Role of the Immune System in Hypertension: Modulation by Dietary Antioxidants

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ABSTRACT

Hypertension is a major health problem worldwide. Individuals with hypertension are at an increased risk for stroke, heart disease, and kidney failure. Although the etiology of essential hypertension has a genetic component, lifestyle factors such as diet play an important role. Insulin resistance is a common feature of hypertension in both humans and animal models affecting glucose and lipid metabolism producing excess aldehydes including methylglyoxal. These aldehydes react with proteins to form conjugates called advanced glycation end products (AGEs). This alters protein structure and function and can affect vascular and immune cells leading to their activation and secretion of inflammatory cytokines. AGEs also act via receptors for advanced glycation end products on these cells altering the function of antioxidant and metabolic enzymes, and ion channels. This results in an increase in cytosolic free calcium, decrease in nitric oxide, endothelial dysfunction, oxidative stress, peripheral vascular resistance, and infiltration of vascular and kidney tissue with inflammatory cells leading to hypertension. Supplementation with dietary antioxidants including vitamins C, E, or B₆, thiols such as cysteine and lipoic acid, have been shown to lower blood pressure and plasma inflammatory cytokines in animal models and humans with essential hypertension. A well-balanced diet rich in antioxidants that includes vegetables, fruits, low fat dairy products, low salt, and includes whole grains, poultry, fish and nuts, lowers blood pressure and vascular inflammation. These antioxidants may achieve their antihypertensive and anti-inflammatory/immunomodulatory effects by reducing AGEs and improving insulin resistance and associated alterations. Dietary supplementation with antioxidants may be a beneficial, inexpensive, front-line alternative treatment modality for hypertension.

KEYWORDS: Hypertension, immune system, insulin resistance, advanced glycation end products, receptors for advanced glycation end products, renin-angiotensin system, oxidative stress, renal damage, inflammatory cytokines, T cells, macrophages, dietary antioxidants

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It has been estimated that at least 600 million people suffer from essential hypertension worldwide.¹ The prevalence of hypertension is considered to be a public health concern of epidemic proportions, as it leads to an increased risk of both cardiovascular and renal diseases.²⁻⁴ Essential hypertension occurs when there is sustained arterial pressure caused by a combination of acquired or genetic metabolic defects involved in blood pressure regulation that interact with environmental factors such as diet and lifestyle.⁵ There are several metabolic processes which affect blood pressure including insulin resistance, increased oxidative stress, decreased nitric oxide (NO) bioavailability, altered renin-angiotensin system (RAS), endothelial dysfunction, increased peripheral vascular resistance, and reduced sodium excretion (Fig. 1). Malfunction of one or all of these processes can lead to endothelial dysfunction, increased vascular cytosolic free calcium and peripheral vascular resistance, resulting in the development of hypertension.

Figure 1 Mechanism of essential hypertension.

Insulin resistance, due to genetic and lifestyle factors, leads to altered glucose and lipid metabolism with an increase in methylglyoxal and other reactive aldehydes. Excess aldehydes form advanced glycation end products (AGEs) which leads to oxidative stress and activation of the immune system. This leads to decreased nitric oxide (NO), endothelial dysfunction, inflammation, increased inflammatory cytokines, cytosolic free calcium, peripheral vascular resistance, and hypertension. Increased inflammatory cytokines may also lead to insulin resistance. RAS, renin-angiotensin system.

Recent studies in hypertensive humans and animal models of hypertension have suggested that components of the immune system contribute to the development of hypertension. It has been suggested that immune system alterations may cause an inflammatory response in blood vessel walls leading to the development of hypertension.^{6,7} For instance, Sesso et al reported a positive relationship between serum C-reactive protein (CRP) levels and the risk of developing hypertension among participants in the Women's Health Study.⁸ Likewise, cross-sectional studies have shown that compared with normotensive humans, plasma levels of inflammatory markers such as CRP, cytokines such as tumor necrosis factor- α (TNF- α) and interleukin (IL)-6, chemokines such as monocyte chemoattractant protein 1 (MCP-1), and adhesion molecules such as P-selectin and soluble intercellular adhesion molecule-1 (sICAM-1) are elevated in patients with essential hypertension.⁹⁻¹¹ As well, insulin resistance contributes to the activation of the immune system in essential hypertension due to the formation of reactive aldehydes, including methylglyoxal, which go on to react with proteins to form advanced glycation end products (AGEs) and contribute to oxidative stress. This can lead to altered functioning of the RAS and trigger the production of inflammatory cytokines which result in the release of reactive oxygen species (ROS), and reduce NO bioavailability which impairs endothelium-dependent vasodilation.¹² Endothelial dysfunction and vascular damage associated with hypertension also increases the vascular production of ROS in the vascular wall. This may lead to oxidatively modified proteins that serve as "neoantigens" and cause the activation of the immune system and recruitment of inflammatory cells, such as T cells and macrophages, to the walls of blood vessels and infiltration into target organs such as the kidney where they excrete inflammatory cytokines.

The present article will review recent findings on the role of the immune system as it relates to the mechanism of essential hypertension. It will also discuss evidence found in humans and animal models of hypertension which demonstrate how the immune system and inflammation are implicated in the pathogenesis of essential hypertension. It will also provide evidence reported in the literature on the ability of dietary antioxidants to attenuate or control blood pressure and inflammation, and discuss possible mechanism of their antihypertensive and anti-inflammatory effects.

MECHANISM OF HYPERTENSION

Under normal physiological conditions, insulin, RAS, and the NO pathway are major factors involved in a complex biochemical network that maintains vascular homeostasis. Alteration to any one of these factors potentially affects all others, leading to impaired blood

pressure regulation and hypertension. Insulin resistance and its downstream effects including altered glucose and lipid metabolism resulting in formation of methylglyoxal, glyoxal and other aldehydes, and formation of AGEs, activation of RAS, and oxidative stress, as well as loss of bioavailable NO and endothelial dysfunction with increase in vascular smooth muscle cells (VSMC) cytosolic free calcium and peripheral vascular resistance resulting in hypertension.^{13–32}

INSULIN RESISTANCE AND THE FORMATION OF AGEs

Insulin is a hormone that regulates lipid and carbohydrate metabolism, and stimulates protein synthesis. Insulin binds to its receptors on the cell surface and initiates a signaling cascade which promotes glucose uptake and is involved in mechanisms which help regulate blood pressure such as NO-mediated vasodilation and angiotensin-II signaling.^{33–38} Insulin resistance is a metabolic disorder characterized by the impaired transport of glucose into the peripheral tissues at a given concentration of insulin due to an impairment of the nonoxidative, glycolytic pathways of intracellular glucose metabolism.^{5,13} As glucose levels rise, the pancreas continues to secrete insulin. This increases arterial pressure as insulin stimulates the reabsorption of sodium ions from the proximal tubule of the kidney causing an increase in blood pressure.

Altered glucose metabolism due to insulin resistance, results in increased production of aldehydes, including methylglyoxal.^{39,40} Under normal conditions, glucose is metabolized to glyceraldehyde-3-phosphate (G3P) via glycolysis. G3P is then converted to 1,3-diphosphoglycerate by glyceraldehydes-3-phosphate dehydrogenase (GAPDH), which is upregulated and G3P is further metabolized to form pyruvate. During insulin resistance, poorly functioning insulin may result in down regulation of GAPDH, slowing glucose metabolism and increasing metabolism via the polyol pathway. This can result in a build-up of G3P, leading to an increased concentration of methylglyoxal, a highly reactive aldehyde.^{13,40,41}

Methylglyoxal itself has been shown to inhibit GAPDH, resulting in further abnormalities in glucose metabolism.⁴² As well, methylglyoxal alters aldose reductase, an enzyme that stimulates glucose flux through the polyol pathway, with further methylglyoxal formation.⁴³ Typical North American diets, which are high in fructose- and fat-enriched processed food products, may also contribute to this altered glucose and lipid metabolism, leading to an increase in reactive aldehyde levels.⁴⁴

AGEs are formed when aldehydes react non-enzymatically with free sulfhydryl (–SH) and amino groups of protein amino acids, including cysteine, arginine, and lysine.^{40,44,45} This direct modification

of protein structure results in functional changes of proteins and enzymes such as nitric oxide synthase (NOS), GAPDH, glutathione (GSH) reductase, Ca^{2+} channels, transcription factors, lipoproteins, and membrane receptors.^{42,46–49} AGE-modified proteins also stimulate receptors for advanced glycation end products, called RAGEs, and various scavenger receptors to modify protein function and enzyme expression activity.^{47,50}

OXIDATIVE STRESS AND HYPERTENSION

Oxidative stress occurs when there is an imbalance between the activity of oxidant enzymes and the body's antioxidant capacity, or when the dietary intake of antioxidants does not keep pace with antioxidant requirements or appropriate combinations of nutrients are not included in the diet.⁵¹ This can lead to the formation of ROS and reactive nitrogen species (RNS), both of which are the undesirable by-products of aerobic metabolism, and includes compounds such as superoxide (O_2^-), hydroxyl radical ($\cdot\text{OH}$), H_2O_2 , and peroxynitrite (ONOO^-).⁵¹ Antioxidant vitamins and other reducing compounds such as GSH and cysteine are able to reduce ROS and RNS nonenzymatically.^{20,52}

In hypertensive patients, an imbalance of ROS production and degradation is often observed, especially in those patients with severe hypertension, since there is an insufficient amount of antioxidant compounds to counterbalance the damage caused by ROS.⁵¹ Hypertensive patients with cardiovascular disease also show low plasma levels of vitamins E and C.^{53–58} High-salt diets, also implicated in hypertension, have been shown to increase oxidative stress.⁵⁹ Both smoking and high ethanol intake, which are lifestyle risk factors associated with hypertension, are also associated with increased oxidative stress.^{60,61}

OXIDATIVE STRESS AND AGEs

Oxidative stress is partially controlled by antioxidant enzymes glutathione peroxidase (GPx) and glutathione reductase (GRed), which have –SH and amino groups at their catalytic sites.^{62–64} Increased oxidative stress results when aldehydes modify these groups to form AGEs or reaction with AGE-modified proteins which can inhibit the activity of these enzymes.^{42,64–66} Methylglyoxal and other aldehydes are normally kept at a low level by catabolism via the glutathione-dependent glyoxalase enzyme system or by binding to cysteine to be excreted in bile and urine. Excess methylglyoxal may lead to the depletion of cysteine and glutathione, resulting in decreased antioxidant capacity.⁴⁰ AGEs may also have an ROS-mediated effect on genetic expression as the activation of RAGEs by AGEs results in a nicotinamide adenine dinucleotide phosphate

(NADPH) oxidase-mediated increase in intracellular reactive oxygen intermediates, extracellular H_2O_2 , and cell surface expression of vascular cell adhesion molecule-1 in human endothelial cells.⁵⁰

Methylglyoxal induces a significant generation of O_2^- and $ONOO^-$ in VSMC in vitro.⁶⁷ In VSMCs cultured from spontaneously hypertensive rats (SHR), increased levels of methylglyoxal, AGEs, and oxidative stress have been observed, along with a reduction in GSH levels, and GPx and GRed activity.⁶⁵ SHRs with elevated plasma methylglyoxal show an increase in aortic AGEs and oxidative stress, and a decrease in GSH levels and antioxidant enzyme activity.²⁷

OXIDATIVE STRESS AND ALTERATION OF MEMBRANE ENZYMES, RECEPTORS, AND IONIC CHANNELS

Proteins and lipids are cellular targets for oxidation, which alters their structure and function (Table 1). There are several ways by which ROS can cause these alterations resulting in pathogenic changes. First, the oxidation of critical amino acids residues, such as cysteine residues, can alter enzyme activity where $-SH$ groups are in the catalytic domain or may affect transcriptional activities if they are within the binding site of the transcription factors.^{42,68-72} Second, intra- or intermolecular conformational changes, such as the formation of disulfide

Table 1 Effect of Oxidative Stress on Various Biochemical Parameters in Hypertension

Affected Component	Action of ROS	Effect of Oxidative Damage (Reference)
GPx and GRed	Inhibition of enzyme activity	A decrease in ability to degrade or neutralize ROS, resulting in an increase in oxidative stress. This lead to lipid peroxidation and formation of secondary aldehydes, setting up a cycle of ROS and AGEs. ^{27,65,74,79}
GAPDH	Inhibition of enzyme activity	Exacerbation of insulin resistance, resulting in an increase in aldehydes and AGEs, and a perpetuation of oxidative stress. ^{40,42,238}
eNOS	Inhibition of enzyme activity and - alteration causing uncoupling of enzyme	Decreased production of NO resulting in an increase in vasoconstriction, blood pressure, platelet aggregation, VSMC proliferation and activation of monocyte adhesion molecules. ^{83-86,239}
Endothelial NO	Degradation into peroxynitrite and peroxynitrous acid	Decreased NO and an increase in reactive nitrogen species, resulting in an increase in blood pressure, inflammation, and nitrosative damage. ^{69,87,240,241}
Membrane NADPH oxidase	Activation of enzyme activity	Increase in oxidative stress at the surface of endothelium, possibly leading to membrane modification that contributes to atherosclerotic processes. ^{90,91,242-244}
PTP	Inhibition of enzyme activity resulting in an increase in PTK	Abnormal intracellular cell signal transduction, resulting in VSMC proliferation, apoptosis, contraction, and inflammation. ^{70,78,88}
ACE	Activation of enzyme activity	Increase in production of angiotensin II, resulting in vasoconstriction and increased blood pressure, hypertrophy, proliferation and apoptosis, and activation of NADPH oxidase with an increase in oxidative stress. ^{88-91,242,245}
Calcium channels and calcium regulatory enzymes and proteins	Modification of vascular calcium channels, inhibition of Ca^{2+} -ATPase, and alteration of transport activating proteins	Increased free cytosolic calcium, either from an increased influx or release from intracellular stores, resulting in an increase in oxidative stress, peripheral vascular resistance and blood pressure, and stimulation of cellular proliferation, apoptosis and inflammation. ^{68,69,93-95,246}
Structural lipids and proteins	Modification to lipid and/or proteins of structural membranes	Alters integrity of endothelial membrane ⁷⁴⁻⁷⁶

ROS, reactive oxygen species; GPx, glutathione peroxidase; GRed, glutathione reductase; AGEs, advanced glycation end products; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; VSMC, vascular smooth muscle cell; NADPH, nicotinamide adenine dinucleotide phosphate; PTP, Protein tyrosine phosphatase; PTK, protein tyrosine kinase; ACE, angiotensin converting enzyme.

bridges between or within proteins, also may result in an alteration of protein activity or function.^{71,73} Next, metal-catalyzed oxidative reactions may cause modifications to membrane proteins and lipids, leading to degradation processes.⁷⁴ Fourth, peroxidation reactions may lead to degradation of membrane lipids with loss of membrane integrity. These reactions may also release aldehydes that modify membrane proteins, possibly altering ion channel or receptor function.^{74–76} Finally, overstimulation of ROS-mediated signaling pathways may result in gene transcription activities that regulate inflammatory factor expression, increased vascular cell proliferation, and apoptosis.^{77,78}

The modification of enzymes involved in cellular function by oxidation can result in adverse effects that contribute to the development of hypertension. GPx and GRed are antioxidant enzymes that protect cells from oxidative damage. These enzymes demonstrate a loss of activity when subjected to oxidative modification, resulting in a decrease in antioxidant defense and perpetuation of oxidative stress.⁷⁹ In SHR, it has been shown that there is an increase in ROS levels in vascular tissue with a decrease in GSH levels and antioxidant enzyme activity.^{27,65} Increased ROS levels and antioxidant capacity have also been reported in humans with essential hypertension.^{56,80–82}

GAPDH also has an important role in insulin resistance. The oxidative modification of GAPDH may contribute to the development and severity of insulin resistance, creating a biochemical loop that perpetuates an oxidizing environment. GAPDH activity is inhibited when O_2^- induced peptide and protein peroxides decrease the free thiols of GAPDH.⁴²

The enzyme endothelial nitric oxide synthase (eNOS) is important for regulating vascular tone. eNOS acts on arginine to produce NO and is an SH-dependent enzyme with a cysteine residue (C184) at its active site. A loss of catalytic activity occurs when there are alterations to this residue.⁸³ As well, oxidation of tetrahydrobiopterin, an eNOS cofactor, causes eNOS to uncouple, resulting in decreased NO production and increased O_2^- production.^{84–86} Reducing the bioavailability of NO by either decreasing eNOS or NO while continuing ROS production, results in endothelial dysfunction.^{86,87}

The function of protein tyrosine phosphatases is to regulate protein tyrosine kinases (PTKs) through phosphorylation/dephosphorylation. Protein tyrosine phosphatases (PTPs) have cysteine residues at their active sites. PTKs initiate the cell signaling pathways which regulate cell growth and inflammatory responses. The activity of PTKs is increased when PTP activity is inhibited by oxidative modification. This results in abnormal intracellular signal transduction, which leads to vascular cell proliferation, apoptosis and contraction, and proinflammatory processes.^{70,78,88}

When ROS act on angiotensin converting enzyme (ACE), it increases its catalytic activity, causing an increase in the production of angiotensin II, a potent vasoconstrictor.⁸⁹ Angiotensin II binds to its type I receptor, causing an increase in contraction, hypertrophy, proliferation, and apoptosis. This may be due to activation of receptor and nonreceptor kinases. The stimulation of type I receptors by angiotensin II also increases O_2^- production by NADPH oxidase.^{88,90} The angiotensin II-included oxidative stress by NADPH oxidase may be partly responsible for the reduction in NO bioavailability and endothelial dysfunction associated with hypertension.^{91,92}

ALTERATION OF CYTOSOLIC FREE CALCIUM IN VSMC

Calcium has an important role in maintaining normal cell homeostasis. It is responsible for regulating contraction in VSMCs, transcription activities, and expression of cell growth factors. The level of calcium within the cytoplasm and intracellular organelles is controlled in several ways including membrane ion channels, transporters, and ATPase pumps. ROS can increase intracellular calcium levels by increasing calcium transport from outside of the cell or causing calcium release from intracellular stores.^{93,94}

Calcium channels in the vascular membrane are dependent on –SH groups for normal functioning.⁹³ When oxidation of these –SH groups occur, it results in a cross-linkage reaction which allows for an increased influx of calcium into the cytosol as the calcium channels are kept in an open state.⁷³ The level of cytosolic free calcium can also be increased when Ca^{2+} -ATPase is inhibited when cysteine and lysine in the enzyme are modified by oxidation.^{68,69,95}

ROS, specifically O_2^- , inhibits Ca^{2+} -ATPase found in the sarcoplasmic reticulum, resulting in the release of calcium from the sarcoplasmic reticulum, and initiates signal transduction processes.⁹⁵ In SHR, H_2O_2 has been shown to increase cytosolic free calcium and contractile responses. This is caused by the increased calcium from upregulated calcium channels.⁹⁴ An increase in cytosolic calcium can also cause an increase in ROS, resulting in a perpetuation of oxidative stress and cellular damage.⁹⁶ Thus, the dysregulation of calcium transport in the membrane caused by ROS leads to an increase in cytosolic free calcium, peripheral vascular resistance, cell proliferation and apoptosis, as well as inflammation and further increase in oxidative stress.

RAS AND HYPERTENSION

The RAS has a significant role in the regulation of blood volume and arterial pressure.⁹⁷ Angiotensinogen is found in many tissues and in the blood. Renin, an

enzyme produced by the kidneys, acts to convert angiotensinogen to angiotensin I, which is then further converted to angiotensin II by ACE. Angiotensin II acts on angiotensin type 1 (AT₁) receptors in the vascular tissues to cause vasoconstriction. Angiotensin II also causes the release of aldosterone from adrenal glands to increase renal sodium and water retention, which increases blood volume and blood pressure.

Angiotensin II infusion in animal models has been shown to increase blood pressure and cause oxidative stress-related insulin resistance as binding of angiotensin II to AT₁ receptors inhibits insulin signaling, decreasing the insulin-induced phosphorylation of the insulin receptor substrate 1 (IRS-1), and p85 binding to IRS-1. Angiotensin II also increases the production of O₂⁻ by the enzyme NADPH oxidase in VSMC in culture, and causes the release of inflammatory factors from vascular endothelial cells.^{98,99}

T cells have been shown to have a functional RAS with the ability to produce angiotensin II, which has a direct effect on T-cell function including activation, expression of tissue homing markers, modulation of NADPH oxidase activity, and the production of the proinflammatory cytokine TNF- α .¹⁰⁰ Jurewicz et al showed that T cells and natural killer (NK) cells have RAS components along with the ability to synthesize their own angiotensin II and deliver it to the sites of inflammation.¹⁰¹ A study conducted using Agtr1a^{-/-} mice (mice that lack AT_{1A} receptors for angiotensin II), found that the interaction between angiotensin II and AT_{1A} receptors on immune cells, causes the proliferation of lymphocytes.¹⁰² This suggests that the malfunctioning of the RAS associated with hypertension may be linked to the increased state of inflammation found in humans and animals with hypertension.

ROLE OF THE SYMPATHETIC NERVOUS SYSTEM AND HYPERTENSION

There is strong evidence for a role of increased sympathetic nervous system (SNS) activity in the pathogenesis of hypertension in humans and animal models of hypertension.¹⁰³ This role is greater in magnitude when hypertension and obesity coexist.¹⁰⁴ Catecholamines are the leading candidates to be both the pressor mechanism that initiates the rise in blood pressure and the trophic mechanism that maintains hypertension via vascular hypertrophy. The data suggest that the number of α -adrenergic receptors in hypertension is regulated by catecholamines, dietary salt intake, and genetic factors. Increase in the number of renal α -adrenergic receptors are also involved in genetic forms of essential hypertension as shown in SHR and Dahl salt-sensitive rats.¹⁰⁵ In addition, the results of recent studies in SHR strongly suggest that central sympathetic outflow is increased in hypertension. This increase in sympa-

thetic activity in peripheral vascular tissue, renal vessels, and central nervous system (CNS) is due to increased oxidative stress in these animal models of hypertension.¹⁰⁶

As discussed above, insulin resistance leads to oxidative stress which may lead to an increase in SNS activity in hypertension. The SNS stimulates the release of proinflammatory cytokines, and sympathetic nerves may also serve as a source of cytokines. There is also experimental evidence that proinflammatory cytokines may activate the SNS.¹⁰⁷ Importantly, many immune cells including macrophages, dendritic cells, and lymphocytes, express adrenergic receptors and can receive signals from the SNS, and leads to increased formation of proinflammatory cytokines, which affect vascular function and endothelium-derived factors involved in blood pressure regulation.¹⁰⁸⁻¹¹⁰

ROLE OF THE IMMUNE SYSTEM IN HYPERTENSION

Animal Studies

Animal studies have demonstrated that there is a positive relationship between the development of essential hypertension and activation of the immune system (Table 2). Schmid-Schönbein et al and Arndt et al showed that total leukocyte count was 50 to 100% higher in hypertensive SHR, a genetic model of hypertension, compared with Wistar Kyoto (WKY) rats, as well, the number of circulating monocytes, activated monocytes, and lymphocytes were also increased in SHR.^{111,112} Rodriguez-Iturbe et al reported that the renal infiltration of lymphocytes and macrophages was significantly correlated with systolic blood pressure (SBP) in SHR.¹¹³

RAG1^{-/-} knockout mice, mice lacking T and B cells, did not show signs of hypertension following angiotensin II administration. When the missing B cells were replaced from healthy donors it did not restore the ability of animals to become hypertensive following angiotensin II administration. However, the transfer of normal T cells resulted in the development of hypertension and increased vascular O₂⁻ response to angiotensin II infusion.¹¹⁴ It was suggested that T cells are of primary importance in the development of angiotensin II-induced hypertension. Further evidence that T cells are involved came from a study by Bataillard et al who used thymectomized Lyon strain rats.¹¹⁵ The increase in blood pressure response to angiotensin I and phenylephrine observed in Lyon hypertensive rats was abolished by neonatal thymectomy. Histological study showed that kidneys from Lyon hypertensive rats showed arterial wall hypertrophy, segmental hyalinization of the glomeruli, and infiltration by mononuclear cells. All of these features of renal injury were reduced in thymectomized rats. The response of isolated perfused

Table 2 Relationship between the Immune System and Hypertension in Animal Models of Hypertension

Animal Model	Investigation	Results	Reference
Uninephrectomized SHR	Treated with lisinopril (5 mg/kg/d) or ICI8731 (ACE inhibitor) (5 mg/kg/d). Immune cell infiltration determined by immunostaining.	Lisinopril and ICI 8731 normalized BP and significantly reduced infiltrating macrophage and T cells in the kidney of SHR compared with control rats at 2, 4, and 6 mo.	Geiger et al, 1997 ¹⁶²
SHR	Given 20 mg/kg body weight MMF or vehicle for 4 wk. BP measured. Immunohistology used to quantify immune cells. O ₂ ⁻ production measured in renal cells.	MMF normalized BP, reduced lymphocyte and macrophage infiltration, and reduced oxidative stress.	Rodríguez-Iturbe et al, 2002 ¹¹⁹
SHR	Fed regular diet or antioxidant-rich test diet for 7 mo. SBP, plasma H ₂ O ₂ and level of immune cell infiltration was measured.	SBP was reduced. Infiltration of lymphocytes, macrophages and angiotensin II positive cells significantly correlated with SBP.	Rodríguez-Iturbe et al, 2003 ¹¹³
DSS rats	Fed 4.0% NaCl diet +30 mg/kg/d MMF for 3 wk. Renal tissue analyzed for cell-specific marks of T and B cells.	MMF decreased MAP and cell-specific markers of T and B cells by 50–60% in the kidneys.	Mattson et al, 2006 ¹²²
SHR	Received 200 mg/kg/d NAC and 250 mg/kg/d Tempol (superoxide dismutase mimetic) intraperitoneally for 1 wk. Renal inflammation and oxidative stress measured in renal tissue.	NAC and Tempol did not reduce BP, but did decrease oxidative stress and macrophage infiltration.	Biswas & de Faria, 2007 ²⁴⁷
SD rats	Angiotensin II infusion (435 ng/kg/min) for 2 wk, plus 30 mg/kg/d MMF. Fed 4% NaCl diet for 3 wk. Measured BP and interstitial inflammation.	MMF had no effect on BP, but MMF did decrease interstitial inflammation.	Franco et al, 2007 ²⁴⁸
RAG1 ^{-/-} mice	Angiotensin II infusion (490 ng/min/kg) for 14 d. BP measured. Adoptive transfer of T and B cells.	Angiotensin II-induced hypertension was blunted in RAG ^{-/-} mice. Transfer of B cells had little effect on BP. Transfer of T cells restored hypertension and increased vascular O ₂ ⁻ response to angiotensin II.	Guzik et al, 2007 ¹¹⁴
DSS rats	Received 8% Na, 8% Na+ MMF (20 mg/kg body weight), 0.3% Na or 0.3% Na+ MMF diet for 5 wks. Measured arterial pressure, monocyte/macrophage infiltration and renal NF-κB activation.	MMF significantly decreased arterial pressure, decreased renal NF-B and renal monocyte/macrophage infiltration.	Tian et al, 2007 ¹²⁰
Agtr1a ^{-/-} mice	Angiotensin II infusion (100 ng/kg/d)/ Fed 6% NaCl diet + 100 mg/kg/d MMF for 28 days. Measured SBP and renal lymphocyte infiltration.	MMF reduced renal lymphocyte infiltration but had no effect on BP.	Crowley et al, 2008 ²⁴⁹

SHR, spontaneously hypertensive rats; ACE, angiotensin converting enzyme; BP, blood pressure; MMF, mycophenolate mofetil; SBP, systolic blood pressure; DSS rats, DOCA salt-sensitive rats; MAP, mean arterial pressure; NAC, N-acetylcysteine; SD rats, Sprague-Dawley rats; RAG^{-/-} mice, T- and B-cell deficient mice; NF-B, nuclear factor B.

kidneys from thymectomized rats showed improved renal perfusion flow and sodium excretion with a decrease in renal vascular resistance. They suggested that the pathological importance of thymus-dependent immunological reactions in these animals by providing evidence for a possible role of T cells in the abnormal vascular responsiveness to pressor agents, in the decreased pressure natriuresis, and in the formation of glomerular lesions that accompany hypertension in this model.¹¹⁵

The evidence for the involvement of monocytes and macrophages in the development of hypertension has also been supported from observations of changes in peripheral-blood monocytes in several different animal models of hypertension.¹¹⁶ The major site of cellular infiltration in renin-angiotensin-aldosterone-induced hypertension appears to be the kidney. This cellular infiltration appears to dissipate following therapeutic treatment which results in normalization of blood pressure and associated adverse renal changes. Angiotensin II has also been shown to cause the release of the chemokine MCP-1 by smooth muscle cells from male Sprague-Dawley rats, which then results in the migration and activation of macrophages, and further production of proinflammatory cytokines by these cells.^{117,118}

Cyclophosphamide, an immunosuppressive agent that targets lymphoid cells and macrophages, was shown to reduce the blood pressure of hypertensive Lyon rats.¹¹⁵ Mycophenolate mofetil (MMF), an immunosuppressive drug, that targets lymphocyte proliferation, when given to hypertensive SHR,^{119,120} hypertensive obese Zucker rats,¹²¹ hypertensive Dahl salt-sensitive rats,¹²² salt-sensitive Sprague-Dawley rats,^{123,124} and hypertensive deoxycorticosterone acetate salt-treated rats,¹²⁵ suppressed lymphocyte function, reduced immune cell infiltration in kidney and lowered blood pressure.

By following various inflammatory markers, including changes in the levels of proinflammatory cytokines, IL-1, IL-6, TNF- α , and IFN- γ , chemokines such as MCP-1, CRP, and cellular adhesion molecules associated with inflammatory cell infiltration in salt-sensitive hypertensive rats, a strong relationship between the development of hypertension and inflammation was found.¹²⁶

Several animal studies have also documented increased level of systemic inflammation and vascular and renal damage due to fructose-induced hypertension.^{127,128} Gersch et al found higher levels of MCP-1 with increased number of ED-1 (monoclonal antibody against rat macrophages) positive cells indicating infiltration of macrophages in renal tissue of fructose-fed Sprague-Dawley rats.¹²⁹ Fructose feeding also induced serum and kidney inflammatory intercellular adhesion molecules 1 (ICAM-1) in male Sprague-Dawley rats.¹³⁰ Fructose also induced ICAM-1 expression in human aortic endothelial cells and MCP-1 in proximal tubular

renal cells in culture. In *in vitro* studies, mesenteric venules when incubated with fructose lead to increased number of adherent leukocytes to the venule. NO donor spermine (SNO) and lipoic acid treatment prevented these changes suggesting fructose induced inflammation via ROS generation. Tan et al showed that aortic expressions of ICAM and vascular cell adhesion molecule (VCAM) were significantly increased with chronic feeding of fructose in male Sprague-Dawley rats.¹³¹ This was also accompanied by the increased activity of the nuclear factor kappa-B (NF- κ B). Fructose-fed hypertensive rats also showed increased CD68-positive macrophages in the aortic wall. Felodipine, a calcium channel blocker, treatment decreased blood pressure, ICAM-1, VCAM-1, and macrophages in the aortic wall. NF- κ B activation was also inhibited suggesting that the modulation of vascular inflammatory response by calcium channel blocker in this model of hypertension.¹³¹ Nyby et al found increased vascular reactivity in mesenteric vessels and increased expression of ACE and angiotensin type 1 receptor, NOX4 subunit of NADPH oxidase, and VCAM in aortic tissue of fructose-fed hypertensive male CD:IGS rats.¹³² Captopril, an ACE inhibitor, treatment prevented these alterations. It was suggested that vascular RAS is upregulated in fructose-induced hypertension, leading to vascular dysfunction and oxidative stress.¹³²

RAGEs are expressed on mature T cells, monocytes, and macrophages, and are involved in inflammation.¹³³ Similarly AT₁ receptors, which are believed to be involved in chemotaxis, having structural features of the chemokine/rhodopsin receptor family, and excitatory functions, leading to the nonantigenic stimulation of inflammation processes.^{134,135} Increased levels of aldehyde conjugates/AGEs in aorta and kidney with adverse renal vascular changes has been shown in SHR, Dahl salt-sensitive, and fructose-induced hypertensive WKY rats. It is suggested that AGEs act on RAGEs of T cells, monocytes, and macrophages, leading to their direct activation resulting in production of inflammatory responses within the vascular tissue in hypertension. Furthermore, it has been shown that RAGEs are involved in the differentiation of T-helper-1T-cells which are proinflammatory.¹³⁶

Human Studies

In addition to the evidence from animal studies, the results from human studies also indicate a positive relationship between the development of hypertension and changes in the immune system (Table 3). Various markers of inflammation have been used to show the activation of the immune system in hypertension.

In newly diagnosed, never-treated hypertensive patients plasma CRP levels were significantly higher compared with healthy control patients.¹⁰ Also, plasma

Table 3 Relationship between the Immune System and Hypertension in Humans

Human Subjects	Investigation	Results	Reference
Untreated hypertensive males and females	BP and blood levels of TNF- α , IL-1 β , and IL-6 cytokine secretion from monocytes were measured.	Secretion of IL-1 β and TNF- α from monocytes was significantly increased in hypertensive patients after stimulation by lipopolysaccharide.	Dörffel et al, 1999 ¹⁴⁰
Healthy males	BP and plasma levels of sICAM-1 and IL-6 were measured.	BP was significantly associated with plasma levels of sICAM-1 and IL-6.	Chae et al, 2001 ¹¹
Hypertensive males and females	BP and blood levels of TNF- α , IL-1 β , IL-1ra, and IL-6 were determined.	Circulating concentration of TNF- α , IL-1 β , and IL-6 did not differ between hypertensive and normotensive patients. IL-1ra was higher in hypertensive patients.	Peeters et al, 2001 ²⁵⁰
Untreated hypertensive males and females	Blood PMN Ca ²⁺ _i concentration was measured.	Hypertensive patients had higher Ca ²⁺ _i concentration. PMN Ca ²⁺ _i concentration correlated with MAP indicating activation.	Sela et al, 2002 ²⁵¹
Hypertensive males and females	BP and serum concentration of CRP levels measured after 12 wk of treatment with 8 mg/d candesartan.	CRP concentration and BP was reduced by candesartan.	Dohi et al, 2003 ¹⁴³
Untreated hypertensive males and females	BP and plasma CRP levels concentration were measured.	Plasma CRP concentration was greater in hypertensive patients. CRP concentration associated with SBP and pulse pressure.	Schillaci et al, 2003 ¹⁰
Normotensive females	BP and plasma CRP levels were measured.	CRP levels were significantly associated with the risk of developing hypertension.	Sesso et al, 2003 ⁸
Normotensive males	BP and serum CRP concentration was measured.	CRP concentration was associated with risk of developing hypertension.	Niskanen et al, 2004 ¹³⁷
Hypertensive males	BP and in vitro monocyte TNF- α release after lipopolysaccharide stimulation was assessed.	Hypertensive males showed 11% higher lipopolysaccharide-stimulated TNF- α release than normotensive males.	Wirtz et al, 2004 ¹⁴¹
Males and females with mild arterial hypertension	BP and plasma levels of MCP-1, hsCRP, IL-6, TNF- α , and IL-10 were measured.	Hypertension was associated with increased levels of hsCRP, IL-6, TNF- α , and MCP-1.	Stumpf et al, 2005 ⁹
Young males and females	BP and CRP concentration measured at baseline, and 7 and 15 y follow-ups.	After adjusting for BMI, CRP concentration was not associated with hypertension in young adults.	Lakoski et al, 2006 ²⁵²
Healthy females	BP and IL-6 and CRP levels in blood were determined at baseline and at 10 y follow-up.	Only CRP levels were strongly associated with hypertension development.	Sesso et al, 2007 ¹³⁸
Untreated, hypertensive males and females	BP and serum levels of CXCL10 were measured.	Serum chemokine CXCL10 levels were increased in hypertensive patients.	Antonelli et al, 2008 ²⁵³
Normotensive males and females	BP and plasma concentration of IL-1 β and IL-1ra was measured.	IL-1 β and IL-1ra concentration were associated with increased risk of developing hypertension.	Vanhala et al, 2008 ¹⁴⁷

BP, blood pressure; TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; sICAM-1, intercellular adhesion molecule-1; IL-1ra, interleukin 1-receptor antagonist; PMN, polymorphonuclear leukocyte; MAP, mean arterial pressure; CRP, C-reactive protein; SBP, systolic blood pressure; MCP-1, monocyte chemoattractant protein-1; IL-10, interleukin-10; hsCRP, high sensitivity CRP; BMI, body mass index; CXCL10, CXC chemokine ligand 10.

CRP had a direct association with SBP and pulse pressure, but not diastolic blood pressure (DBP).¹⁰ In normotensive males and females, elevated plasma CRP levels were found to be significantly associated with an increased risk of future development of hypertension, suggesting that hypertension is an inflammatory disorder.^{8,137-139}

Dörffel et al investigated the involvement of peripheral blood monocytes in hypertension in untreated hypertensive patients.¹⁴⁰ It was found that secretion of IL-1 β , TNF- α , and IL-6, which are all proinflammatory cytokines produced mainly by activated monocytes or macrophages, was significantly increased in peripheral blood monocytes from hypertensive patients compared with healthy control patients after stimulation with lipopolysaccharide.^{140,141}

In studies, treatment with antihypertensive drugs angiotensin II receptor blockers, such as olmesartan, candesartan, and valsartan, have found that lowering of blood pressure was associated with attenuation of inflammatory markers. Fliser et al gave olmesartan to hypertensive patients and were able to reduce blood pressure and serum levels of high sensitivity CRP (hsCRP), TNF- α , IL-6, and MCP-1 after 6 weeks of therapy.¹⁴² Studies using candesartan demonstrated reduced inflammation in hypertensive patients shown by decreased blood levels of CRP as well as reduced blood pressure.^{143,144} Using valsartan, Manabe et al were able to significantly reduce blood pressure and serum levels of TNF- α and IL-6, but not CRP, in hypertensive patients.¹⁴⁵ By combining treatment of angiotensin II receptor blocker, olmesartan with the calcium channel blocker azelnidipine, Ishimitsu et al showed lowering of blood pressure and plasma levels of hsCRP in patients with essential hypertension.¹⁴⁶

Blood pressure has been found to be significantly associated with plasma levels of sICAM-1, IL-6, IL-1 β , and IL-1-receptor antagonist (IL-1ra) in healthy, normotensive males and females.^{9,11,147} These studies show that even in the early stages of hypertension, inflammatory pathways are activated.

Compared with salt-resistant patients, salt-sensitive hypertensives have been shown to have age-adjusted, increased levels of P-selectin and MCP-1 levels in serum. Salt-sensitive subjects also exhibited decreased serum levels of mature metalloproteinases type 1, 2, and 9, and tissue inhibition of metalloproteinase type 1. These serum markers of inflammation, especially selectins and chemokines, as well as markers of vascular remodeling are altered in salt-sensitive hypertension in humans.¹⁴⁸

Epidemiologic studies have linked fructose intake to the development of hypertension.^{149,150} Elevated levels of serum uric acid were also found in some of these studies. In *in vitro* studies, incubation of human epithelial tubular cells with fructose or uric acid showed increased production of MCP-1.¹⁵¹ The ability of fruc-

tose and uric acid to stimulate this proinflammatory cytokine was suggested to be the cause of tubular injury associated with hypertension.¹⁵¹ MCP-1 is an inflammatory cytokine that mediates macrophage accumulation in experimental models of chronic kidney disease. All this information supports the role of various immune cells in hypertension and associated vascular damage.

The Mechanism

Both genetic and lifestyle factors, including high dietary fructose and salt and/or deficiencies in antioxidant vitamins, have been linked to essential hypertension. Interaction of these factors leads to insulin resistance. The poor functioning of insulin downregulates GAPDH, slows glucose metabolism through the glycolytic pathway, and increases metabolism via the polyol pathway, resulting in a build-up of G3P and increased methylglyoxal with increased formation of AGEs.^{13,40,41} Oxidative stress can also increase the formation of methylglyoxal and other aldehydes through lipid peroxidation and auto-oxidation of glucose, and further contribute to the formation of AGEs.¹⁵² The modification of proteins, lipids, and enzymes by AGEs and oxidative stress, leads to alteration of RAS, decreased NO, endothelial dysfunction, increased vascular cytosolic free calcium, peripheral vascular resistance, and hypertension. The formation of AGEs also leads to the development of neoantigens and causes overactivation of the immune system. AGEs also act on T cells, monocytes, and macrophages, leading to their activation and formation of various proinflammatory cytokines and chemokines contributing to hypertension.

Analysis of perivascular tissues has shown that the perivascular fat, which is adjacent to the adventitia of the aorta and mesenteric vessels, becomes infiltrated with T cells during the development of hypertension.¹¹⁴ The infiltration of tissues by T cells is also accompanied by the infiltration by other leukocytes including NK cells and macrophages. In the absence of T cells, the perivascular accumulation of leukocytes does not occur, suggesting that T cells have a key role in mediating the cellular infiltration and overall inflammatory response.¹¹⁴ Based on a study conducted in IL-17^{-/-} mice (IL-17 deficient mice), Madhur et al suggested that T cells found in the perivascular fat release cytokines, such as IL-17, TNF- α , and IL-6, which diffuse into nearby VSMC and enhance O₂⁻ production, reduce endothelium-dependent vasodilation and promote vasoconstriction, thereby increasing blood pressure.¹⁵³ These cells are known as T-helper 17 (Th17) cells which regulate inflammatory responses.

When free fatty acids accumulate in adipocytes, it causes oxidative stress at the level of the mitochondrion, resulting in decreased triglycerol synthesis, increased lipolysis, and release of free fatty acids.¹⁵⁴ It has been

suggested that free fatty acids exert a proinflammatory action in adipocytes and macrophages through the activation of toll-like receptor 4 (TLR4), a pattern recognition receptor of the innate immunity which activates NF- κ B and stimulates the release of cytokines and modulates T cell function.^{155,156} This activation of NF- κ B has been demonstrated by coculturing adipocytes and macrophages.¹⁵⁵ As well, chronic activation of NF- κ B has been shown to be associated with insulin resistance in liver and skeletal muscle.^{157–159} The TLR4/NF- κ B pathway has been shown to mediate insulin resistance and vascular inflammation caused by saturated free fatty acids and diet-induced obesity in animal models.^{160,161} In TLR4 mutant mice (C3H/HeJ) fed a high fat diet or the inactivation of TLR4 result in increased insulin sensitivity and lipolysis.^{160,161} These observations strongly implicate over activity of immune system due to insulin resistance leading to vascular inflammation.

T cells have been shown to have a functional RAS with the ability to produce angiotensin II, which has a direct effect on T cell function including activation, expression of tissue homing markers, modulation of NADPH oxidase activity, and the production of TNF- α .¹⁰⁰ Jurewicz et al showed that T cells have RAS components and the ability to synthesize angiotensin II and deliver this to sites of inflammation.¹⁰¹ A study conducted using SHR showed that the treatment of SHR with lisinopril (ACE inhibitor) and ICI D8731 (angiotensin-II blocker) normalized blood pressure and significantly reduced infiltrating macrophages and T cells in renal tissue of these animals compared with the control rats at 2, 4, and 6 months.¹⁶²

ROLE OF CYTOKINES IN HYPERTENSION

Several proinflammatory cytokines have been linked, in both animal models and human studies, to hypertension. These cytokines include, but are not limited to, TNF- α , IL-1 β , IL-6, MCP-1, and more recently IL-17. These cytokines have also been shown to be upregulated by RAS, SNS, oxidative stress, and salt-sensitive mechanisms. Proinflammatory cytokines are responsible for the migration and activation of leukocytes and the resulting injury by infiltrating leukocytes into tissues.¹⁶³

Angiotensin II has been shown to possess inflammatory properties and its formation can lead to the local and systemic release of cytokines such as IL-6, IL-1, and TNF- α from vascular tissue and circulating leukocytes.^{164,165} Angiotensin II receptor blockers can reduce the levels of these cytokines and subsequently reduce their inflammatory affects.¹⁶⁶ Dendritic cell activation and production of IL-6, transforming growth factor- β (TGF- β), IL-22, and IL-23 are important in the differentiation and formation of Th17 cells and the eventual release of IL-17 and several chemokines, enhancing the infiltration of leukocytes into surrounding tissues and

subsequently vascular dysfunction and hypertension.^{153,167} Blocking the formation of angiotensin II with ACE inhibitors has been shown to lead to the production of T_{regs} and the modulation of Th1 and Th17 T cells in several inflammatory diseases with reversal of both inflammation and hypertension.¹⁶⁸

It has been proposed that activation of adrenergic receptors on cells, including macrophages and neutrophils, also mediate the production of proinflammatory cytokines. Use of adrenergic receptor blockade to reduce hypertension has been shown to modulate the production of these cytokines and reduce inflammatory responses in animal models and humans.^{169,170}

Oxidative stress, physiologically speaking, can lead to several processes that can result in hypertension. In particular, oxidative stress can lead to endothelial dysfunction, inflammatory cytokine release, and inflammation in vascular tissues. Cytokines produced include IL-6, IL-1, TNF- α , and MCP-1.¹⁶⁶ The benefits of exercise and diet on oxidative stress and reduction of proinflammatory cytokine levels has been shown to improve redox homeostasis, restore NO production, improve vascular function, and attenuate the development of hypertension in rat models.¹⁷¹

Finally salt-induced hypertension in Dahl salt-sensitive rats has been shown to result in elevated cytokine production involving IL-6, TNF- α , and MCP-1, and antioxidant vitamins such as E and C were shown to reduce TNF- α and MCP-1 and restore blood pressure and reduce leukocyte infiltration into the kidney and tissue damage.¹⁷² Some of these cytokines have also been shown to activate smooth muscle cells causing further release of cytokines, cell migration and proliferation, increased cytoplasmic calcium levels, and smooth muscle contraction resulting in hypertension.^{173–177}

MECHANISMS OF INFLAMMATORY CYTOKINES CAUSING HYPERTENSION

We have provided evidence that insulin resistance, activation of the RAS and adrenergic system, and oxidative stress all lead to increased blood pressure and formation of inflammatory cytokines, including IL-1, IL-6, TNF- α , and IL-17. In the following section we will provide available evidence that some of these cytokines positively affect these systems causing further increase in blood pressure.

CYTOKINES CAUSE INSULIN RESISTANCE, REGULATE eNOS, AND ENDOTHELIAL DYSFUNCTION

In healthy lean male volunteers, TNF- α infusion into the brachial artery inhibited insulin-mediated forearm blood flow response. This inhibition was larger during

insulin infusion but was not increased by eNOS inhibitors. It was suggested that TNF- α inhibition of the combined response to insulin and acetylcholine was likely mediated through inhibition of NO production.¹⁷⁸ In rodent models of obesity-associated insulin resistance, TNF- α is overexpressed in adipose tissue and treatment with anti-TNF- α antibodies or if performed in TNF- α -knockout animals or its receptor improves insulin sensitivity. TNF- α affects intracellular insulin signaling in adipose tissue, skeletal muscle, and other insulin responsive tissues by inhibiting protein kinase B (PKB) activity in the proximal part of the insulin-signaling pathway. A similar signaling pathway in vascular endothelium results in the production of NO which is necessary for insulin-stimulated vasodilation. In the rat model of obesity associated insulin resistance, vascular insulin signaling is compromised and in cultured endothelial cells TNF- α inhibits insulin signaling and NO production.

In three animal models of obesity (ob/ob mice, fa/fa rats, and high fat diet-induced obese [DIO] mice), eNOS expression and mitochondrial biogenesis was reduced in brown and white adipose tissue and soleus muscle. The genetic deletion of TNF- α receptor in these animal models restored eNOS expression and mitochondrial biogenesis. Furthermore, TNF- α downregulated eNOS expression and mitochondrial biogenesis in cultured white and brown adipocytes and muscle satellite cells of mice. NO donors prevented the induction of mitochondrial biogenesis.¹⁷⁹ These observations suggested that TNF- α is a causative factor in the induction of insulin resistance and induction of hypertension. Several potential mechanisms could explain the apparent mechanism of insulin resistance due to TNF- α . First, TNF- α can inhibit the expression of key genes in adipose tissue and muscle, such as glucose transporter 4 (GLUT 4), in relation to a decrease in glucose transport. Second, TNF- α has a direct effect on insulin signaling, such as to promote serine phosphorylation of the IRS, resulting in a net decrease in insulin receptor-mediated signaling which impairs glucose metabolism, eNOS, and vascular function.^{180,181}

IL-6 has also been shown to induce hepatic insulin resistance, and the loss of IL-6 selectively improves insulin action in obese mice. IL-6-induced insulin resistance is mediated in this model by increased expression of suppressor of cytokine signaling 3 (SOCS3), a protein that binds and inhibits the IRS protein and directs this to proteasomal degradation.^{182–186} Chronic treatment of mice with IL-6 reduced hepatic insulin receptor autophosphorylation and tyrosine phosphorylation of the insulin receptor-1 and 2 causing hepatic insulin resistance.¹⁸⁷ IL-6 treatment of human aortic endothelial cells decreased steady state levels of eNOS mRNA and protein.¹⁸⁸ IL-6 has also been shown to impair the insulin signaling pathway in human um-

bilical vein endothelial cells with inhibition of eNOS activity and vasodilatory effect.^{189,190}

Chronic treatment with IL-1 β reduced the insulin-induced glucose uptake in cultured mouse adipocytes. This inhibitory effect was due to a decrease in the amount of IRS-1. Activation of PKB after tyrosine kinase phosphorylation of IRS-1 and IRS-2 is a crucial step for insulin-induced glucose transport and GLUT-4 translocation. PKB-induced phosphorylation of AS160, a protein containing a Rab GTPase activating domain promotes GLUT-4 translocation to the plasma membrane. The decrease IRS-1 also resulted in a reduction in tyrosine phosphorylation, alteration of insulin-induced PKB activation, and AS160 phosphorylation. Targeting IRS-1 and IL-1 β impairs insulin signaling and action, and participates in concert with other cytokines, in the development of insulin resistance.¹⁹¹

CYTOKINES CAUSE ACTIVATION OF RAS AND OXIDATIVE STRESS

Angiotensinogen encodes the only known precursor of angiotensin II. The transcription of angiotensinogen is increased by TNF- α via NF- κ B-like protein binding to an inducible enhancer of an acute phase response element, C1 and C2 complex was shown using human hepatoblastoma cell line in earlier studies.¹⁹² Later using rat cardiac fibroblasts, TNF- α and IL-1 β were shown to upregulate angiotensin II type 1 A (AT_{1A}) receptor transcription and splicing with increase in AT₁ receptor protein levels.¹⁹³ More recently using a human hepatic cell line it was shown that ACE, angiotensinogen, and angiotensin AT₁ receptor mRNA expression were upregulated by TNF- α .¹⁹⁴

Stimulation of cultured rat aortic VSMC with IL-6 lead to upregulation of AT₁ receptor mRNA and protein expression. Preincubation of VSMC with IL-6 resulted in an enhanced angiotensin II-induced production of ROS. Treatment of mice with IL-6 for 18 days increased vascular AT₁ receptor expression and angiotensin II-induced vasoconstriction, enhanced vascular superoxide production, and impaired endothelium-dependent vasodilation. These effects were completely blocked in AT₁ receptor knockout mice (AT_{1A}^{-/-} mice). These studies suggest that upregulation of vascular AT₁ receptor expression in vitro and in vivo is involved in IL-6-induced propagation of oxidative stress and endothelial dysfunction.¹⁹⁵ In male IL-6 knockout mice, angiotensin II infusion and a 4% salt diet caused increased blood pressure which was 30 mm Hg lower than in control mice (IL-6 intact) with similar treatment suggesting that IL-6 contributes to angiotensin II-induced hypertension.¹⁹⁶

In another study, angiotensin II infusion response to cause increase in SBP was similar in both wild type and IL-17 knockout mice, but this increase was not

maintained in IL-17 knockout mice which was lower by 30 mm Hg by 4 weeks of angiotensin II infusion. Vessels from IL-17 knockout mice displayed preserved vascular function, decreased superoxide production, and reduced T-cell infiltration in aorta and mesenteric vessels in response to angiotensin II. Gene array analysis of cultured human aortic smooth muscle cells revealed that IL-17, in conjunction with TNF- α -modulated expression of >30 genes while TNF- α alone altered 6 genes. IL-17 levels in serum were significantly higher in diabetic hypertensive than diabetic normotensives.¹⁹⁷

In cerebral microvascular endothelial cells from newborn pigs and mice TNF- α treatment increased ROS generation, including hydrogen peroxide and superoxide radicals. The TNF- α response was inhibited by superoxide dismutase and catalase.¹⁹⁸ TNF- α has been shown to inhibit the vasodilator effect of insulin in cremaster resistance arteries.¹⁹⁹ In aorta from diabetic TNF- α null mice, acetylcholine-induced vasorelaxation was greater than in diabetic control mice. Incubation of aorta from diabetic control mice with TNF- α impaired endothelial-dependent vasorelaxation. Diabetic TNF- α null mice showed decreased superoxide radical production and NAD(P)H oxidase activity compared with diabetic control mice aorta.²⁰⁰

TNF- α treatment of human aortic VSMC in culture-induced proliferation was inhibited by Emodin, a natural anthraquinone compound that is present in many vegetables including cabbage, lettuce, rhubarb, and peas. Emodin has been reported to inhibit TNF- α -induced NF- κ B degradation in vascular endothelial cells. This report also provided evidence suggesting that Emodin inhibits TNF- α -induced human aortic smooth muscle cell proliferation via caspase and a mitochondrial-dependent apoptotic pathway. It is suggested that TNF- α may control the increase in blood pressure by stimulating VSMC proliferation observed in hypertension.²⁰¹ In a recent study, Apo e + IL-1R1 null mice were given a high fat diet for 8 weeks and then blood pressure and atherosclerosis development were measured. Apo e + IL-1R1 null mice had reduced blood pressure and significantly less atheroma than Apo e null control mice, thus suggesting a contribution of IL-1 in causing the increase in blood pressure.²⁰²

CYTOKINES CAUSE STIMULATION OF ADRENERGIC PATHWAYS

The effects of several proinflammatory cytokines have been studied for their activity on the adrenergic system. IL-6 was shown to stimulate both the CNS and SNS, which may result in hypertension.²⁰³ Administration of IL-6 leads to the elevation in heart rate and serum norepinephrine levels in humans.²⁰⁴ Both IL-1 α and IL-1 β were shown to stimulate production of epinephrine levels when added to primary cultures of dispersed rat

adrenal cells.²⁰⁵ Furthermore, in Sprague-Dawley rats, TNF- α administered intravenously or into the ipsilateral carotid artery activated the paraventricular nucleus of hypothalamus and rostral ventrolateral nucleus, two critical brain regions regulating sympathetic drive and this caused increased sympathetic activity, arterial pressure, and heart rate. Response to TNF- α was substantially reduced by injection of the cyclooxygenase inhibitor ketorolac into lateral ventricle. This suggested that systemic cytokines stimulate cardiovascular and renal vascular response via a prostaglandin-dependent mechanism operating at the hypothalamic level.²⁰⁶

We have illustrated above the mounting evidence that proinflammatory cytokines play a major role in not only causing the pathological changes that lead to hypertension but also act synergistically with other factors in enhancing and maintaining these changes (see Fig. 1). Thus mechanisms targeting inflammation or the cytokines that cause it may be beneficial in the prevention and treatment of hypertension. These could include the use of anticytokine antibodies or cytokine receptor antagonists, or small inhibitory molecules that target specific signaling pathways in cytokine activation sequences. Finally, diet may control and prevent the inflammatory process and reduce inflammation by modulating insulin resistance, RAS or adrenergic or cytokine-induced oxidative stress, or directly regulating proinflammatory cytokine production by normalizing glucose and lipid metabolism. The next section on diet and hypertension explores this possibility.

MODULATION OF THE IMMUNE SYSTEM AND HYPERTENSION WITH DIETARY ANTIOXIDANTS

There is increasing evidence showing that various foods and their nutrients have anti-inflammatory and antihypertensive effects (Fig 2). A study by Lopez-Garcia et al, demonstrated this using female participants from the Nurses' Health Study.²⁰⁷ Dietary intake was assessed using a food-frequency questionnaire and blood samples were taken to measure the levels of CRP, IL-6, E-selectin, sICAM-1, and sVCAM-1. It was found that women who consumed a diet high in fruits, vegetables, legumes, fish, poultry, and whole grains have lower plasma concentrations of CRP and E-selectin.²⁰⁷ Whereas those who consumed a diet high in red and processed meats, sweets, desserts, French fries, and refined grains resulted in a positive correlation between their diet and levels of CRP, IL-6, E-selectin, sICAM-1, and sVCAM-1.²⁰⁷

Studies that investigated the effect of specific foods or nutrients on inflammation and hypertension have found similar results. A study that investigated if fruit and vegetable intake were associated with markers of oxidative stress and inflammation in adolescent boys

Figure 2 Mechanism of antihypertensive and immunomodulatory action of dietary antioxidants.

Antioxidants work directly to neutralize reactive oxygen species (ROS) and indirectly by improving insulin resistance and decreasing aldehyde and advanced glycation end product (AGE) formation. This leads to normalization of the immune system, decreased inflammation, improved endothelial function, and decreased blood pressure. NO, nitric oxide.

and girls found that CRP levels were inversely associated with fruit intake, while IL-6 levels were inversely associated with vegetable and legume intake.²⁰⁸ Likewise, a study conducted using participants from the Washington County, MD, Prospective Study found that fruit, vegetable, and antioxidant intake was associated with lower risk of cardiovascular disease.²⁰⁹ As well, protein intake higher than currently recommended guidelines (1.2 g/kg/d) can help promote insulin sensitivity, NO production, reduce oxidative stress, and modulate immune functions.²¹⁰ Nuts have also been shown to protect against inflammation and insulin resistance due to components found in nuts such as magnesium, fiber, α -linolenic acid, arginine, antioxidants, and monounsaturated fatty acids.^{211,212} Fiber has also been shown to have an effect on CRP levels. A study that measured CRP levels in humans given either diet naturally rich in fiber (30 g/d) or a

fiber-supplemented diet (30 g/d) found that both diets can reduce levels of CRP.²¹³

Supplementation with antioxidants, including vitamins C, E, or B₆, thiols such as lipoic acid and cysteine, have been shown to lower blood pressure in animal models and humans with essential hypertension (Fig. 2).⁵² These vitamins and oxidants have been shown to have immunomodulatory and anti-inflammatory effects. In a recent Boston Puerto Rican Health Study, a longitudinal study on stress, nutrition, health, and aging, a strong inverse association between plasma pyridoxal-5-phosphate (PLP), a marker of dietary vitamin B₆ and CRP, was found in 1205 (age range, 45 to 75 years) Puerto Ricans living in Massachusetts. Low plasma PLP was also significantly associated with higher urinary 8-hydroxydeoxyguanosine (8-HdG), a marker of oxidative DNA damage.²¹⁴ The measurement of PLP and CRP levels in blood samples collected from participants in the 2000 to 2004 NHANES study also found an inverse relationship of these two parameters in the general U.S. population. This study also indicated the protective effect of dietary vitamin B₆ on tissue inflammation.²¹⁵ In a cross-sectional study using data from monitoring of trends and determinants in cardiovascular disease (MONICA/KORA) survey 1994/95 from population-based samples of 2045 women and 2172 men (age range, 25 to 72 years), intake of dietary supplements containing vitamins and supplements was associated with lower CRP levels in women.²¹⁶ Especially vitamin E in combination with other vitamins such as C, B₁, B₂, B₆, and B₁₂, niacin, folic acid, and pantothenic acid and selenium, was significantly associated with lower CRP levels.²¹⁶

In human subjects and animal models, α -tocopherol, a major form of vitamin E, has been shown to have potent anti-inflammatory properties. α -tocopherol supplementation in human subjects and animal models has been shown to decrease lipid peroxidation and superoxide (O_2^-) production by impairing the assembly of NADPH oxidase as well as by decreasing the expression of scavenger receptors that are important in the formation of foam cells. α -Tocopherol has been shown to decrease the release of proinflammatory cytokines, the chemokine IL-8, and plasminogen activator inhibitor, as well as decrease the adhesion of monocytes to endothelium. It has also been shown to decrease CRP levels in patients with cardiovascular disease.²¹⁷ In a randomized, placebo-controlled double-blind study, a combination of gamma-tocopherol plus α -tocopherol (800 mg each per day for 6 weeks) decreased plasma levels of CRP, TNF, and lipid peroxides in subjects with metabolic syndrome.²¹⁸ Wu et al reported anti-inflammatory properties of tocotrienol-rich fraction (vitamin E) of palm oil in vitro studies using human monocytic cells by measuring lipopolysaccharide-induced iNOS, COX-2, and NF- κ B expression.²¹⁹

Dietary vitamin E and plasma vitamin C were significantly and inversely associated with the concentration of CRP level and tissue plasminogen activation in men aged 40 to 59 years in the British Regional Heart Study.²²⁰ In another study, a negative relationship between dietary vitamin C intake and the levels of PGF2d, CRP, IL-6, and F2-isoprostanes was observed.²²¹ The result of both in vivo and in vitro studies in animal models of inflammation and dietary intake of vitamin C in humans have suggested an anti-inflammatory and immunomodulatory effect of vitamin C.^{222,223}

Dietary supplementation of cysteine and *N*-acetylcysteine (NAC) has been reported to have anti-inflammatory and immunomodulatory effects in humans and in animal models of inflammatory disease. L-cysteine supplementation lowered blood glucose, glycated hemoglobin, CRP, MCP-1, and oxidative stress and inhibited NF- κ B activation in the livers of Zucker diabetic rats.²²⁴ NAC treatment minimized macrophage/microglia activation, pericyte loss, and endothelial/perivascular cell changes in streptozotocin-induced diabetic rats. TNF- α , a plasma marker of inflammation, was also lowered with NAC.²²⁵ L-cysteine supplementation attenuated weight loss and intestinal permeability, reduced local chemokine expression and neutrophil influx, and markedly improved colon histology in porcine models of colitis. Furthermore, cysteine significantly reduced the expression of proinflammatory cytokines including TNF- α , IL-6, IL-12 p40, IL-1 β in the colon. Infiltration of lymphocytes into the mucosal tissue assessed by markers of myeloperoxidase activity was also reduced by L-cysteine.²²⁶ In a prospective randomized study in burn injury patients, NAC treatment decreased expression of CD markers CD11a, CD18, and CD97 on the granulocytes and CD49d on lymphocytes and monocytes. These adhesion molecules play a role in tight cell to cell (leucocyte-endothelium) interaction and are involved in lymphocyte, eosinophil, and monocyte adhesion and migration.²²⁷

Lipoic acid is an antioxidant that is highly effective in treating the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Several laboratories have demonstrated that lipoic acid can completely suppress clinical and pathological EAE by preventing migration of pathogenic T cells and monocytes into the spinal cord.^{228–230} This is associated with decreased expression of cellular adhesion markers ICAM-1 and VCAM-1 on renal endothelial cells.²³¹ Lipoic acid has also been shown to decrease the migration of human T cells in vitro. A pilot study in multiple sclerosis patients demonstrated that oral administration of lipoic acid for 2 weeks resulted in decreased serum levels of matrix metalloproteinase-9 and sICAM-1, which are markers of inflammatory activity.²³² In in vitro studies, lipoic acid treatment inhibited IL-6 and IL-17 production and decreased T-cell proliferation and

activation.²³³ Other in vitro experiments have found lipoic acid to increase cAMP in T cells and NK cells by stimulating adenylate cyclase, which suggested that some of the dramatic effect of lipoic acid on EAE was due to its effect on increase in cyclic AMP in these cells. Lipoic acid has also been shown to have a protective effect in trinitrobenzene sulfonic acid-induced colitis in Sprague-Dawley rats. Treatment with lipoic acid in this animal model normalized the elevated levels of plasma proinflammatory cytokine TNF- α , IL-1 β , and IL-6. Lipoic acid also had a modulatory effect on accumulation of inflammatory cells in colonic tissue in patients with colitis.²³⁴

Two studies in 1997 and 2001 that investigated Dietary Approaches to Stop Hypertension (DASH) demonstrated that a diet rich in fruit, vegetables, and low-fat dairy products, that includes whole grains, modest portions of lean meat and nuts, and that is low in salt and sugar significantly reduced blood pressure in both hypertensive and normotensive people.^{235,236} Fruits, nuts, vegetables, and whole grains are major sources of antioxidant vitamins including vitamins B, C, and E, as well as coenzyme Q10 and lipoic acid.²³⁷ Lean meats and low-fat dairy may provide ample amounts of dietary protein containing the antioxidant amino acid cysteine. These nutrients and antioxidants may be important contributing factors to the antihypertensive, anti-inflammatory, and immunomodulatory effects of these diets (Fig. 2).

CONCLUSION

Essential hypertension is caused by a combination of genetic and/or acquired metabolic defects that interact with environmental factors such as diet and lifestyle, which lead to the development of insulin resistance, cytokine imbalance, and AGEs formation. This results in altered RAS, oxidative stress, decreased NO bioavailability, reduced sodium excretion, endothelial dysfunction, increased vascular cytosolic free calcium, peripheral vascular resistance, and hypertension. Evidence from studies in hypertensive humans and animal models of hypertension has suggested a link between altered immune system activity initiated through insulin resistance which contributes to hypertension and associated vascular damage. Release of proinflammatory cytokines leads to endothelial dysfunction and vascular damage associated with hypertension and increased vascular ROS production, which further lead to the recruitment of immune cells, such as T cells and macrophages, to blood vessel walls and infiltration into target organs, such as the kidney, production of more proinflammatory cytokines and tissue injury. There is strong evidence in animal and human studies that diets rich in antioxidants modulate insulin resistance, oxidative damage, overactive immune system, and vascular inflammation, and

thus lowers blood pressure. A diet that is low in sugar, fat, and salt, and includes an abundance of fruits and vegetables which is rich in antioxidants and adequate amounts of proteins similar to the DASH diet will prevent hypertension, inflammation, and other associated vascular damage.

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