamental anomaly. Insulin signaling involves the cascade which is initiated by binding of the hormone to its receptor, followed by autophosphorylation of the receptor, activation of receptor tyrosine kinases with consequent phosphorylation of tyrosine on the insulin receptor substrate (IRSs; IRS1, IRS2, IRS3, IRS4, Gab1 and Shc) (80). IRS then bind to the regulatory subunit of PI3K, which then phosphorylates PIP2. This is followed by the activation of 3-phosphoinositide-dependent protein kinases, PDK-1 and -2, and the activation of protein kinase B (PKB)/Akt as well as PKCgamma/zeta. Activated Akt phosphorylates it substrate which then stimulates the translocation of insulin-mediated GLUT4 from intracellular vesicles to the plasma membrane, thereby enabling the fundamental cellular glucose transport mechanism.

Insulin resistance is the inability of insulin to produce its numerous actions, despite the unimpaired secretion of the hormone from the pancreas (81). Over the years, a large body of evidence has accumulated that indicates that insulin resistance contributes to the development of cardiovascular diseases. For example, it has been reproducibly demonstrated that lean type II diabetics and obese normal glucose tolerant patients, are both resistant to insulin and that this resistance primarily affects the glycogen synthetic pathway (82). While the molecular mechanisms that underlie this phenomenon are very complex, in the context of systemic or vascular inflammation, some specific information is relevant to this discussion. Obesity appears to be a very common cause of insulin resistance. A potential mechanism for this relationship is the accumulation of lipid. Obesity is also associated with a systemic chronic inflammatory response which is characterized by abnormal cytokine synthesis and anomalous activation of inflammatory signaling pathways (83). Several reports have linked this abnormal inflammatory response to the development of insulin resistance. The mechanistic explanation behind this observation is thought to be twofold. First, activation of inflammatory signaling intermediates may be directly involved in the serine phosphorylation of IRS-1 within insulin-sensitive cells. Second, the infiltration of inflammatory cells within adipose tissue may be involved in altering lipid metabolism in the adipocyte as well as

altering the synthesis of cytokines by adipose tissue (83). Inflammatory cytokines such as TNFalpha and IL-6 have been linked to insulin resistance. Expression of TNFalpha, for example, is increased in adipose tissue in obese rodents as well as obese humans, and suppressing signaling by this molecule either by knocking it out genetically or by infusing antagonists, can reduce insulin resistance in obese rodents (84). On the other hand, it has also been shown that TNFalpha antibody infusion in humans did not alter insulin sensitivity, and this has lent some uncertainty about the biological relevance of this pathway, at least in human insulin-resistant states (85). It has been proposed that FFA-induced serine phosphorylation of IRS-1, might be mediated by IkappaBK-beta. This hypothesis has been supported by studies that show that inhibition of IkappaK-beta by high doses of salicylates, or by deletion of the IkappaK-beta gene, can attenuate insulin resistance in obese rodents (86). The JNK-1 kinase has also been identified as a potential mechanistic link behind insulin resistance in that it was shown to induce serine phosphorylation of IRS-1 (87). Yet another link has been described that could connect inflammation with insulin resistance. This involves the suppressor of cytokine signaling 3 (SOCS-3), which is thought to participate in negative feedback loops in cytokine signaling. This signaling system is usually amplified by the activation of signal transducers and activators of NFkappaB (88). In *in vitro* studies, SOCS3 was shown to interact directly with the insulin receptor, thereby inhibiting IRS-1 tyrosine phosphorylation and leading to producing insulin-stimulated glycogen synthesis in cultured myotubes (89). Thus, overall, it appears that inflammation and insulin resistance are mechanistically linked in ways that are relevant for diabetic patients, and these have largely been validated in diabetic animal models and appear to synergize with the pathogenesis of cardiovascular disease, particularly atherosclerosis.

5.7. Endothelial dysfunction

In recent years, the concepts of endothelial physiology and pathology have undergone significant changes. It has now been established that ED, induced by elevated LDL, increased generation of free radicals, the effect of infectious microorganisms, shear stress from blood flow, high blood pressure, and the prevalence of toxins in the circulation, combine to induce a compensatory inflammatory response in the vasculature (90,91). ED is characterized by reduced bioavailability of NO, local oxidation of circulating lipoproteins and a predominance of vaso-constrictive hormones and signaling molecules in the milieu of the vessel wall (92). Although a precise definition of vascular inflammation, which encompasses all vascular pathologies that are known to have inflammatory components, is impossible, a general description can be articulated. At sites of inflammation, infection or injury, pro-inflammatory stimuli can make the vascular endothelial surface attractive to circulating leukocytes (93). This response is triggered by closely regulated signaling cascades which involve specific consecutive steps of interactions between the circulating cells and the endothelium. Initial contact between endothelial cells and leukocytes causes a rolling action whereby leukocytes

move over the endothelial surface in a rolling motion mediated through a bond which is dependent on selectins (93). Subsequent to this, the leukocytes are activated by chemokines that are secreted by the endothelial lining, resulting in the activation of integrins on the leukocyte itself and leads to arrest of leukocytes on the endothelial surface. Once firmly attached to the endothelial surface, leukocytes utilize a transmigration processes to pass across the endothelial barrier. One is the *transcellular* route which takes the leukocytes through the endothelial cell body itself. The other is the *paracellular* route involving passage of the cell through endothelial junctions. In addition to leukocytes, other circulating cells such as platelets also arrive at the site of injury/inflammation under the influence of chemotactic signals and contribute to a pro-coagulant and pro-atherogenic state as well as to enhancement of the local immune response, partly facilitated by leukocyte-endothelial interactions. These initial and very early events characterize the vascular inflammatory process that underlies a variety of systemic pathologies and their vascular correlates and counterparts. Of these, atherosclerosis, is a key example (Figure 1).

6. INFLAMMATORY MEDIATORS IMPLICATED IN ATHEROSCLEROSIS AND DIABETES

Historically, the concept that inflammation is related to metabolic conditions such as obesity and insulin resistance, began with the seminal publication by Hotamisligil *et al*, nearly 20 years ago, which demonstrated that adipocytes constitutively express the cytokine TNFalpha, and that the expression of this molecule in obese animals is markedly increased (this was demonstrated in ob/ob, db/db, and fa/fa Zucker mice) (83). Compellingly, these researchers demonstrated that neutralization of TNFalpha led to a decrease in insulin resistance in these animal models (83). Subsequent published data also showed that adipose tissue in humans also expressed TNFalpha in an obese background and these expression levels declined with weight-loss (94). Furthermore, in the 1990s, Crook *et al* and Pickup *et al,* independently proposed that type II diabetes was also an inflammatory condition being characterized by elevated concentrations of acute phase inflammatory reactions in the plasma such as sialic acid, and the proinflammatory cytokine IL-6 (95,96). Several subsequent studies have confirmed that the presence of inflammation is not simply a biomarker for metabolic disorders, it also predicts the development of type II diabetes. For example, Schimdt *et al* pioneered the demonstration that the presence of inflammatory mediators predicted the future occurrence of type II diabetes in adults (97) and their data was published as part of the larger Atherosclerosis Risk in Communities Study (ARIC). Novel data has also recently been published that confirms that concomitant presence of promoter polymorphisms of TNFalpha and IL-6 in obese subjects with impaired glucose tolerance carries two times the risk of conversion to type II diabetes when compared with other genotypes (98-100). In this regard, it is interesting to note that a state of insulin resistance promotes inflammation. This appears to be secondary to the fact that insulin exerts an anti-inflammatory effect at the cellular and molecular level,

both *in vitro* and *in vivo* (94). For example, low-dose infusion of insulin reduces the generation of ROS by mononuclear cells suppresses, NADPH oxidase expression, attenuates intra-nuclear NFkappaB binding, induces IkappaB expression, and suppresses, ICAM-1 and MCP-1 concentrations (94).

It is now widely accepted that diabetes causes atherosclerotic lesions, regardless of other factors (101). For example, it has been shown the diabetic mice, have significantly higher cholesterol even when they are maintained on a cholesterol free diet as compared with nondiabetic mice (101).in another study data has been presented that suggests that hyperglycemia is responsible for the development of atherosclerosis (102). Furthermore, researchers have also shown the lipoprotein lipase synthesized by macrophages in the vascular wall favors the development of atherosclerosis through the promotion of lipid accumulation within the atherosclerotic lesion. Sartippour *et al* have, quite intriguingly, demonstrated that high glucose concentrations stimulated *in vitro* murine and human macrophage production of lipoprotein lipase (103). Thus, several studies have proven useful in elucidating multiple mechanisms by which glucose might provoke atherogenesis. However, because of the inherent limitations of animal models, these mechanisms have to be validated in *in vivo* models of atherosclerosis. Indeed, *in vivo* work has also confirmed the cause-and-effect relationship between glucose and atherosclerosis. For example, following glucose infusion, leukocyte rolling and adhesion to the endothelium was shown to be stimulated in the rat (73). Most compellingly, this effect was seen to be normalized by treatment with insulin (104). In other studies, for example, overexpression of the glucose transporter GLUT1 in arterial smooth muscle cells was shown to attenuate apoptosis following vascular injury (73).

In diabetic and/or obese patients, increased levels of circulating inflammatory markers have been noted. These include CRP, TNFalpha, IL-6 and ICAM (105,106). Increased levels of inflammatory markers in patients also predict cardiovascular risk in diabetes (107).

NFkappaB, is not only a key regulator of inflammatory signaling, but is also critical in the regulation of endothelial activation and has been linked to the pathogenesis of insulin resistance (108). NFkappaB is activated by FFAs, inflammatory cytokines as well as the RAGE (109-111). NFkappaB activation involves the phosphorylation and subsequent degradation of the inhibitory subunit IkappaB by the IkappaB kinase, IKK-beta. This signaling step allows translocation of the regulatory subunits of NFkappaB, p50 and p65, to the nucleus, where they promote expression of inflammatory genes. It has been demonstrated, that in skeletal muscle for example, TNFalpha or overexpression of IKK-beta, can both produce insulin resistance (112). Genetic suppression or pharmacological inhibition of IKK-beta has also been shown to prevent insulin resistance, which further validates this paradigm (108). Many studies in cultured endothelial cells with support from animal studies, have firmly established links between activation of NFkappaB,

development of an inflammatory phenotype, insulin resistance, and endothelial dysfunction (113).

In many human studies as well, the mechanistic link and interaction between diabetes and inflammation has been established. As an example, when obese human subjects were treated with anti-inflammatory compounds, along with the reduction in circulating markers of inflammation, insulin sensitivity was also markedly improved (23). In addition, increased expression of p65 accompanied with decreased expression of IκB, has been observed in endothelial cells isolated from elderly obese individuals (114).

PKCbeta is a member of a family of serine/threonine kinases that act at the plasma membrane and regulate signal transduction in a variety of cell types. PKCbeta is an important member of this family and is the predominant isoform in endothelial cells. In the cells, PKCbeta is activated by DAG when the cell is confronted with conditions of increased glucose and increased fatty acid concentration (115). Activation of this kinase, may explain the links between inflammation, endothelial dysfunction, insulin resistance, and diabetes. PKCbeta inhibits PI3K and Akt which leads to reducing eNOS phosphorylation (116). PKCbeta also activates NFkappaB (117). When PKCbeta is inhibited, NO bioavailability is improved, and activation of the inflammatory cascades in the endothelium is attenuated, in several different experimental models (118,119). Intriguingly, in humans, treatment with a PKCbeta pharmacological antagonist has been shown to prevent the development of endothelial dysfunction after glucose infusion in healthy volunteers (120). Overall, it is becoming widely accepted that the impact of diabetes on the vasculature has a strong inflammatory basis and the "vascular inflammation paradigm" also successfully explains the mechanisms of vascular disease and vascular complications in diabetes.

Based on data from a large number of *in vitro* and *in vivo* studies, a consensus has emerged that the keys cellular constituents in the progression from a normal blood vessel to plaque rupture and all the intermediate phases, are thought to be endothelial cells, monocyte-derived macrophages, and arterial smooth muscle cells (20). In endothelial cells, there is an increased expression of inflammatory factors or adhesion molecules. In these cells, against the backdrop of atherogenic risk factors, there is also increased expression of inhibitors of endothelium-dependent vasodilation as well as the increased incidence of endothelial cell death. In macrophages, sterol and cholesterol transport are disturbed and adhesion molecules and inflammatory cytokine expression is increased. In smooth muscle cells, there is a predilection for proliferation that leads to altered composition of the extracellular matrix under atherogenic pressure.

Studies in isolated cells have demonstrated that hyperglycemia increases adhesion of monocytes to the endothelium and this results in heightened expression of adhesion factors on both the surface of endothelial cells as well as monocyte-macrophages (121). Hyperglycemia has

also been shown to increase the expression of NFkappaB in endothelial cells as well as macrophages along with increased production of O_2^- and ROS, thus amplifying oxidative stress (122). In turn, increased oxidative stress leads to increased production of oxidized LDL in the blood vessel wall, which further exacerbates atherogenicity. In addition, hyperglycemia has also been shown to impair NO production, which is critical for endothelium-dependent vasodilation. Furthermore, proteins modified by AGE have also been documented to interrupt key steps in normal cholesterol transport (123). Overall, it has become quite clear that changes in endothelial cells in an atherosclerotic phenotype correlate directly to hyperglycemia as well as a atherogenic lipoprotein profile. In these animal models, the notion that hyperglycemia is directly injurious to the vessel wall, has been validated many times. For example, endothelial cells express receptors that recognize AGE proteins, and signaling via these receptors appears to activate proinflammatory genes (20). Several groups have reported that modifying signaling via the AGE receptors can lead to reducing the formation of these and products as well as attenuating atherosclerosis in mouse models of diabetes (124,125). Exposure to glucose alone can also affect monocytes and macrophages to activate *in vitro*. For example, monocytes grown in high glucose conditions show evidence of increased expression of the cytokines IL-1beta and IL-6 (32). These inflammatory changes are also associated with induction of PKC, activation of NFkappaB and increased synthesis of ROS such as superoxide, and these further participate in glucose mediated oxidative stress.

While *in vitro* studies have proven useful in elucidating multiple mechanisms by which glucose might provoke atherogenesis, because of the inherent limitations of animal models, these mechanisms have to be validated in *in vivo* models of atherosclerosis. Indeed, *in vivo* work has also confirmed the cause-and-effect relationship between glucose and atherosclerosis. For example, following glucose infusion, leukocyte rolling and adhesion to the endothelium was shown to be stimulated in the rat (73). Most compellingly, this effect was seen to be normalized by treatment with insulin (104). In other studies, for example, overexpression of the glucose transporter GLUT-1 in arterial smooth muscle cells was shown to attenuate apoptosis following vascular injury (73).

6.1. Reactive oxygen species

The generation of O_2^-, also appears to be central in the formation of the atherosclerotic plaques. O_2^- Promotes the formation of reactive intermediates including $ONOO^-$, hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). O_2^- is derived both from vascular sources as well as macrophages. Vascular sources of this highly reactive radical include mitochondrial oxidases, myeloperoxidase, xanthine oxidase, NOS and NAD(P)H oxidase (126). In the inflammatory milieu, cytokines enhance ROS production in the vasculature. As an example, MCS-F, which regulates proliferation, differentiation and chemotaxis of macrophages, has been shown to stimulate the raft-associated NAD(P)H-oxidase (127). Collectively, these molecules accelerate atherogenesis via oxidation of LDL

and enhanced foam cell formation. Oxidized LDL can further lead to production of ROS. It appears that positive feedback mechanisms also exist in the vasculature, which significantly contribute to the genesis of pathological change. An important example of this, is the self-propagating phenomena involving H_2O_2 and NAD(P)H oxidase, which was recently described and proposed to contribute to atherogenesis (126). ROS also play a very significant role in the regulation of NFkappaB. NFkappaB controls more than 160 gene products including those involved in cell proliferation, apoptosis and inflammation. Many reports have implicated ROS-driven induction of NFkappaB by oxidized LDL and H_2O_2 via inhibition of IkappaB (128,129). As described above, NFkappaB promotes the synthesis of pro-inflammatory cytokines, cyclooxygenase-2 (COX-2) and MMPs (130). COX isoforms promote the oxygenation and transformation of arachidonic acid into eicosanoids that possess both pro-PGE_2 (Prostaglandin) and anti-PGI_2 properties, thereby providing a homeostatic balance which is likely to be critical to the vascular endothelium and the vascular milieu. PGE_2 derived from COX-2, for example, stimulates EP-4 receptor-mediated enhancement of plaque formation as well as induction of MMP-9 expression. In later stages, this process contributes to plaque destabilization (131-133). Experimental evidence has shown that a reduction in oxidative stress in the endothelium could be beneficial in animal models of atherosclerosis. For example, anti-oxidants were shown to suppress atherosclerotic lesions in ApoE-deficient as well as ApoE/LDL-receptor-deficient mice (134). It is interesting to note, that several commonly used cardiovascular drugs (such as the Statins) possess antioxidant properties and these have been demonstrated to target redox-sensitive transcription factors, as well as to reduce the expression of VCAM-1, ICAM-1 and selectins in human aortic endothelial cells (135). In recent years, the development of ROS inhibitors that are specific for various cellular sources of ROS, have also shown promise. For example, antioxidants targeted at mitochondria-derived ROS, appeared to confer marked protection against mitochondrial oxidative damage in atherosclerosis (136,137).

6.2. Eicosanoids

Eicosanoids are signaling molecules made by oxidation of twenty-carbon essential fatty acids. They exert complex control over many bodily systems, mainly in inflammation and the immune response. Upon binding of growth factors and cytokines to their receptors, they activate several phospholipases which act on membrane phospholipids to release arachidonic acid (138). Arachidonic acid leads to the formation of a number of precursor molecules that are metabolized to bioactive lipid mediators through the P450 monooxygenase pathway (CYP). In addition to the P450 pathway, the cyclooxygenase (COX) pathway and the lipooxygenase pathway, are also very critical in the eicosanoid signaling system. There appears to be compelling evidence that shows involvement of the COX derived lipid mediators in the pathogenesis of atherosclerosis. In monocytes, the expression of COX-2 is stimulated by IL-1, TNFalpha, lipopolysaccharide, Transforming growth factor beta (TGF-

beta) , EGF (epidermal growth factor), PDGF (platelet derived growth factor) and FGF (fibroblast growth factor) (139). These cytokines and growth factors are known to be present in the initial phases of atherosclerotic lesion formation. Once COX-2 is induced in activated macrophages within the wall of the artery, it can promote atherosclerosis through several mechanisms. These include activation of chemotaxis, induction of vascular permeability, promotion of cytokine signaling, and stimulation of VSMC migration. Furthermore, expression of COX-2 in macrophages has pro-inflammatory effects because in the cells it couples with, prostaglandin synthase and leads to synthesis of PGE2, which is a prostanoid with pro-inflammatory properties (140). Most compellingly, inhibition of COX-2 has been demonstrated to have potent anti-inflammatory effects, which leads to interference with monocyte recruitment in LDLr deficient mice along with reduction in the formation of fatty streaks by the selective COX-2 inhibitor, rofecoxib (141,142). However, other studies regarding the role of COX-2 in the pathogenesis of atherosclerosis have led to less obvious results, and these point to the complexity of its role, which may be due in part to the fact that it can couple with a variety of different downstream mediators leading to opposing actions on the genesis of atherosclerotic plaques (140).

Thromboxanes are also a member of the eicosanoid family. They are synthesized by Thromboxane-A synthase, an enzyme found in platelets, which converts the arachidonic acid derivative prostaglandin H_2 to Thromboxane. The two major thromboxanes are thromboxane A_2 and thromboxane B_2. These molecules act by binding to their specific receptors, which are G-protein coupled. They are potent vasoconstrictors and facilitate platelet aggregation. Thromboxane A_2 is produced by activated platelets, has thrombotic properties, and further stimulates activation of platelets as well as enhancing platelet aggregation on the vascular endothelium. It has been shown that the thromboxane A_2 receptor (TPr), when stimulated induces hypertrophy in vascular smooth muscle cells. In this process, evidence has been reported that the activation of AMPK is stimulated by the receptor, and AMPK in turn limits the synthesis of the receptor in vascular smooth muscle cells (143). As has been found that hypertrophy of smooth muscle cells is a critical element in vascular remodeling that is so often associated with cardiovascular disease and atherosclerosis. The role of TPr has also been defined in the context of insulin signaling in the endothelium. Zou and colleagues have reported recently that the receptor activates a Rho-associated kinase/LKB1/PTEN signaling pathway to suppress insulin signaling in the vascular endothelium (144). Interestingly, a fresh report from this group has also implicated TPr in the activation of NAD(P)H oxidase, culminating in uncoupling of eNOS in bovine aortic endothelial cells with experiments validated in gp91(phox) knockout mice (145). Overall, there appears to be abundant evidence implicating eicosanoids in the pathogenesis of atherosclerotic plaque. However, the role of inhibitors of the eicosanoid synthetic pathways has remained controversial, largely because of lack of demonstrable efficacy in a clinical setting.

The 5-lipoxygenase pathway was initially ascribed a role in atherosclerosis from studies which identified a locus on the mouse chromosome 6, and which conferred almost total prevention of atherogenesis (146). Subsequently, when mice were generated to be deficient in 5-lipoxygenase, their resistance to atherogenesis was reproduced. However, in subsequent investigations in mice lacking this enzyme, this early reproducibility was not observed and instead, this pathway was linked to hyperlipidemia dependent inflammation of the arterial wall and to the pathogenesis of aortic aneurysms (147). On the other hand, the role of 5-lipoxygenase in atherogenesis has been supported by findings that the expression of this enzyme correlates with disease severity (148). As further examples, 5-lipoxygenase, Src Like Adapter Protein (SLAP), and leukotriene A4 (LTA4) hydrolase, are localized in macrophages of human atherosclerotic lesions and the number of these cells reportedly increases in advanced lesions (148). Furthermore, 5-lipoxygenase dependent synthesis of the leukotriene LTB4 signaling through the BLT1 receptor (the high affinity receptor for this leukotriene) has recently been reported as being essential for MMP-2 and MMP-9 as well as the activation of the vascular smooth muscle cells via 4-hydroxynonenal (149).

7. CURRENT AND POTENTIAL FUTURE THERAPEUTIC TARGETS FOR VASCULAR INFLAMMATION IN ATHEROSCLEROSIS

7.1. Insulin

Insulin is a central therapeutic agent used predominantly in the treatment and management of T1DM. In certa*in situ*ations, insulin can also be used to manage hyperglycemia in T2DM. It has been reported that insulin has a potent anti-inflammatory effect. For example, this has been observed in human aortic endothelial cells (HAECs) (150). Overall, at physiological concentrations, insulin causes suppression of NFkappaB, ICAM-1 and MCP-1. It has been hypothesized that these effects could be related to insulin's ability to induce the release of NO and/or to enhance the expression of constitutively expressed NOS (151,152). Furthermore, infusion of insulin and low dosages in obese human subjects, has been demonstrated to suppress NFkappaB, decrease p47[phox] [a subunit of NAD(P)H oxidase] and decrease CRP (153). It is thought that these effects of insulin are rapid and have very potent anti-inflammatory as well as anti-atherogenic consequences. Insulin has also been shown to suppress activator protein-1 (AP-1), which is the transcription factor modulating MMPs, expressed in plaques and which are largely responsible for plaque rupture (154,155). Interestingly, the successful use of insulin in acute myocardial infarction, with or without the use of thrombolytics in diabetics as well as non-diabetics, has been shown to improve clinical outcomes. This most likely reflects the profound anti-inflammatory as well as anti-thrombotic properties of this hormone (156). These and similar findings in the primary literature, not only attribute an additional therapeutic role to insulin, they also underscore the importance of inflammation in cardiovascular disease and atherogenesis.

7.2. Metformin

Metformin is the only member of the biguanide family of drugs with current FDA approval. It is a popular compound used in the treatment of T2DM and decreases blood glucose concentration by mechanisms which are distinct from those of insulin or sulfonylureas. It lowers, rather than increases, the concentration of insulin in the fasting state and acts by enhancing insulin sensitivity, by increasing the preferred uptake of glucose, and by decreasing the output of glucose from the liver (157). In the large United Kingdom prospective diabetes study (UKPDS; undertaken in the United Kingdom between 1977 and 1997), 4075 patients were recruited in 15 centers, all with newly diagnosed T2DM without symptoms of hyperglycemia. In this study, two groups of patients were studied. In one, metformin was used as the primary modality and in the other conventional therapy was instituted. In this landmark study, metformin showed a variety of beneficial effects in T2DM, which included a 32% reduction in risk for any diabetes-related endpoint, 42% for diabetes-related death and 36% for all-cause mortality. These and many other subsequent findings and studies have led to metformin becoming a very popular drug of choice in T2DM. One key mode of action of metformin is the activation of the AMP-activated protein kinase (AMPK). AMPK is a major cellular energy sensor and key regulator of metabolic homeostasis (158). A number of physiologically relevant processes have been shown to activate AMPK and these include conditions that change the AMP to ATP ratio such as hypoxia and glucose deprivation. In addition, AMPK also affects calcium concentrations in the cell and modulates the action of various hormones, cytokines, and adipokines. Once activated, AMPK is responsible for metabolic changes via the phosphorylation of various downstream substrates. Overall, the net effect of AMPK activation is shifting tissues and organs from an energy-consuming state to an energy-producing profile in order to restore energy balance (158). As stated, vascular inflammation is a feature of endothelial dysfunction, and NFkappaB activation is a major player in this process. It is therefore very intriguing that recent reports suggest that AMPK is a master regulator of macrophage differentiation in an anti-inflammatory phenotype (159). Cacicedo *et al* have reported that pharmacological or genetic activation of AMPK blunts palmitate- or TNF-induced NFkappaB activation and consequent expression of adhesion molecules in endothelial cells (160). AICAR has also been shown to reduce NOS-II synthesis by adipocytes, macrophages, myocytes, and glial cells (161,162). Similarly, metformin is reported to inhibit NFkappaB activation in vascular endothelial cells and suppress inflammation via AMPK activation (163). Therefore it is becoming more accepted that AMPK appears to be a natural suppressor of NFkappaB and vascular inflammation in endothelial cells. In specific mechanistic terms, AMPK is also implicated in upregulating mitochondrial uncoupling protein-2 (UCP-2) in endothelial cells which attenuates ROS stress in these cells against a diabetic phenotype (164). The kinase has also been reported to mediate the suppression of oxidative stress by RNS afforded by statin drugs (Simvastatin in this report) in cultured endothelial cells (165) as well protecting

from endoplasmic reticulum stress (ER stress) in an *in vivo* model of atherosclerosis where the alpha2 subunit of AMPK was found to be critical (166). These findings, as well as reports that AMPK in skeletal muscle is activated in response to exercise, have led to an increased interest in developing AMPK activators as potential therapies for T2DM as well as obesity (158). Recent studies have shown that metformin can stimulate AMPK activity and enhances the peroxisome proliferator-activated receptor-gamma co-activator-one alpha (PGC-1alpha) (167). The drug has also been shown to have favorable effects on certain key inflammatory markers such as CRP (168). The impact of metformin on oxidative stress has also been investigated in a large number of studies. It has been shown for example that metformin can quench ROS in insulin resistance and diabetic states. For example, Rosen *et al* have reported that in the GK rat model of T2DM, metformin can reduce mitochondrial derived oxidative stress (169). The drug has also been shown to reduce ROS levels in human leukocytes by directly scavenging free radicals as well as modulating their synthesis in the cell (170). Metformin has been noted to reduce oxidant stress through inhibition of the PKC and NAD(P)H oxidase pathways as well (171). Furthermore, it has been observed to exert anti-proliferative effects on VSMC through the inhibition of the PKC pathway, which can also inhibit the rate of formation of atherosclerotic plaque (172). In a very comprehensive study by Lund and colleagues, levels of TNFalpha, plasminogen activator inhibitor−1 (PAI-1), tissue plasminogen activator (tPA) antigen, von Willebrand factor, ICAM-1 and E-selctin, were all significantly lower following metformin treatment versus treatment with repaglinide (a member of the meglitinide family of hypoglycemic agents that are widely used in diabetes therapy) in patients with T2DM (173). Thus, it is now widely accepted that this popular and effective drug not only appears to attenuate high blood glucose levels via direct mechanisms, but also has profound anti-inflammatory properties which might account for its wider beneficial effects in patients with diabetes (The impact of metformin and AMPK activation is summarized in Figure 3).

7.3. Peroxisome proliferation-activated receptors (ppars) and thazolidinediones

PPARs are ligand-activated transcription factors, which belong to the nuclear hormone receptor superfamily (174). Three isotypes of these factors have been identified in mammals: PPARalpha (also called NR1C1), PPARbeta/delta (NR1C2) and PPARgamma (NR1C3). These receptors have different tissue distributions and functions as well as different ligand specificities. They form heterodimers with the retinoid X receptor (RXR) and activate transcription by binding to a specific DNA element called the peroxisome proliferator response element (PPRE). These elements are found in the regulatory regions of a variety of genes encoding proteins that are involved in critical cell functions involving lipid metabolism and energy homeostasis (175). PPARs are also expressed in macrophages, B-lymphocytes, T-lymphocytes as well as other members of the cellular immune response family. PPARalpha is also found in endothelial cells where it regulates the expression of leukocyte adhesion molecules.

Illustrative example of AMPK as a therapeutic target in vascular inflammation and vascular pathology
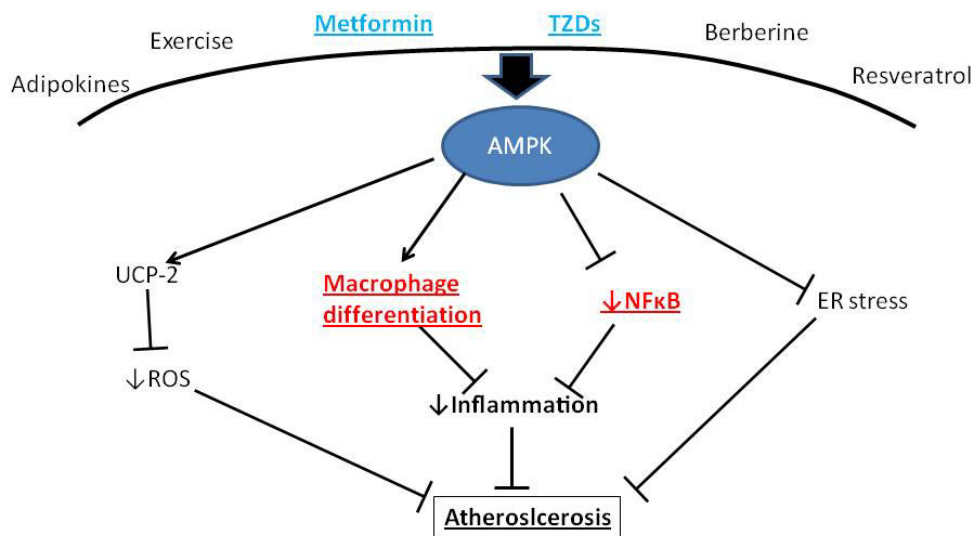
Figure 3. AMPK as a therapeutic target treating vascular complications in diabetes. Activators of AMPK shown in green are commonly used in clinical practice in the management of T2DM. Arrows indicate enhancing/promoting/modulating effects. 'T-ended' bars indicate suppressing/inhibiting effects or pathways. TZD: Thiazolidinediones; Berberine: Plant alkaloid thought to exert beneficial effects in cardiovascular disease; Resveratrol: Polyphenol commonly found in grapes and wine and thought to have beneficial effects in cardiovascular disease.

In mutant mice, that are deficient in PPARalpha, an imbalance occurs between Th1/Th2 (T-lymphocyte subsets) cells pushing them toward a more pro-inflammatory Th1 phenotype (176). Upon ligand activation, PPARalpha down-regulates components of the pro-inflammatory response such as chemokines and cytokines, by decreasing the expression of the Th1 transcription factor, T-bet (T box expressed in T cells) and increasing the expression of GATA-3 (guanosine adenosine thymidine adenosine 3). Both of these are known as positive regulators of Th2 cells (177). PPARalpha agonists also inhibit the activity of NFkappaB, AP-1 (activated protein 1), GATA and NFAT (nuclear factor of activated T cells), thereby mediating the induction of genes that are responsible for inflammation (176). In mice that are deficient in PPAR, a severe inflammatory phenotype has been observed which suggests an anti-inflammatory role for unliganded PPAR or PPAR activated by endogenous ligands (178). In chronic inflammation, PPARalpha, when bound to its ligand, suppresses the production of pro-inflammatory cytokines such as IF-gamma and IL-17. In macrophages, PPARgamma ligands attenuate the expression of the subset of the toll-like receptor (TLR) by a mechanism that has been called "ligand-dependent transrepression" (179). PPAR ligands also suppress the expression of cell adhesion molecules on the surface of endothelial cells as well as reducing the secretion of chemokines by the cells and decreasing the recruitment of leukocytes to the site of vascular inflammation (180).

The thiazolidinediones are a class of drugs, which are synthetic ligands for PPARgamma. They are used as insulin sensitizers in the treatment of diabetes. In recent reports, these drugs have been reported to decrease the cellular response observed as part of the insulin resistance syndrome (181,182). A key member of this drug family, pioglitazone, promotes apoptosis in adipose tissue macrophages as well as improvement of insulin sensitivity (183). These drugs have also been shown to regulate inflammatory cytokines like TNFalpha, and CRP, PAI and Adiponectin (182,184-186). In a report by Hwang and colleagues, rosiglitazone (another member of this family) reduced NADPH oxidase activity and superoxide generation in the vasculature, taken from obese, diabetic, leptin receptor deficient mice (187). Thus, PPAR-agonists have shown remarkable promise as adjunct therapy in diabetes and appear to act, in part, via anti-inflammatory mechanisms.

7.4. Statins

Statins, constitute the best characterized class of drugs with lipid-modulating as well as anti-inflammatory properties in primary and secondary prevention of coronary artery disease. Clinical evidence for direct anti-inflammatory effect of statins comes from the post-hoc CRP studies on several trials which include PROVE-IT, TIMI 22, A TO Z and REVERSAL (188-190). These studies documented that statin-induced reductions of CRP and LDL cholesterol levels were weakly correlated whereas reduction in CRP was markedly correlated with reduction in the progression of atherosclerotic lesions, independent of the drugs' LDL and cholesterol lowering properties. In the important JUPITER trial, it was prospectively confirmed that in primary prevention of patients with elevated CRP,

similar effects of statins were noted along with lowering of LDL cholesterol (188). The ARMYDA trial has also shown that administration of high doses of statins prior to revascularization in patients with acute coronary stenosis, reduces major adverse cardiovascular events (191). While Statins have no direct effect on the insulin profile of diabetes or sensitivity of peripheral tissues to this hormone, their influence on cardiovascular risk is immeasurable. Thus in patients with metabolic syndrome and overt lipid anomalies like hypercholesterolemia and hypertriglyceridemia, they have shown such remarkable efficacy in preventing cardiovascular disease that their use is now considered virtually indispensible.

8. CONCLUSIONS

Overall, *in vitro, in vivo*, as well as human data all point to a role for acute and chronic inflammatory change in the vasculature as a possible mechanistic explanation underlying atherosclerosis. Several clinical findings are also consistent with this hypothesis. For example, an increased incidence of myocardial infarction has been noted in patients diagnosed with chronic inflammatory conditions which include rheumatoid arthritis, systemic lupus erythematosus, psoriasis, and gout (192-195). In addition, the discovery of a link between polymorphisms in the promoter region of MHC class II transactivator and rheumatoid arthritis, multiple sclerosis and myocardial infarction, has lent this idea strong additional support. Expression of MHC class II molecules is also reported to be induced by the pro-inflammatory cytokine IFN-gamma on several different cell types which include VSMCs, endothelial cells and macrophages, all of which a relevant to atherosclerosis and cardiovascular disease. Research in chronic inflammatory diseases, has further refined the identity of specific markers and mediators of inflammation which include the tumor necrosis factor superfamily and the IL-1 superfamily (196). Interestingly, coronary artery disease appears to share features of both autoimmune and inflammatory diseases. For example, in patients with coronary artery disease, who present with acute myocardial infarction, several compounds have been discovered to be elevated in the circulation and these include members of the tumor necrosis factor superfamily such as TNFalpha, CD40L, LIGHT, RANKL, OPG and TRAIL as well as members of the IL-1 superfamily which include IL-1beta, IL-18 and IL-33 (197-205). The levels of these compounds correlated with the subsequent risk of cardiovascular death or congestive heart failure.

The fact that diabetes is clearly associated with an increased incidence of atherosclerosis and that atherosclerosis is fundamentally a consequence of vascular inflammation, is relatively undisputed. However, because of any demonstrable lack of morphological or structural difference between the atherosclerotic plaques found in the non-diabetic individuals and those with diabetes, it is often difficult to comprehend why diabetes predisposes individuals to atherosclerotic vascular disease. It is fundamentally in this regard that the inflammatory hypothesis of atherosclerosis provides a compelling link

between this pathology and diabetes. Specifically, it is because increased of vascular inflammation is generally observed in diabetic mouse models of macrovascular disease, and there is evidence linking hyperglycemia to increased vascular inflammation in the absence of hyperlipidemia (77), that the inflammatory hypothesis has been entertained. As described, systemic expression of inflammatory markers such as C-reactive protein and several proinflammatory cytokines appear to be a common feature in humans with type II diabetes (206). Inflammation is also a compelling candidate as a mediator of vascular complications in diabetes because it links the concomitant occurrence of macro- and micro-vascular disease frequently observed in diabetic patients, a phenomenon absent in those free of diabetic pathology (207). Therefore, overall, a number of different signaling systems have been implicated in endothelial dysregulation by hyperglycemia resulting in atherosclerotic change in the vasculature. These include increased flux of glucose through the polyol pathway leading to increased consumption of NAD(P)H (a critical co-factor for several antioxidant enzymes), activation of RAGE by advanced glycation end products (AGEs) leading to expression of proinflammatory cytokines, stimulation of PKC by high intracellular glucose resulting in increased transcription of several proinflammatory and vasoactive genes, and increased flux through the Hexosamine pathway leading to formation of N-acetyl glucoseamine that influences phosphorylation of transcription factor regulators (for example, the PAI-1 and TGF-beta genes). Furthermore, atherosclerosis is primarily characterized by an oxidative attack on lipoprotein lipids in the vascular extracellular matrix. Oxidized lipoproteins in the subendothelial space induce a number of proinflammatory genes within the vessel wall, and these collectively help to recruit, retain, and activate inflammatory cells in the vessel wall (208-210) (these events are summarized in figure 2). Thus, the role of inflammation as a candidate integrating mechanism in diabetic vascular pathology, particularly atherosclerosis, is both elegant and compelling.

Despite the bulk of such evidence, the results of clinical trials aimed at inhibiting inflammation in cardiovascular disease have not been extremely promising. For example, the data has been inconclusive in clinical trials aimed at attenuating inflammation during reperfusion injury in a non-selective manner using steroids or by targeting neutrophil recruitment (211,212). This suggests that the focus thus far has been on time points that may not be relevant to the formation of atherosclerotic lesions. It has been accepted that patients with coronary artery disease are at high risk for future coronary events, despite currently available treatments, as well as strong evidence of inflammation in the vasculature. However, this cohort does represent the most promising study population for novel anti-inflammatory therapies. For example, a trial termed Cardiovascular Inflammation Reduction Trial (CIRT) has recently been designed. This trial will aim to compare the effects of the drug methotrexate at very low dosages against placebo in addition to standard treatments, in secondary prevention in patients with coronary artery disease. In this and similar trials, cardiovascular death should be the primary endpoint and data derived and

Inflammatory model linking diabetes and atheroslcerotic cardiovascular disease
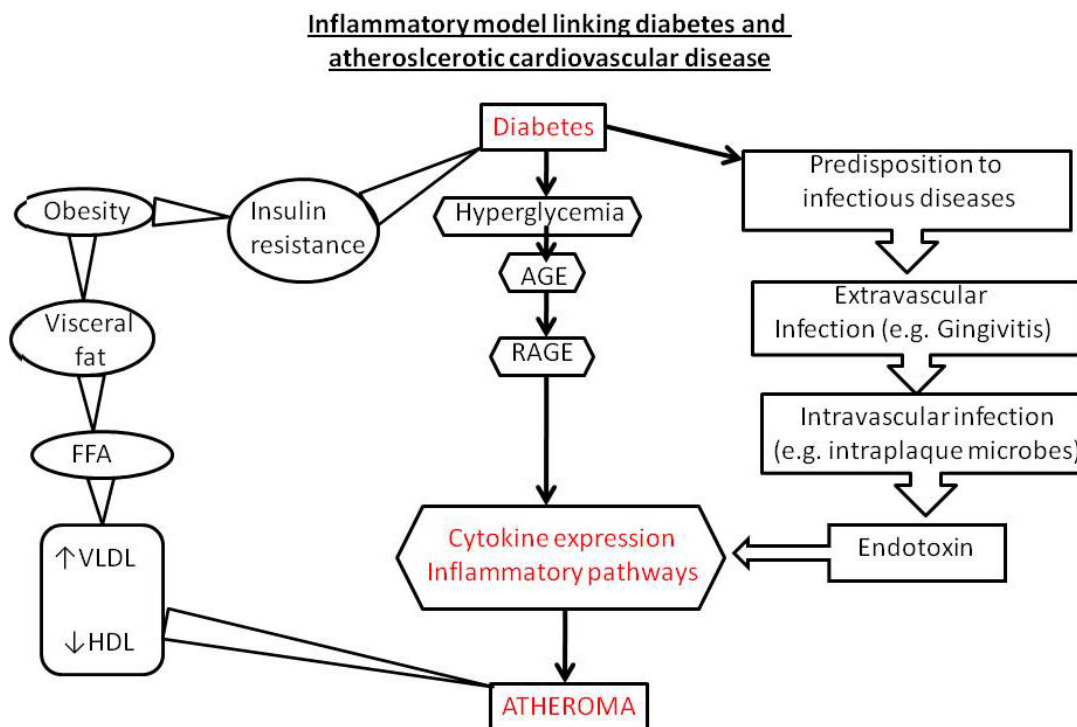
Figure 4. Hypothesized model of inflammation as the causal link between diabetes and atherosclerotic cardiovascular disease.

analyzed from these studies will likely yield important clinical information in addition to the discovery of new biomarkers that are modifiable by anti-inflammatory therapies as well as vascular imaging for adequate therapeutic monitoring (213). Any candidates for successful and novel therapies that target the inflammatory process in atherosclerosis will need to be designed for direct interference with endothelium/smooth muscle/atherosclerotic plaque inflammatory signaling without interfering with the cardiovascular risk profile (such as the lipid profile) of patients with cardiovascular disease. Another compelling strategy, in the light of similarities between cardiovascular disease from atherosclerosis and many autoimmune and inflammatory diseases, would be to design clinical trials using novel immunomodulatory compounds and registering cardiovascular endpoints to include traditional risk profiles for postpartum analysis (213). In an excellent review by Klingenberg and Hansson, the authors have suggested that the immunomodulatory agent Fingolimod used in the treatment of multiple sclerosis, could be such a candidate. This is especially compelling since some experimental data is already available on Fingolimod's role in atherosclerosis.

Atherosclerotic pathology underlies several serious human diseases like myocardial infarction and stroke. Despite the success of statin drugs, prevention of atherosclerosis remains a major challenge in human medicine. Emerging new evidence clearly indicates the inflammatory process and its various anomalies, might be linked to the changes in the endothelium and vascular smooth muscle and these changes ultimately lead to the formation of atherosclerotic plaques and acute vascular occlusion causing potentially deadly outcomes. Inflammation also appears to play a decisive role in the progression of atherosclerosis that leads to plaque destabilization which converts a chronic process into an acute disorder by virtue of thromboembolic consequences. It appears that during atherosclerosis, immune cells such as macrophages and T-cells, infiltrate the wall of the vessel triggered by an initial phase of endothelial dysfunction, and locally interact in a manner that is synergistic and insidious. Macrophages transform into foam cells after uptake of oxidized LDL and these foam cells then secrete matrix metalloproteinase which predisposes the plaque to subsequent rupture. Our proposed paradigm of inflammation being the potential causal link between diabetes and cardiovascular disease is summarized in Figure 4. It appears that immune modulation techniques, pharmacological antagonism and blockade of key molecules of receptors as well as the management of a variety of risk factors, holds the potential for effectively controlling, managing, and possibly curing the human population of atherosclerosis and its attendant complications. To achieve these lofty goals, we foresee several years if not decades of intense research, some of which would be directed against teasing out further details of the complex inflammatory mechanisms that underlie vascular dysfunction and atherosclerosis in the world's diabetic populations. This work and its inevitable challenges is clearly a worthy undertaking, not least of all because it can potentially save millions of lives given that both diabetes and its vascular sequelae, are on an alarming upswing worldwide.

9. REFERENCES

1. A. R. Brasier, A. Recinos, 3rd and M. S. Eledrisi: Vascular inflammation and the renin-angiotensin system. *Arterioscler Thromb Vasc Biol*, 22(8), 1257-66 (2002)

2. R. Ross: Atherosclerosis--an inflammatory disease. *N Engl J Med*, 340(2), 115-26 (1999)

3. D. F. van Wijk and S. M. Boekholdt: Improving risk stratification for cardiovascular disease. *Expert Rev Cardiovasc Ther*, 8(8), 1091-3 (2010)

4. P. Libby, P. M. Ridker and G. K. Hansson: Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol*, 54(23), 2129-38 (2009)

5. T. Munzel and T. Gori: Lipoprotein-associated phospholipase A(2), a marker of vascular inflammation and systemic vulnerability. *Eur Heart J*, 30(23), 2829-31 (2009)

6. D. De Keyzer, S. A. Karabina, W. Wei, B. Geeraert, D. Stengel, J. Marsillach, J. Camps, P. Holvoet and E. Ninio: Increased PAFAH and oxidized lipids are associated with inflammation and atherosclerosis in hypercholesterolemic pigs. *Arterioscler Thromb Vasc Biol*, 29(12), 2041-6 (2009)

7. P. M. Ridker, E. Danielson, F. A. Fonseca, J. Genest, A. M. Gotto, Jr., J. J. Kastelein, W. Koenig, P. Libby, A. J. Lorenzatti, J. G. MacFadyen, B. G. Nordestgaard, J. Shepherd, J. T. Willerson and R. J. Glynn: Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*, 359(21), 2195-207 (2008)

8. G. Biasillo, M. Leo, R. Della Bona and L. M. Biasucci: Inflammatory biomarkers and coronary heart disease: from bench to bedside and back. *Intern Emerg Med*, 5(3), 225-33 (2010)

9. C. W. Hamm, C. Heeschen, B. Goldmann, A. Vahanian, J. Adgey, C. M. Miguel, W. Rutsch, J. Berger, J. Kootstra and M. L. Simoons: Benefit of abciximab in patients with refractory unstable angina in relation to serum troponin T levels. c7E3 Fab Antiplatelet Therapy in Unstable Refractory Angina (CAPTURE) Study Investigators. *N Engl J Med*, 340(21), 1623-9 (1999)

10. J. R. Lee, J. K. Kim, S. J. Lee and K. P. Kim: Role of protein tyrosine nitration in neurodegenerative diseases and atherosclerosis. *Arch Pharm Res*, 32(8), 1109-18 (2009)

11. L. Zheng, B. Nukuna, M. L. Brennan, M. Sun, M. Goormastic, M. Settle, D. Schmitt, X. Fu, L. Thomson, P. L. Fox, H. Ischiropoulos, J. D. Smith, M. Kinter and S. L. Hazen: Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest*, 114(4), 529-41 (2004)

12. H. C. Stary, A. B. Chandler, S. Glagov, J. R. Guyton, W. Insull, Jr., M. E. Rosenfeld, S. A. Schaffer, C. J. Schwartz, W. D. Wagner and R. W. Wissler: A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*, 89(5), 2462-78 (1994)

13. A. J. Merched, K. Ko, K. H. Gotlinger, C. N. Serhan and L. Chan: Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators. *FASEB J*, 22(10), 3595-606 (2008)

14. Y. Zhang, W. J. Cliff, G. I. Schoefl and G. Higgins: Plasma protein insudation as an index of early coronary atherogenesis. *Am J Pathol*, 143(2), 496-506 (1993)

15. H. Loppnow, K. Werdan and M. Buerke: Vascular cells contribute to atherosclerosis by cytokine- and innate-immunity-related inflammatory mechanisms. *Innate Immun*, 14(2), 63-87 (2008)

16. G. K. Hansson and P. Libby: The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol*, 6(7), 508-19 (2006)

17. P. Libby: Inflammation and cardiovascular disease mechanisms. *Am J Clin Nutr*, 83(2), 456S-460S (2006)

18. R. De Caterina: Endothelial dysfunctions: common denominators in vascular disease. *Curr Opin Clin Nutr Metab Care*, 3(6), 453-67 (2000)

19. C. D. Stehouwer, J. Lambert, A. J. Donker and V. W. van Hinsbergh: Endothelial dysfunction and pathogenesis of diabetic angiopathy. *Cardiovasc Res*, 34(1), 55-68 (1997)

20. T. Mazzone: Intensive glucose lowering and cardiovascular disease prevention in diabetes: reconciling the recent clinical trial data. *Circulation*, 122(21), 2201-11 (2010)

21. M. Y. Donath and S. E. Shoelson: Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol* (2011)

22. C. M. Larsen, M. Faulenbach, A. Vaag, A. Volund, J. Ehses, B. Seifert, T. Mandrup-Poulsen and M. Donath: [Interleukin-1 receptor antagonist-treatment of patients with type 2 diabetes]. *Ugeskr Laeger*, 169(45), 3868-71 (2007)

23. A. Fleischman, S. E. Shoelson, R. Bernier and A. B. Goldfine: Salsalate improves glycemia and inflammatory parameters in obese young adults. *Diabetes Care*, 31(2), 289-94 (2008)

24. H. Dominguez, H. Storgaard, C. Rask-Madsen, T. Steffen Hermann, N. Ihlemann, D. Baunbjerg Nielsen, C. Spohr, L. Kober, A. Vaag and C. Torp-Pedersen: Metabolic and vascular effects of tumor necrosis factor-alpha blockade with etanercept in obese patients with type 2 diabetes. *J Vasc Res*, 42(6), 517-25 (2005)

25. J. Lo, L. E. Bernstein, B. Canavan, M. Torriani, M. B. Jackson, R. S. Ahima and S. K. Grinspoon: Effects of TNF-alpha neutralization on adipocytokines and skeletal muscle

adiposity in the metabolic syndrome. *Am J Physiol Endocrinol Metab*, 293(1), E102-9 (2007)

26. D. Brealey and M. Singer: Hyperglycemia in critical illness: a review. *J Diabetes Sci Technol*, 3(6), 1250-60 (2009)

27. S. S. Korshunov, V. P. Skulachev and A. A. Starkov: High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett*, 416(1), 15-8 (1997)

28. S. Dhindsa, D. Tripathy, P. Mohanty, H. Ghanim, T. Syed, A. Aljada and P. Dandona: Differential effects of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor-kappaB in mononuclear cells. *Metabolism*, 53(3), 330-4 (2004)

29. A. Aljada, J. Friedman, H. Ghanim, P. Mohanty, D. Hofmeyer, A. Chaudhuri and P. Dandona: Glucose ingestion induces an increase in intranuclear nuclear factor kappaB, a fall in cellular inhibitor kappaB, and an increase in tumor necrosis factor alpha messenger RNA by mononuclear cells in healthy human subjects. *Metabolism*, 55(9), 1177-85 (2006)

30. L. Monnier, E. Mas, C. Ginet, F. Michel, L. Villon, J. P. Cristol and C. Colette: Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA*, 295(14), 1681-7 (2006)

31. A. Jacob, M. L. Steinberg, J. Yang, W. Dong, Y. Ji and P. Wang: Sepsis-induced inflammation is exacerbated in an animal model of type 2 diabetes. *Int J Clin Exp Med*, 1(1), 22-31 (2008)

32. M. R. Dasu, S. Devaraj and I. Jialal: High glucose induces IL-1beta expression in human monocytes: mechanistic insights. *Am J Physiol Endocrinol Metab*, 293(1), E337-46 (2007)

33. G. Boden and X. Chen: Effects of fat on glucose uptake and utilization in patients with non-insulin-dependent diabetes. *J Clin Invest*, 96(3), 1261-8 (1995)

34. G. Boden, B. Lebed, M. Schatz, C. Homko and S. Lemieux: Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. *Diabetes*, 50(7), 1612-7 (2001)

35. S. I. Itani, N. B. Ruderman, F. Schmieder and G. Boden: Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes*, 51(7), 2005-11 (2002)

36. G. Boden, P. She, M. Mozzoli, P. Cheung, K. Gumireddy, P. Reddy, X. Xiang, Z. Luo and N. Ruderman: Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver. *Diabetes*, 54(12), 3458-65 (2005)

37. C. Yu, Y. Chen, G. W. Cline, D. Zhang, H. Zong, Y. Wang, R. Bergeron, J. K. Kim, S. W. Cushman, G. J. Cooney, B. Atcheson, M. F. White, E. W. Kraegen and G. I. Shulman: Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem*, 277(52), 50230-6 (2002)

38. L. Chang, S. H. Chiang and A. R. Saltiel: Insulin signaling and the regulation of glucose transport. *Mol Med*, 10(7-12), 65-71 (2004)

39. T. Inoguchi, P. Li, F. Umeda, H. Y. Yu, M. Kakimoto, M. Imamura, T. Aoki, T. Etoh, T. Hashimoto, M. Naruse, H. Sano, H. Utsumi and H. Nawata: High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes*, 49(11), 1939-45 (2000)

40. O. A. MacDougald and C. F. Burant: The rapidly expanding family of adipokines. *Cell Metab*, 6(3), 159-61 (2007)

41. H. Zhang, J. Cui and C. Zhang: Emerging role of adipokines as mediators in atherosclerosis. *World J Cardiol*, 2(11), 370-6 (2010)

42. D. E. Moller and K. D. Kaufman: Metabolic syndrome: a clinical and molecular perspective. *Annu Rev Med*, 56, 45-62 (2005)

43. J. E. Manson, G. A. Colditz, M. J. Stampfer, W. C. Willett, B. Rosner, R. R. Monson, F. E. Speizer and C. H. Hennekens: A prospective study of obesity and risk of coronary heart disease in women. *N Engl J Med*, 322(13), 882-9 (1990)

44. G. S. Hotamisligil: Inflammation and metabolic disorders. *Nature*, 444(7121), 860-7 (2006)

45. V. Z. Rocha, E. J. Folco, G. Sukhova, K. Shimizu, I. Gotsman, A. H. Vernon and P. Libby: Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. *Circ Res*, 103(5), 467-76 (2008)

46. H. Wu, S. Ghosh, X. D. Perrard, L. Feng, G. E. Garcia, J. L. Perrard, J. F. Sweeney, L. E. Peterson, L. Chan, C. W. Smith and C. M. Ballantyne: T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. *Circulation*, 115(8), 1029-38 (2007)

47. C. Lohmann, N. Schafer, T. von Lukowicz, M. A. Sokrates Stein, J. Boren, S. Rutti, W. Wahli, M. Y. Donath, T. F. Luscher and C. M. Matter: Atherosclerotic mice exhibit systemic inflammation in periadventitial and visceral adipose tissue, liver, and pancreatic islets. *Atherosclerosis*, 207(2), 360-7 (2009)

48. N. Ouchi, S. Kihara, Y. Arita, K. Maeda, H. Kuriyama, Y. Okamoto, K. Hotta, M. Nishida, M. Takahashi, T. Nakamura, S. Yamashita, T. Funahashi and Y. Matsuzawa: Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation*, 100(25), 2473-6 (1999)

49. Y. Arita, S. Kihara, N. Ouchi, K. Maeda, H. Kuriyama, Y. Okamoto, M. Kumada, K. Hotta, M. Nishida, M. Takahashi, T. Nakamura, I. Shimomura, M. Muraguchi, Y. Ohmoto, T. Funahashi and Y. Matsuzawa: Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation*, 105(24), 2893-8 (2002)

50. N. Ouchi, S. Kihara, Y. Arita, M. Nishida, A. Matsuyama, Y. Okamoto, M. Ishigami, H. Kuriyama, K. Kishida, H. Nishizawa, K. Hotta, M. Muraguchi, Y. Ohmoto, S. Yamashita, T. Funahashi and Y. Matsuzawa: Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation*, 103(8), 1057-63 (2001)

51. L. Tian, N. Luo, R. L. Klein, B. H. Chung, W. T. Garvey and Y. Fu: Adiponectin reduces lipid accumulation in macrophage foam cells. *Atherosclerosis*, 202(1), 152-61 (2009)

52. K. Tsubakio-Yamamoto, F. Matsuura, M. Koseki, H. Oku, J. C. Sandoval, M. Inagaki, K. Nakatani, H. Nakaoka, R. Kawase, M. Yuasa-Kawase, D. Masuda, T. Ohama, N. Maeda, Y. Nakagawa-Toyama, M. Ishigami, M. Nishida, S. Kihara, I. Shimomura and S. Yamashita: Adiponectin prevents atherosclerosis by increasing cholesterol efflux from macrophages. *Biochem Biophys Res Commun*, 375(3), 390-4 (2008)

53. Y. Okamoto, E. J. Folco, M. Minami, A. K. Wara, M. W. Feinberg, G. K. Sukhova, R. A. Colvin, S. Kihara, T. Funahashi, A. D. Luster and P. Libby: Adiponectin inhibits the production of CXC receptor 3 chemokine ligands in macrophages and reduces T-lymphocyte recruitment in atherogenesis. *Circ Res*, 102(2), 218-25 (2008)

54. X. J. Cai, L. Chen, L. Li, M. Feng, X. Li, K. Zhang, Y. Y. Rong, X. B. Hu, M. X. Zhang, Y. Zhang and M. Zhang: Adiponectin inhibits lipopolysaccharide-induced adventitial fibroblast migration and transition to myofibroblasts via AdipoR1-AMPK-iNOS pathway. *Mol Endocrinol*, 24(1), 218-28 (2010)

55. N. Luo, J. Liu, B. H. Chung, Q. Yang, R. L. Klein, W. T. Garvey and Y. Fu: Macrophage adiponectin expression improves insulin sensitivity and protects against inflammation and atherosclerosis. *Diabetes*, 59(4), 791-9 (2010)

56. Y. Okamoto, S. Kihara, N. Ouchi, M. Nishida, Y. Arita, M. Kumada, K. Ohashi, N. Sakai, I. Shimomura, H.

Kobayashi, N. Terasaka, T. Inaba, T. Funahashi and Y. Matsuzawa: Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation*, 106(22), 2767-70 (2002)

57. M. Zeadin, M. Butcher, G. Werstuck, M. Khan, C. K. Yee and S. G. Shaughnessy: Effect of leptin on vascular calcification in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*, 29(12), 2069-75 (2009)

58. P. F. Bodary, S. Gu, Y. Shen, A. H. Hasty, J. M. Buckler and D. T. Eitzman: Recombinant leptin promotes atherosclerosis and thrombosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*, 25(8), e119-22 (2005)

59. S. Taleb, O. Herbin, H. Ait-Oufella, W. Verreth, P. Gourdy, V. Barateau, R. Merval, B. Esposito, K. Clement, P. Holvoet, A. Tedgui and Z. Mallat: Defective leptin/leptin receptor signaling improves regulatory T cell immune response and protects mice from atherosclerosis. *Arterioscler Thromb Vasc Biol*, 27(12), 2691-8 (2007)

60. R. Z. Yang, Q. Huang, A. Xu, J. C. McLenithan, J. A. Eisen, A. R. Shuldiner, S. Alkan and D. W. Gong: Comparative studies of resistin expression and phylogenomics in human and mouse. *Biochem Biophys Res Commun*, 310(3), 927-35 (2003)

61. A. Fukuhara, M. Matsuda, M. Nishizawa, K. Segawa, M. Tanaka, K. Kishimoto, Y. Matsuki, M. Murakami, T. Ichisaka, H. Murakami, E. Watanabe, T. Takagi, M. Akiyoshi, T. Ohtsubo, S. Kihara, S. Yamashita, M. Makishima, T. Funahashi, S. Yamanaka, R. Hiramatsu, Y. Matsuzawa and I. Shimomura: Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science*, 307(5708), 426-30 (2005)

62. A. R. Moschen, A. Kaser, B. Enrich, B. Mosheimer, M. Theurl, H. Niederegger and H. Tilg: Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. *J Immunol*, 178(3), 1748-58 (2007)

63. M. Brownlee: Advanced protein glycosylation in diabetes and aging. *Annu Rev Med*, 46, 223-34 (1995)

64. E. D. Schleicher, E. Wagner and A. G. Nerlich: Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. *J Clin Invest*, 99(3), 457-68 (1997)

65. M. U. Ahmed, E. Brinkmann Frye, T. P. Degenhardt, S. R. Thorpe and J. W. Baynes: N-epsilon-(carboxyethyl)lysine, a product of the chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. *Biochem J*, 324 (Pt 2), 565-70 (1997)

66. S. Miyata and V. Monnier: Immunohistochemical detection of advanced glycosylation end products in diabetic tissues using monoclonal antibody to pyrraline. *J Clin Invest*, 89(4), 1102-12 (1992)

67. K. Ienaga, K. Nakamura, T. Hochi, Y. Nakazawa, Y. Fukunaga, H. Kakita and K. Nakano: Crosslines, fluorophores in the AGE-related cross-linked proteins. *Contrib Nephrol*, 112, 42-51 (1995)

68. S. Tanaka, G. Avigad, B. Brodsky and E. F. Eikenberry: Glycation induces expansion of the molecular packing of collagen. *J Mol Biol*, 203(2), 495-505 (1988)

69. R. Bucala, K. J. Tracey and A. Cerami: Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest*, 87(2), 432-8 (1991)

70. J. el Khoury, C. A. Thomas, J. D. Loike, S. E. Hickman, L. Cao and S. C. Silverstein: Macrophages adhere to glucose-modified basement membrane collagen IV via their scavenger receptors. *J Biol Chem*, 269(14), 10197-200 (1994)

71. T. Kislinger, C. Fu, B. Huber, W. Qu, A. Taguchi, S. Du Yan, M. Hofmann, S. F. Yan, M. Pischetsrieder, D. Stern and A. M. Schmidt: N(epsilon)-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. *J Biol Chem*, 274(44), 31740-9 (1999)

72. S. F. Yan, R. Ramasamy, Y. Naka and A. M. Schmidt: Glycation, inflammation, and RAGE: a scaffold for the macrovascular complications of diabetes and beyond. *Circ Res*, 93(12), 1159-69 (2003)

73. A. Chait and K. E. Bornfeldt: Diabetes and atherosclerosis: is there a role for hyperglycemia? *J Lipid Res*, 50 Suppl, S335-9 (2009)

74. R. G. Gerrity, R. Natarajan, J. L. Nadler and T. Kimsey: Diabetes-induced accelerated atherosclerosis in swine. *Diabetes*, 50(7), 1654-65 (2001)

75. L. Park, K. G. Raman, K. J. Lee, Y. Lu, L. J. Ferran, Jr., W. S. Chow, D. Stern and A. M. Schmidt: Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nat Med*, 4(9), 1025-31 (1998)

76. P. Reaven, S. Merat, F. Casanada, M. Sutphin and W. Palinski: Effect of streptozotocin-induced hyperglycemia on lipid profiles, formation of advanced glycation endproducts in lesions, and extent of atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol*, 17(10), 2250-6 (1997)

77. C. B. Renard, F. Kramer, F. Johansson, N. Lamharzi, L. R. Tannock, M. G. von Herrath, A. Chait and K. E. Bornfeldt: Diabetes and diabetes-associated lipid abnormalities have distinct effects on initiation and progression of atherosclerotic lesions. *J Clin Invest*, 114(5), 659-68 (2004)

78. R. K. Vikramadithyan, Y. Hu, H. L. Noh, C. P. Liang, K. Hallam, A. R. Tall, R. Ramasamy and I. J. Goldberg: Human aldose reductase expression accelerates diabetic atherosclerosis in transgenic mice. *J Clin Invest*, 115(9), 2434-43 (2005)

79. S. M. Haffner, M. P. Stern, H. P. Hazuda, B. D. Mitchell and J. K. Patterson: Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA*, 263(21), 2893-8 (1990)

80. K. Choi and Y. B. Kim: Molecular mechanism of insulin resistance in obesity and type 2 diabetes. *Korean J Intern Med*, 25(2), 119-29 (2010)

81. L. Duvnjak and M. Duvnjak: The metabolic syndrome - an ongoing story. *J Physiol Pharmacol*, 60 Suppl 7, 19-24 (2009)

82. R. A. DeFronzo: Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. *Diabetologia*, 53(7), 1270-87 (2010)

83. G. S. Hotamisligil, N. S. Shargill and B. M. Spiegelman: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*, 259(5091), 87-91 (1993)

84. K. T. Uysal, S. M. Wiesbrock, M. W. Marino and G. S. Hotamisligil: Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature*, 389(6651), 610-4 (1997)

85. F. Ofei, S. Hurel, J. Newkirk, M. Sopwith and R. Taylor: Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes*, 45(7), 881-5 (1996)

86. J. K. Kim, Y. J. Kim, J. J. Fillmore, Y. Chen, I. Moore, J. Lee, M. Yuan, Z. W. Li, M. Karin, P. Perret, S. E. Shoelson and G. I. Shulman: Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest*, 108(3), 437-46 (2001)

87. J. Hirosumi, G. Tuncman, L. Chang, C. Z. Gorgun, K. T. Uysal, K. Maeda, M. Karin and G. S. Hotamisligil: A central role for JNK in obesity and insulin resistance. *Nature*, 420(6913), 333-6 (2002)

88. K. Ueki, T. Kondo and C. R. Kahn: Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol*, 24(12), 5434-46 (2004)

89. D. B. Savage, K. F. Petersen and G. I. Shulman: Mechanisms of insulin resistance in humans and possible links with inflammation. *Hypertension*, 45(5), 828-33 (2005)

90. R. Ross: Atherosclerosis is an inflammatory disease. *Am Heart J*, 138(5 Pt 2), S419-20 (1999)

91. G. Stoll and M. Bendszus: Inflammation and atherosclerosis: novel insights into plaque formation and destabilization. *Stroke*, 37(7), 1923-32 (2006)

92. J. Davignon and P. Ganz: Role of endothelial dysfunction in atherosclerosis. *Circulation*, 109(23 Suppl 1), III27-32 (2004)

93. H. F. Langer and T. Chavakis: Leukocyte-endothelial interactions in inflammation. *J Cell Mol Med*, 13(7), 1211-20 (2009)

94. P. Dandona, A. Aljada and A. Bandyopadhyay: Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol*, 25(1), 4-7 (2004)

95. M. A. Crook, P. Tutt and J. C. Pickup: Elevated serum sialic acid concentration in NIDDM and its relationship to blood pressure and retinopathy. *Diabetes Care*, 16(1), 57-60 (1993)

96. J. C. Pickup, M. B. Mattock, G. D. Chusney and D. Burt: NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia*, 40(11), 1286-92 (1997)

97. M. I. Schmidt, B. B. Duncan, A. R. Sharrett, G. Lindberg, P. J. Savage, S. Offenbacher, M. I. Azambuja, R. P. Tracy and G. Heiss: Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet*, 353(9165), 1649-52 (1999)

98. A. Kubaszek, J. Pihlajamaki, V. Komarovski, V. Lindi, J. Lindstrom, J. Eriksson, T. T. Valle, H. Hamalainen, P. Ilanne-Parikka, S. Keinanen-Kiukaanniemi, J. Tuomilehto, M. Uusitupa and M. Laakso: Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. *Diabetes*, 52(7), 1872-6 (2003)

99. A. Kubaszek, J. Pihlajamaki, P. Karhapaa, I. Vauhkonen and M. Laakso: The K121Q polymorphism of the PC-1 gene is associated with insulin resistance but not with dyslipidemia. *Diabetes Care*, 26(2), 464-7 (2003)

100. A. Kubaszek, J. Pihlajamaki, K. Punnonen, P. Karhapaa, I. Vauhkonen and M. Laakso: The C-174G promoter polymorphism of the IL-6 gene affects energy expenditure and insulin sensitivity. *Diabetes*, 52(2), 558-61 (2003)

101. R. K. Mathur: Role of diabetes, hypertension, and cigarette smoking on atherosclerosis. *J Cardiovasc Dis Res*, 1(2), 64-8 (2010)

102. V. V. Kunjathoor, D. L. Wilson and R. C. LeBoeuf: Increased atherosclerosis in streptozotocin-induced diabetic mice. *J Clin Invest*, 97(7), 1767-73 (1996)

103. M. R. Sartippour, A. Lambert, M. Laframboise, P. St-Jacques and G. Renier: Stimulatory effect of glucose on macrophage lipoprotein lipase expression and production. *Diabetes*, 47(3), 431-8 (1998)

104. G. Booth, T. J. Stalker, A. M. Lefer and R. Scalia: Elevated ambient glucose induces acute inflammatory events in the microvasculature: effects of insulin. *Am J Physiol Endocrinol Metab*, 280(6), E848-56 (2001)

105. J. F. Keaney, Jr., J. M. Massaro, M. G. Larson, R. S. Vasan, P. W. Wilson, I. Lipinska, D. Corey, P. Sutherland, J. A. Vita and E. J. Benjamin: Heritability and correlates of intercellular adhesion molecule-1 in the Framingham Offspring Study. *J Am Coll Cardiol*, 44(1), 168-73 (2004)

106. A. Festa, R. D'Agostino, Jr., G. Howard, L. Mykkanen, R. P. Tracy and S. M. Haffner: Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation*, 102(1), 42-7 (2000)

107. M. B. Schulze, E. B. Rimm, T. Li, N. Rifai, M. J. Stampfer and F. B. Hu: C-reactive protein and incident cardiovascular events among men with diabetes. *Diabetes Care*, 27(4), 889-94 (2004)

108. S. E. Shoelson, J. Lee and A. B. Goldfine: Inflammation and insulin resistance. *J Clin Invest*, 116(7), 1793-801 (2006)

109. F. Kim, B. Gallis and M. A. Corson: TNF-alpha inhibits flow and insulin signaling leading to NO production in aortic endothelial cells. *Am J Physiol Cell Physiol*, 280(5), C1057-65 (2001)

110. A. Bierhaus, T. Illmer, M. Kasper, T. Luther, P. Quehenberger, H. Tritschler, P. Wahl, R. Ziegler, M. Muller and P. P. Nawroth: Advanced glycation end product (AGE)-mediated induction of tissue factor in cultured endothelial cells is dependent on RAGE. *Circulation*, 96(7), 2262-71 (1997)

111. A. Bierhaus, S. Chevion, M. Chevion, M. Hofmann, P. Quehenberger, T. Illmer, T. Luther, E. Berentshtein, H. Tritschler, M. Muller, P. Wahl, R. Ziegler and P. P. Nawroth: Advanced glycation end product-induced activation of NF-kappaB is suppressed by alpha-lipoic acid in cultured endothelial cells. *Diabetes*, 46(9), 1481-90 (1997)

112. C. de Alvaro, T. Teruel, R. Hernandez and M. Lorenzo: Tumor necrosis factor alpha produces insulin resistance in skeletal muscle by activation of inhibitor kappaB kinase in a p38 MAPK-dependent manner. *J Biol Chem*, 279(17), 17070-8 (2004)

113. F. Kim, K. A. Tysseling, J. Rice, M. Pham, L. Haji, B. M. Gallis, A. S. Baas, P. Paramsothy, C. M. Giachelli, M. A. Corson and E. W. Raines: Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKKbeta. *Arterioscler Thromb Vasc Biol*, 25(5), 989-94 (2005)

114. A. J. Donato, I. Eskurza, A. E. Silver, A. S. Levy, G. L. Pierce, P. E. Gates and D. R. Seals: Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. *Circ Res*, 100(11), 1659-66 (2007)

115. T. Inoguchi, R. Battan, E. Handler, J. R. Sportsman, W. Heath and G. L. King: Preferential elevation of protein kinase C isoform beta II and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation. *Proc Natl Acad Sci U S A*, 89(22), 11059-63 (1992)

116. B. Tesfamariam, M. L. Brown and R. A. Cohen: Elevated glucose impairs endothelium-dependent relaxation by activating protein kinase C. *J Clin Invest*, 87(5), 1643-8 (1991)

117. C. Rask-Madsen and G. L. King: Proatherosclerotic mechanisms involving protein kinase C in diabetes and insulin resistance. *Arterioscler Thromb Vasc Biol*, 25(3), 487-96 (2005)

118. M. A. Cotter, A. M. Jack and N. E. Cameron: Effects of the protein kinase C beta inhibitor LY333531 on neural and vascular function in rats with streptozotocin-induced diabetes. *Clin Sci (Lond)*, 103(3), 311-21 (2002)

119. K. Naruse, C. Rask-Madsen, N. Takahara, S. W. Ha, K. Suzuma, K. J. Way, J. R. Jacobs, A. C. Clermont, K. Ueki, Y. Ohshiro, J. Zhang, A. B. Goldfine and G. L. King: Activation of vascular protein kinase C-beta inhibits Akt-dependent endothelial nitric oxide synthase function in obesity-associated insulin resistance. *Diabetes*, 55(3), 691-8 (2006)

120. J. A. Beckman, A. B. Goldfine, M. B. Gordon, L. A. Garrett and M. A. Creager: Inhibition of protein kinase Cbeta prevents impaired endothelium-dependent vasodilation caused by hyperglycemia in humans. *Circ Res*, 90(1), 107-11 (2002)

121. A. Otsuka, K. Azuma, T. Iesaki, F. Sato, T. Hirose, T. Shimizu, Y. Tanaka, H. Daida, R. Kawamori and H. Watada: Temporary hyperglycaemia provokes monocyte adhesion to endothelial cells in rat thoracic aorta. *Diabetologia*, 48(12), 2667-74 (2005)

122. R. Piga, Y. Naito, S. Kokura, O. Handa and T. Yoshikawa: Short-term high glucose exposure induces monocyte-endothelial cells adhesion and transmigration by increasing VCAM-1 and MCP-1 expression in human aortic endothelial cells. *Atherosclerosis*, 193(2), 328-34 (2007)

123. N. Ohgami, A. Miyazaki, M. Sakai, A. Kuniyasu, H. Nakayama and S. Horiuchi: Advanced glycation end products (AGE) inhibit scavenger receptor class B type I-mediated reverse cholesterol transport: a new crossroad of AGE to cholesterol metabolism. *J Atheroscler Thromb*, 10(1), 1-6 (2003)

124. J. M. Forbes, L. T. Yee, V. Thallas, M. Lassila, R. Candido, K. A. Jandeleit-Dahm, M. C. Thomas, W. C. Burns, E. K. Deemer, S. R. Thorpe, M. E. Cooper and T. J. Allen: Advanced glycation end product interventions reduce diabetes-accelerated atherosclerosis. *Diabetes*, 53(7), 1813-23 (2004)

125. L. G. Bucciarelli, T. Wendt, W. Qu, Y. Lu, E. Lalla, L. L. Rong, M. T. Goova, B. Moser, T. Kislinger, D. C. Lee, Y. Kashyap, D. M. Stern and A. M. Schmidt: RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. *Circulation*, 106(22), 2827-35 (2002)

126. H. Cai: NAD(P)H oxidase-dependent self-propagation of hydrogen peroxide and vascular disease. *Circ Res*, 96(8), 818-22 (2005)

127. R. Salvayre, N. Auge, H. Benoist and A. Negre-Salvayre: Oxidized low-density lipoprotein-induced apoptosis. *Biochim Biophys Acta*, 1585(2-3), 213-21 (2002)

128. K. Brand, T. Eisele, U. Kreusel, M. Page, S. Page, M. Haas, A. Gerling, C. Kaltschmidt, F. J. Neumann, N. Mackman, P. A. Baeurele, A. K. Walli and D. Neumeier: Dysregulation of monocytic nuclear factor-kappa B by oxidized low-density lipoprotein. *Arterioscler Thromb Vasc Biol*, 17(10), 1901-9 (1997)

129. B. D. Lamon and D. P. Hajjar: Inflammation at the molecular interface of atherogenesis: an anthropological journey. *Am J Pathol*, 173(5), 1253-64 (2008)

130. M. P. de Winther, E. Kanters, G. Kraal and M. H. Hofker: Nuclear factor kappaB signaling in atherogenesis. *Arterioscler Thromb Vasc Biol*, 25(5), 904-14 (2005)

131. A. M. Fulton, X. Ma and N. Kundu: Targeting prostaglandin E EP receptors to inhibit metastasis. *Cancer Res*, 66(20), 9794-7 (2006)

132. T. Ulven and E. Kostenis: Targeting the prostaglandin D2 receptors DP and CRTH2 for treatment of inflammation. *Curr Top Med Chem*, 6(13), 1427-44 (2006)

133. S. Pavlovic, B. Du, K. Sakamoto, K. M. Khan, C. Natarajan, R. M. Breyer, A. J. Dannenberg and D. J. Falcone: Targeting prostaglandin E2 receptors as an alternative strategy to block cyclooxygenase-2-dependent extracellular matrix-induced matrix metalloproteinase-9 expression by macrophages. *J Biol Chem*, 281(6), 3321-8 (2006)

134. W. J. Zhang, K. E. Bird, T. S. McMillen, R. C. LeBoeuf, T. M. Hagen and B. Frei: Dietary alpha-lipoic acid supplementation inhibits atherosclerotic lesion development in apolipoprotein E-deficient and apolipoprotein E/low-density lipoprotein receptor-deficient mice. *Circulation*, 117(3), 421-8 (2008)

135. Y. H. Chen, S. J. Lin, Y. L. Chen, P. L. Liu and J. W. Chen: Anti-inflammatory effects of different drugs/agents

with antioxidant property on endothelial expression of adhesion molecules. *Cardiovasc Hematol Disord Drug Targets*, 6(4), 279-304 (2006)

136. M. Milagros Rocha and V. M. Victor: Targeting antioxidants to mitochondria and cardiovascular diseases: the effects of mitoquinone. *Med Sci Monit*, 13(7), RA132-45 (2007)

137. V. M. Victor and M. Rocha: Targeting antioxidants to mitochondria: a potential new therapeutic strategy for cardiovascular diseases. *Curr Pharm Des*, 13(8), 845-63 (2007)

138. M. Hersberger: Potential role of the lipoxygenase derived lipid mediators in atherosclerosis: leukotrienes, lipoxins and resolvins. *Clin Chem Lab Med*, 48(8), 1063-73 (2010)

139. L. J. Crofford: COX-1 and COX-2 tissue expression: implications and predictions. *J Rheumatol Suppl*, 49, 15-9 (1997)

140. F. Cipollone, G. Cicolini and M. Bucci: Cyclooxygenase and prostaglandin synthases in atherosclerosis: recent insights and future perspectives. *Pharmacol Ther*, 118(2), 161-80 (2008)

141. M. E. Burleigh, V. R. Babaev, J. A. Oates, R. C. Harris, S. Gautam, D. Riendeau, L. J. Marnett, J. D. Morrow, S. Fazio and M. F. Linton: Cyclooxygenase-2 promotes early atherosclerotic lesion formation in LDL receptor-deficient mice. *Circulation*, 105(15), 1816-23 (2002)

142. R. Langenbach, C. Loftin, C. Lee and H. Tiano: Cyclooxygenase knockout mice: models for elucidating isoform-specific functions. *Biochem Pharmacol*, 58(8), 1237-46 (1999)

143. M. Zhang, Y. Dong, J. Xu, Z. Xie, Y. Wu, P. Song, M. Guzman, J. Wu and M. H. Zou: Thromboxane receptor activates the AMP-activated protein kinase in vascular smooth muscle cells via hydrogen peroxide. *Circ Res*, 102(3), 328-37 (2008)

144. P. Song, M. Zhang, S. Wang, J. Xu, H. C. Choi and M. H. Zou: Thromboxane A2 receptor activates a Rho-associated kinase/LKB1/PTEN pathway to attenuate endothelium insulin signaling. *J Biol Chem*, 284(25), 17120-8 (2009)

145. M. Zhang, P. Song, J. Xu and M. H. Zou: Activation of NAD(P)H oxidases by thromboxane A2 receptor uncouples endothelial nitric oxide synthase. *Arterioscler Thromb Vasc Biol*, 31(1), 125-32 (2011)

146. M. Mehrabian, J. Wong, X. Wang, Z. Jiang, W. Shi, A. M. Fogelman and A. J. Lusis: Genetic locus in mice that blocks development of atherosclerosis despite extreme hyperlipidemia. *Circ Res*, 89(2), 125-30 (2001)

147. L. Zhao, M. P. Moos, R. Grabner, F. Pedrono, J. Fan, B. Kaiser, N. John, S. Schmidt, R. Spanbroek, K. Lotzer, L. Huang, J. Cui, D. J. Rader, J. F. Evans, A. J. Habenicht and C. D. Funk: The 5-lipoxygenase pathway promotes pathogenesis of hyperlipidemia-dependent aortic aneurysm. *Nat Med*, 10(9), 966-73 (2004)

148. H. Qiu, A. Gabrielsen, H. E. Agardh, M. Wan, A. Wetterholm, C. H. Wong, U. Hedin, J. Swedenborg, G. K. Hansson, B. Samuelsson, G. Paulsson-Berne and J. Z. Haeggstrom: Expression of 5-lipoxygenase and leukotriene A4 hydrolase in human atherosclerotic lesions correlates with symptoms of plaque instability. *Proc Natl Acad Sci U S A*, 103(21), 8161-6 (2006)

149. K. W. Seo, S. J. Lee, C. E. Kim, M. R. Yun, H. M. Park, J. W. Yun, S. S. Bae and C. D. Kim: Participation of 5-lipoxygenase-derived LTB(4) in 4-hydroxynonenal-enhanced MMP-2 production in vascular smooth muscle cells. *Atherosclerosis*, 208(1), 56-61 (2010)

150. A. Aljada, H. Ghanim, R. Saadeh and P. Dandona: Insulin inhibits NFkappaB and MCP-1 expression in human aortic endothelial cells. *J Clin Endocrinol Metab*, 86(1), 450-3 (2001)

151. G. Zeng and M. J. Quon: Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J Clin Invest*, 98(4), 894-8 (1996)

152. A. Aljada and P. Dandona: Effect of insulin on human aortic endothelial nitric oxide synthase. *Metabolism*, 49(2), 147-50 (2000)

153. P. Dandona, A. Aljada and P. Mohanty: The anti-inflammatory and potential anti-atherogenic effect of insulin: a new paradigm. *Diabetologia*, 45(6), 924-30 (2002)

154. J. F. Woessner, Jr.: Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J*, 5(8), 2145-54 (1991)

155. Z. S. Galis, G. K. Sukhova, M. W. Lark and P. Libby: Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest*, 94(6), 2493-503 (1994) doi:10.1172/JCI117619

156. F. Fath-Ordoubadi and K. J. Beatt: Glucose-insulin-potassium therapy for treatment of acute myocardial infarction: an overview of randomized placebo-controlled trials. *Circulation*, 96(4), 1152-6 (1997)

157. United Kingdom Prospective Diabetes Study (UKPDS). 13: Relative efficacy of randomly allocated diet, sulphonylurea, insulin, or metformin in patients with newly diagnosed non-insulin dependent diabetes followed for three years. *BMJ*, 310(6972), 83-8 (1995)

158. B. B. Zhang, G. Zhou and C. Li: AMPK: an emerging drug target for diabetes and the metabolic syndrome. *Cell Metab*, 9(5), 407-16 (2009)

159. D. Sag, D. Carling, R. D. Stout and J. Suttles: Adenosine 5'-monophosphate-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. *J Immunol*, 181(12), 8633-41 (2008)

160. J. M. Cacicedo, N. Yagihashi, J. F. Keaney, Jr., N. B. Ruderman and Y. Ido: AMPK inhibits fatty acid-induced increases in NF-kappaB transactivation in cultured human umbilical vein endothelial cells. *Biochem Biophys Res Commun*, 324(4), 1204-9 (2004)

161. G. Pilon, P. Dallaire and A. Marette: Inhibition of inducible nitric-oxide synthase by activators of AMP-activated protein kinase: a new mechanism of action of insulin-sensitizing drugs. *J Biol Chem*, 279(20), 20767-74 (2004)

162. S. Giri, N. Nath, B. Smith, B. Viollet, A. K. Singh and I. Singh: 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside inhibits proinflammatory response in glial cells: a possible role of AMP-activated protein kinase. *J Neurosci*, 24(2), 479-87 (2004)

163. Y. Hattori, K. Suzuki, S. Hattori and K. Kasai: Metformin inhibits cytokine-induced nuclear factor kappaB activation via AMP-activated protein kinase activation in vascular endothelial cells. *Hypertension*, 47(6), 1183-8 (2006)

164. Z. Xie, J. Zhang, J. Wu, B. Viollet and M. H. Zou: Upregulation of mitochondrial uncoupling protein-2 by the AMP-activated protein kinase in endothelial cells attenuates oxidative stress in diabetes. *Diabetes*, 57(12), 3222-30 (2008)

165. H. C. Choi, P. Song, Z. Xie, Y. Wu, J. Xu, M. Zhang, Y. Dong, S. Wang, K. Lau and M. H. Zou: Reactive nitrogen species is required for the activation of the AMP-activated protein kinase by statin *in vivo*. *J Biol Chem*, 283(29), 20186-97 (2008)

166. Y. Dong, M. Zhang, B. Liang, Z. Xie, Z. Zhao, S. Asfa, H. C. Choi and M. H. Zou: Reduction of AMP-activated protein kinase alpha2 increases endoplasmic reticulum stress and atherosclerosis *in vivo*. *Circulation*, 121(6), 792-803 (2010)

167. G. Zhou, R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M. F. Hirshman, L. J. Goodyear and D. E. Moller: Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*, 108(8), 1167-74 (2001)

168. B. Molavi, N. Rassouli, S. Bagwe and N. Rasouli: A review of thiazolidinediones and metformin in the treatment of type 2 diabetes with focus on cardiovascular complications. *Vasc Health Risk Manag*, 3(6), 967-73 (2007)

169. P. Rosen and N. F. Wiernsperger: Metformin delays the manifestation of diabetes and vascular dysfunction in Goto-Kakizaki rats by reduction of mitochondrial oxidative stress. *Diabetes Metab Res Rev*, 22(4), 323-30 (2006)

170. D. Bonnefont-Rousselot, B. Raji, S. Walrand, M. Gardes-Albert, D. Jore, A. Legrand, J. Peynet and M. P. Vasson: An intracellular modulation of free radical production could contribute to the beneficial effects of metformin towards oxidative stress. *Metabolism*, 52(5), 586-9 (2003)

171. N. Ouslimani, J. Peynet, D. Bonnefont-Rousselot, P. Therond, A. Legrand and J. L. Beaudeux: Metformin decreases intracellular production of reactive oxygen species in aortic endothelial cells. *Metabolism*, 54(6), 829-34 (2005)

172. L. Li, J. C. Mamputu, N. Wiernsperger and G. Renier: Signaling pathways involved in human vascular smooth muscle cell proliferation and matrix metalloproteinase-2 expression induced by leptin: inhibitory effect of metformin. *Diabetes*, 54(7), 2227-34 (2005)

173. S. S. Lund, L. Tarnow, C. D. Stehouwer, C. G. Schalkwijk, T. Teerlink, J. Gram, K. Winther, M. Frandsen, U. M. Smidt, O. Pedersen, H. H. Parving and A. A. Vaag: Impact of metformin versus repaglinide on non-glycaemic cardiovascular risk markers related to inflammation and endothelial dysfunction in non-obese patients with type 2 diabetes. *Eur J Endocrinol*, 158(5), 631-41 (2008)

174. A. Yessoufou and W. Wahli: Multifaceted roles of peroxisome proliferator-activated receptors (PPARs) at the cellular and whole organism levels. *Swiss Med Wkly*, 140, w13071 (2010)

175. B. P. Kota, T. H. Huang and B. D. Roufogalis: An overview on biological mechanisms of PPARs. *Pharmacol Res*, 51(2), 85-94 (2005)

176. A. Yessoufou, A. Hichami, P. Besnard, K. Moutairou and N. A. Khan: Peroxisome proliferator-activated receptor alpha deficiency increases the risk of maternal abortion and neonatal mortality in murine pregnancy with or without diabetes mellitus: Modulation of T cell differentiation. *Endocrinology*, 147(9), 4410-8 (2006)

177. P. Delerive, K. De Bosscher, S. Besnard, W. Vanden Berghe, J. M. Peters, F. J. Gonzalez, J. C. Fruchart, A. Tedgui, G. Haegeman and B. Staels: Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J Biol Chem*, 274(45), 32048-54 (1999)

178. M. Adachi, R. Kurotani, K. Morimura, Y. Shah, M. Sanford, B. B. Madison, D. L. Gumucio, H. E. Marin, J. M. Peters, H. A. Young and F. J. Gonzalez: Peroxisome proliferator activated receptor gamma in colonic epithelial cells protects against experimental inflammatory bowel disease. *Gut*, 55(8), 1104-13 (2006)

179. Y. M. Shah, K. Morimura and F. J. Gonzalez: Expression of peroxisome proliferator-activated receptor-gamma in macrophage suppresses experimentally induced colitis. *Am J Physiol Gastrointest Liver Physiol*, 292(2), G657-66 (2007)

180. D. S. Straus and C. K. Glass: Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms. *Trends Immunol*, 28(12), 551-8 (2007)

181. M. Bajaj, S. Suraamornkul, T. Pratipanawatr, L. J. Hardies, W. Pratipanawatr, L. Glass, E. Cersosimo, Y. Miyazaki and R. A. DeFronzo: Pioglitazone reduces hepatic fat content and augments splanchnic glucose uptake in patients with type 2 diabetes. *Diabetes*, 52(6), 1364-70 (2003)

182. N. Rasouli, U. Raue, L. M. Miles, T. Lu, G. B. Di Gregorio, S. C. Elbein and P. A. Kern: Pioglitazone improves insulin sensitivity through reduction in muscle lipid and redistribution of lipid into adipose tissue. *Am J Physiol Endocrinol Metab*, 288(5), E930-4 (2005)

183. A. M. Bodles, A. Banga, N. Rasouli, F. Ono, P. A. Kern and R. J. Owens: Pioglitazone increases secretion of high-molecular-weight adiponectin from adipocytes. *Am J Physiol Endocrinol Metab*, 291(5), E1100-5 (2006)

184. N. F. Chu, M. H. Shen, D. M. Wu and S. M. Shieh: Plasma TNF-R1 and insulin concentrations in relation to leptin levels among normal and overweight children. *Clin Biochem*, 35(4), 287-92 (2002)

185. H. Yki-Jarvinen: Thiazolidinediones. *N Engl J Med*, 351(11), 1106-18 (2004)

186. N. Rasouli, A. Yao-Borengasser, L. M. Miles, S. C. Elbein and P. A. Kern: Increased plasma adiponectin in response to pioglitazone does not result from increased gene expression. *Am J Physiol Endocrinol Metab*, 290(1), E42-E46 (2006)

187. J. Hwang, D. J. Kleinhenz, H. L. Rupnow, A. G. Campbell, P. M. Thule, R. L. Sutliff and C. M. Hart: The PPARgamma ligand, rosiglitazone, reduces vascular oxidative stress and NADPH oxidase expression in diabetic mice. *Vascul Pharmacol*, 46(6), 456-62 (2007)

188. P. M. Ridker, C. P. Cannon, D. Morrow, N. Rifai, L. M. Rose, C. H. McCabe, M. A. Pfeffer and E. Braunwald: C-reactive protein levels and outcomes after statin therapy. *N Engl J Med*, 352(1), 20-8 (2005)

189. D. A. Morrow, J. A. de Lemos, M. S. Sabatine, S. D. Wiviott, M. A. Blazing, A. Shui, N. Rifai, R. M. Califf and E. Braunwald: Clinical relevance of C-reactive protein during follow-up of patients with acute coronary syndromes in the Aggrastat-to-Zocor Trial. *Circulation*, 114(4), 281-8 (2006)

190. S. E. Nissen, E. M. Tuzcu, P. Schoenhagen, T. Crowe, W. J. Sasiela, J. Tsai, J. Orazem, R. D. Magorien, C.

O'Shaughnessy and P. Ganz: Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *N Engl J Med*, 352(1), 29-38 (2005)

191. G. Patti, V. Pasceri, G. Colonna, M. Miglionico, D. Fischetti, G. Sardella, A. Montinaro and G. Di Sciascio: Atorvastatin pretreatment improves outcomes in patients with acute coronary syndromes undergoing early percutaneous coronary intervention: results of the ARMYDA-ACS randomized trial. *J Am Coll Cardiol*, 49(12), 1272-8 (2007)

192. Y. Asanuma, A. Oeser, A. K. Shintani, E. Turner, N. Olsen, S. Fazio, M. F. Linton, P. Raggi and C. M. Stein: Premature coronary-artery atherosclerosis in systemic lupus erythematosus. *N Engl J Med*, 349(25), 2407-15 (2003)

193. H. K. Choi and G. Curhan: Independent impact of gout on mortality and risk for coronary heart disease. *Circulation*, 116(8), 894-900 (2007)

194. J. M. Gelfand, A. L. Neimann, D. B. Shin, X. Wang, D. J. Margolis and A. B. Troxel: Risk of myocardial infarction in patients with psoriasis. *JAMA*, 296(14), 1735-41 (2006)

195. H. Maradit-Kremers, P. J. Nicola, C. S. Crowson, K. V. Ballman and S. E. Gabriel: Cardiovascular death in rheumatoid arthritis: a population-based study. *Arthritis Rheum*, 52(3), 722-32 (2005)

196. C. A. Dinarello: Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol*, 27, 519-50 (2009)

197. P. M. Ridker, N. Rifai, M. Pfeffer, F. Sacks, S. Lepage and E. Braunwald: Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation*, 101(18), 2149-53 (2000)

198. C. Heeschen, S. Dimmeler, C. W. Hamm, M. J. van den Brand, E. Boersma, A. M. Zeiher and M. L. Simoons: Soluble CD40 ligand in acute coronary syndromes. *N Engl J Med*, 348(12), 1104-11 (2003)

199. K. Otterdal, C. Smith, E. Oie, T. M. Pedersen, A. Yndestad, E. Stang, K. Endresen, N. O. Solum, P. Aukrust and J. K. Damas: Platelet-derived LIGHT induces inflammatory responses in endothelial cells and monocytes. *Blood*, 108(3), 928-35 (2006)

200. W. J. Sandberg, A. Yndestad, E. Oie, C. Smith, T. Ueland, O. Ovchinnikova, A. K. Robertson, F. Muller, A. G. Semb, H. Scholz, A. K. Andreassen, L. Gullestad, J. K. Damas, S. S. Froland, G. K. Hansson, B. Halvorsen and P. Aukrust: Enhanced T-cell expression of RANK ligand in acute coronary syndrome: possible role in plaque destabilization. *Arterioscler Thromb Vasc Biol*, 26(4), 857-63 (2006)

201. T. Omland, T. Ueland, A. M. Jansson, A. Persson, T. Karlsson, C. Smith, J. Herlitz, P. Aukrust, M. Hartford and K. Caidahl: Circulating osteoprotegerin levels and long-

term prognosis in patients with acute coronary syndromes. *J Am Coll Cardiol*, 51(6), 627-33 (2008)

202. P. Secchiero, F. Corallini, C. Ceconi, G. Parrinello, S. Volpato, R. Ferrari and G. Zauli: Potential prognostic significance of decreased serum levels of TRAIL after acute myocardial infarction. *PLoS One*, 4(2), e4442 (2009)

203. T. Waehre, A. Yndestad, C. Smith, T. Haug, S. H. Tunheim, L. Gullestad, S. S. Froland, A. G. Semb, P. Aukrust and J. K. Damas: Increased expression of interleukin-1 in coronary artery disease with downregulatory effects of HMG-CoA reductase inhibitors. *Circulation*, 109(16), 1966-72 (2004)

204. Z. Mallat, P. Henry, R. Fressonnet, S. Alouani, A. Scoazec, P. Beaufils, Y. Chvatchko and A. Tedgui: Increased plasma concentrations of interleukin-18 in acute coronary syndromes. *Heart*, 88(5), 467-9 (2002)

205. M. Shimpo, D. A. Morrow, E. O. Weinberg, M. S. Sabatine, S. A. Murphy, E. M. Antman and R. T. Lee: Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. *Circulation*, 109(18), 2186-90 (2004)

206. S. Jovinge, A. Hamsten, P. Tornvall, A. Proudler, P. Bavenholm, C. G. Ericsson, I. Godsland, U. de Faire and J. Nilsson: Evidence for a role of tumor necrosis factor alpha in disturbances of triglyceride and glucose metabolism predisposing to coronary heart disease. *Metabolism*, 47(1), 113-8 (1998)

207. J. Nilsson, E. Bengtsson, G. N. Fredrikson and H. Bjorkbacka: Inflammation and immunity in diabetic vascular complications. *Curr Opin Lipidol*, 19(5), 519-24 (2008)

208. W. Palinski and J. L. Witztum: Immune responses to oxidative neoepitopes on LDL and phospholipids modulate the development of atherosclerosis. *J Intern Med*, 247(3), 371-80 (2000)

209. J. A. Berliner, M. Navab, A. M. Fogelman, J. S. Frank, L. L. Demer, P. A. Edwards, A. D. Watson and A. J. Lusis: Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. *Circulation*, 91(9), 2488-96 (1995)

210. S. Horkko, C. J. Binder, P. X. Shaw, M. K. Chang, G. Silverman, W. Palinski and J. L. Witztum: Immunological responses to oxidized LDL. *Free Radic Biol Med*, 28(12), 1771-9 (2000)

211. R. Roberts, V. DeMello and B. E. Sobel: Deleterious effects of methylprednisolone in patients with myocardial infarction. *Circulation*, 53(3 Suppl), I204-6 (1976)

212. D. M. Yellon and D. J. Hausenloy: Myocardial reperfusion injury. *N Engl J Med*, 357(11), 1121-35 (2007)

213. R. Klingenberg and G. K. Hansson: Treating inflammation in atherosclerotic cardiovascular disease: emerging therapies. *Eur Heart J*, 30(23), 2838-44 (2009)

Send correspondence to: Ming-Hui Zou, Department of Medicine, University of Oklahoma Health Science Center, 941 Stanton L. Young Blvd., Oklahoma City, OK 73104, USA, Tel: 405-271-3974, Fax: 405-271-3973, E-mail: ming-hui-zou@ouhsc.edu