The Role of Oxidative Stress in the Metabolic Syndrome

Adam Whaley-Connell, DO, MSPH, FAHA, FACP, FASN, FASH,[1] Peter A. McCullough, MD, MPH, FACC, FACP, FAHA, FCCP,[2] James R. Sowers, MD[1]

[1]Diabetes and Cardiovascular Center, Harry S. Truman VA Medical Center, and the University of Missouri-Columbia School of Medicine, Columbia, MO; [2]Department of Medicine, Cardiology Section, St. John Providence Health System, Providence Park Hospital, Novi, MI

Loss of reduction-oxidation (redox) homeostasis and generation of excess free oxygen radicals play an important role in the pathogenesis of diabetes, hypertension, and consequent cardiovascular disease. Reactive oxygen species are integral in routine in physiologic mechanisms. However, loss of redox homeostasis contributes to proinflammatory and profibrotic pathways that promote impairments in insulin metabolic signaling, reduced endothelial-mediated vasorelaxation, and associated cardiovascular and renal structural and functional abnormalities. Redox control of metabolic function is a dynamic process with reversible pro- and anti-free radical processes. Labile iron is necessary for the catalysis of superoxide anion, hydrogen peroxide, and the generation of the damaging hydroxyl radical. Acute hypoxia and cellular damage in cardiovascular tissue liberate larger amounts of cytosolic and extracellular iron that is poorly liganded; thus, large increases in the generation of oxygen free radicals are possible, causing tissue damage. The understanding of iron and the imbalance of redox homeostasis within the vasculature is integral in hypertension and progression of metabolic dysregulation that contributes to insulin resistance, endothelial dysfunction, and cardiovascular and kidney disease.

[Rev Cardiovasc Med. *2011;12(1):21-29 doi: 10.3909/ricm0555*]

Key words: Metabolic syndrome • Oxidative stress • Labile iron • Neutrophil-associated lipocalin • Galectin-3

The prevalence of cardiovascular disease (CVD) and chronic kidney disease (CKD) is progressively increasing in the United States, a phenomenon that closely parallels the epidemics of obesity and the metabolic syndrome.[1-12] Approximately 70 million adults in the United States are obese and another 70 million have hypertension.[1-5] Data from the National Health and Nutrition Examination Survey indicate that the prevalence of hypertension increases progressively with increasing body mass index (BMI).[3-5] Recent work supports the notion that obese patients exhibit more frequent impairments of insulin metabolic signaling, dyslipidemia, microalbuminuria, and hypertension—all components of the metabolic syndrome.[6-9]

The metabolic syndrome is a complex clinical syndrome that is associated with a substantial risk for CVD events such as stroke, congestive heart failure, CKD, and overall mortality.[1-9] CVD in the United States represents a continuing crisis of epidemic proportion. There are an estimated 64.4 million people in the United States who suffer from CVD.[10-12] CVD is often perceived as the disease of the elderly, but recent data suggest approximately 50% of CVD diagnoses and 15% of CVD deaths are in patients younger than 65 years. In addition, the metabolic syndrome has been shown to be independently related to CKD and to the development of acute kidney injury (AKI). CKD is an accepted CVD risk state, meaning that CKD contributes to incident and prevalent CVD. In almost every case, CKD renders the treatment of CVD more difficult and more hazardous. Thus, the metabolic syndrome poses a unique cardiorenal risk on a chronic basis, which is the set-up for acute CVD and AKI events that lead to excesses in end-stage renal disease and premature mortality.[13] The growing incidence of CKD and CVD among the younger population can be attributed to the increasing incidence of obesity in the United States.

Obesity is characterized by the presence of insulin resistance and is now known to herald even greater CVD risk.[6-13] As a result of excess adiposity, insulin resistance is central to the metabolic syndrome and implies impaired biologic and physiologic response to insulin. Insulin resistance is often accompanied by decreased insulin-dependent metabolic signaling and impaired glucose transport and utilization in skeletal muscle, liver, and fat.[14,15] Fatty steatosis (or excesses in intracellular fatty acids and triglycerides), as well as the presence of visceral adipose tissue, are cardinal features of insulin resistance. Thus, accentuation of some actions of insulin metabolic effects with concurrent resistance gives rise to diverse clinical manifestations and sequelae of the metabolic syndrome.

A chronic low-level, proinflammatory/pro-oxidative state often accompanies insulin resistance. As a result of excess adiposity, subclinical elevations of proinflammatory markers from the liver and adipose tissue (including interleukin-6, C-reactive protein, plasminogen activator inhibitor-1, and fibrinogen) are linked with the development of impaired insulin-dependent glucose utilization.[15] The presence of insulin resistance is also associated with inappropriate activation of the renin-angiotensin-aldosterone system (RAAS) due to the presence of angiotensinogen from adipose tissue.[16] Mounting evidence supports the concept that angiotensin II (Ang II), acting through its type 1 receptor (AT_1R), induces insulin resistance by inhibiting the actions of insulin in vascular and skeletal muscle tissue.[17-20] It does so by promoting oxidative stress and endothelial dysfunction through increased nicotinamide adenine dinucleotide H (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and dependent generation of labile iron-dependent reactive oxygen species (ROS). Thereby, oxidative stress contributes to alterations in redox-sensitive insulin-dependent signaling through phosphatidylinositol-3-kinase and protein kinase B signaling. These changes promote metabolic dysregulation observed in obesity that manifests as insulin resistance and hypertension, both prominent features of the metabolic syndrome.

Herein we describe the important mechanisms that contribute to the generation of oxidative stress in the vasculature and kidney that promote endothelial dysfunction and metabolic dysregulation, which contribute to the metabolic syndrome.

A chronic low-level, proinflammatory/pro-oxidative state often accompanies insulin resistance.

The Importance of Insulin Resistance

Obese subjects are noted to have elevated levels of insulin, which is required to maintain glucose and fatty acid metabolism, and often have insulin resistance in traditional insulin-sensitive tissues such as skeletal muscle, liver, and adipose tissue, but also in nontraditional insulin-sensitive tissues such as heart, aorta, and kidney.[21-24] In the insulin-resistant state there is impaired biologic and physiologic response to insulin in tissue. However, this resistance to insulin is not uniform in all tissues. This altered response is often accompanied by decreased insulin-mediated metabolic signaling and impaired glucose transport/utilization, as well as impaired nitric oxide (NO)-induced vasodilatation in skeletal muscle and cardiovascular tissue.[25-28] Thus, accentuation of some actions of insulin metabolic effects, with parallel resistance to other actions, gives rise to diverse clinical manifestations associated with the metabolic syndrome. Sequelae that result from insulin actions on activation of the sympathetic nervous system include salt sensitivity, inappropriate activation of the RAAS, and increases in inflammation and oxidative stress.

There is also mounting evidence that supports the idea that excess

insulin (eg, hyperinsulinemia) as a compensatory mechanism in insulin resistance contributes to further adipose tissue deposition. In several models, excesses of insulin promote available glucose and fatty acid transport into adipocytes, therefore increasing cell size and fat mass. In addition, insulin and stimulated insulin-like growth factors drive the conversion of preadipocytes to mature and growing adipocytes, ready to accumulate more substrate and begin producing adipokines, which worsen the insulin resistance and proinflammatory state.[29]

Role of RAAS in Oxidative Stress and the Metabolic Syndrome

Despite a state of relative volume expansion and salt sensitivity, the RAAS is inappropriately activated in obese individuals who display insulin resistance. Recent work suggests this effect is due to elevated levels of circulating aldosterone associated with increases in visceral adiposity.[30-35] Preclinical and clinical reports support this relationship between BMI and elevations in RAAS markers such as plasma renin activity, plasma angiotensin-converting enzyme (ACE), and circulating Ang II.[34-39]

Mounting data support that adipose tissue displays a local RAAS. Data support that visceral tissue has higher levels of angiotensinogen (AOGEN) than subcutaneous tissue, and also expresses the AT_1R, which suggests an autocrine-paracrine role for visceral adipose tissue.[40] Some investigators postulate that adipose tissue RAAS may actually regulate systemic blood pressure to some degree. This is best illustrated in studies in transgenic AOGEN mice in which production is limited to adipose tissue—support that AOGEN produced in fat cells can enter the circulation and further regulate blood pressure and sodium homeostasis.[40,41]

Traditional doctrine has been that Ang II is the main effector peptide of the RAAS and signals through the AT_1R and angiotensin type 2 receptor (AT_2R) with second messenger signaling that classically oppose each other. Ang II signaling through the AT_1R contributes to the deleterious effects and vasoconstrictor actions of Ang II that are opposed by binding of the AT_2R under routine physiologic conditions. It should also be noted that there are many other effects of Ang II generally considered to have endocrine, exocrine, paracrine, and autocrine effects. Ligand receptor binding of the Ang receptors also leads to G protein versus non–G protein-mediated effects. G protein-mediated effects may be mediated through phospholipase C with formation of 1,4,5-inositol and diacylglycerol, whereas non–G protein-mediated effects happen through stimulation of tyrosine kinases. Both of these pathways eventually contribute to activation of components of NADPH oxidase and other metabolic oxidases, and generation of superoxide anion ($\cdot O_2^-$) and other free radicals observed in the metabolic syndrome.[34-38]

Although Ang II is classically thought to exert many of the profibrotic, proinflammatory, and prooxidative effects, recent work highlights what may be more important—direct actions mediated through Ang II stimulation of aldosterone production. It is now increasingly recognized that aldosterone has actions that are independent of Ang II stimulation. Most notably are aldosterone actions that augment AT_1R signaling that promote vascular production of oxidative stress through the enzyme complex NADPH oxidase. Aldosterone has been shown to act both directly and indirectly through potentiation of an Ang II effect that contributes to impairments in endothelium-dependent relaxation.[15,40,41] This is associated with increased vascular oxidative stress resulting in reductions in bioavailable NO.

Aldosterone independently promotes vascular injury with attendant increases in collagen synthesis and fibrosis, resulting in arterial stiffness, left ventricular hypertrophy, and renal fibrosis.[40-46] As alluded to above, these maladaptive changes are augmented through a relative cross-talk between aldosterone and Ang II. Analogous to Ang II, aldosterone acts via rapid, nongenomic actions, mediating endothelial dysfunction and cardiac remodeling in animal models and in humans.[42,43] Aldosterone stimulates pericytes and macrophages to secrete galectin-3, an important paracrine factor that signals local fibroblasts to secrete procollagen, which is then cross-linked to collagen in the extracellular matrix.[44] In rodent models of both tissue Ang II and aldosterone excess, antagonism of the target of aldosterone (eg, the mineralocorticoid receptor) has been shown to reduce cardiac tissue oxidative stress through reductions in NADPH oxidase with improvements in interstitial fibrosis, remodeling, hypertrophy, and, ultimately, diastolic function.[45,46]

Aldosterone independently promotes vascular injury with attendant increases in collagen synthesis and fibrosis, resulting in arterial stiffness, left ventricular hypertrophy, and renal fibrosis.

Vascular NADPH Oxidase

The vascular enzyme complex NADPH oxidase, which is induced in

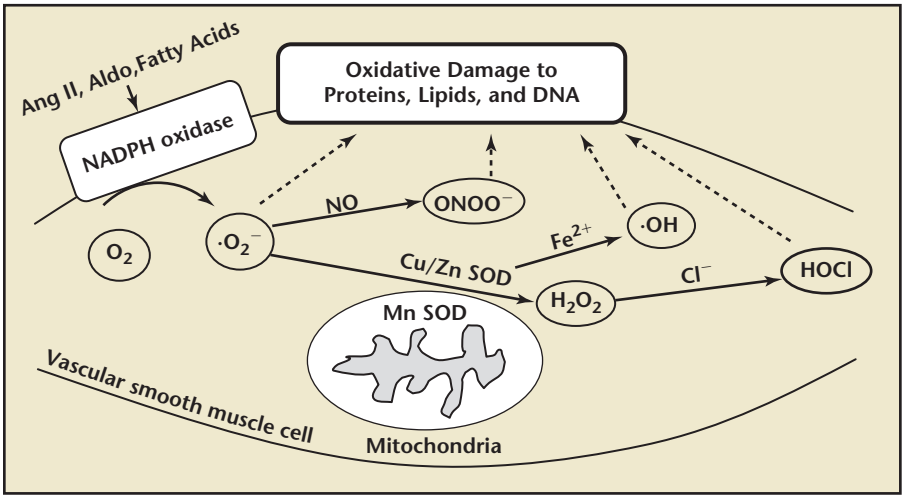


Figure 1. *The role of oxidant stress in endothelial dysfunction and vascular tissue injury. Ang II, angiotensin II; Aldo, aldosterone; GSH, glutathione; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; SOD, superoxide dismutase.*

part by fatty acids, as well as Ang II and aldosterone, is largely considered the major source of ROS production in cardiovascular and kidney tissue (Figure 1).[23-25] NADPH oxidase represents the enzyme responsible for much of the generation of $\cdot O_2^-$ in cardiovascular tissue that contributes to endothelial dysfunction, in addition to cardiac and renal tissue remodeling.[13] The enzyme complex is composed of several membrane and cytosolic subunits that mobilize and activate under various agonists, such as fatty acids, Ang II, and aldosterone.[47,48] The membrane complex consists of two subunits, gp91^{phox} (now referred to as NOX2) and p22^{phox}, which together form a heterodimer known as flavocytochrome b_{558}. The cytosolic subunits include p47^{phox}, which is considered the strategic organizer subunit when activated.[49] Another cytosolic subunit, p67^{phox}, facilitates the transfer of the hydride ion from NADPH to flavin adenine dinucleotide upon interaction with the small G-protein Rac through the tetratricopeptide domain. Rac exists in an inactive state when bound to guanosine diphosphate; however, phosphorylation results in activation (eg, Rac-guanosine triphosphate [GTP]) and translocation to the membrane complex. p40^{phox} then binds to p67^{phox} and facilitates the assembly of p47^{phox}-p67^{phox} at the membrane. The final transfer of an electron to form $\cdot O_2^-$ is facilitated by two heme groups present within the NADPH complex.

Reactive Oxygen Species

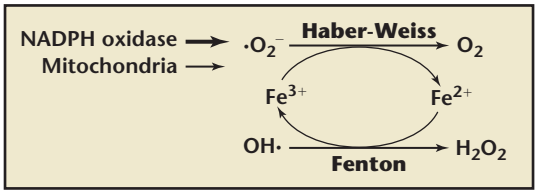
ROS are important to routine physiologic and cellular functions.[13,14] However, it is the imbalance in the metabolism of oxygen and resultant production of excess oxygen-derived free radicals that impose an "oxidant stress" in vascular tissue. There are several steps in generation of ROS, $\cdot O_2^-$ being highly damaging to vascular tissue (Figure 2). Oxygen may be reduced to form $\cdot O_2^-$ (reaction 1). $\cdot O_2^-$ can then either dismutate or go through another reduction reaction by superoxide dismutase to form H_2O_2 (reaction 2). H_2O_2 can then be reduced through several pathways. In total, the net Fritz-Haber reaction (reaction 3) is slow, and in the presence of reduced transition metals such as ferric iron (Fe_3^+), a Haber-Weiss reaction results in the formation of the highly damaging hydroxyl radical (OH•) from $\cdot O_2^-$ (reaction 4). In the presence of ferrous iron (Fe_2^+), a Fenton-type reaction converts H_2O_2 to the highly damaging OH• (reaction 5) (Figure 3). Further reduction of OH^2 leads to the formation of H_2O. H_2O_2 reacts with myeloperoxidase in the presence of chlorine (Cl^-) to form hypochlorous acid (reaction 6). This important acid is a potent oxidant known to activate neutrophil-derived collagenase and gelatinase.

The generation of ROS and free oxygen radicals has been shown to regulate pathways involved in cell signaling, apoptotic pathways, cell and tissue growth, and salt and

Figure 2. *Pathways of generation of reactive oxygen species.*

Reaction 1 → $O_2 + e^- \rightarrow \cdot O_2^-$
Reaction 2 → $\cdot O_2^- + \cdot O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$
Reaction 3 → $H_2O_2 + \cdot O2^- \rightarrow OH^- + OH\cdot + O_2$
Reaction 4 → $Fe^{3+} + \bullet O2^- \rightarrow Fe^{2+} + O_2$
Reaction 5 → $H_2O_2 + Fe_2^+ \rightarrow Fe_3^+ + OH^- + OH\cdot$
Reaction 6 → $H_2O_2 + Cl^- + H^+ \leftrightarrow H_2O + HOCl$

Figure 3. *The Haber-Weiss reaction generates hydroxyl radicals from hydrogen peroxide and superoxide as a source of reaction oxygen species and involves reduction of ferric ion to ferrous. NADPH, nicotinamide adenine dinucleotide phosphate.*



fluid homeostasis. Free radicals such as $\cdot O_2^-$, hydroxyl moiety ($\cdot$OH), hypochlorite (ClO^-), peroxynitrite ($ONOO^-$), and protein and lipid species, are short lived and scavenged by a series of antioxidant enzymes. However, repeated agonist exposure generates an imbalance of redox reactions and thereby contributes to an excess of ROS and results in oxidative stress.

In the context of insulin sensitivity and obesity, Ang II, aldosterone, fatty acids, and even high salt levels contribute to increased production of $\cdot O_2^-$ and H_2O_2. ROS are known promoters of downstream signaling pathways that include transcription factors, tyrosine kinases/phosphatases, ion channels, and mitogenic factors. The impact of ROS generated within various components of the vasculature (eg, heart, aorta, or kidney) is subject to local concentrations and the balance of pro- and antioxidant mechanisms. This is especially pertinent with the increasing interest in the availability of catalytic iron as a potential source for vascular ROS as described above.[50] Although there are a variety of normal molecular mechanisms to manage H_2O_2, there are fewer that can manage the hydroxyl radical, which is generated from H_2O_2 by the catalysis of Fe_2^+ to Fe_3^+. This is especially pertinent in chronic disease, including the various risk components of CMS for CVD and CKD.[13-16] It is interesting to note the recent appreciation of siderocalin, otherwise known as neutrophil gelatinase lipocalin, as a marker of both chronic CVD and CKD.[51] This protein attempts to manage catalytic iron and works as a protective factor against labile iron-associated oxidative stress mechanisms.[52]

Redox Control of Vascular Function

Free radicals are generally divided into ROS and reactive nitrogen species (RNS) generated for specific cellular processes: smooth muscle relaxation, inhibition of platelet adhesion, cell growth and differentiation, and numerous second messenger systems that promote impairments in insulin metabolic signaling.

Although the predominant form of ROS is $\cdot O_2^-$, critical to the metabolic phenotype is the NO radical (NO$\cdot$).[13] NO$\cdot$ is produced by oxidation of one of the terminal nitrogen atoms of L-arginine, a reaction that is then catalyzed by nitric oxide synthase (NOS). Levels of bioavailable NO, a potent vasodilator, diminish as NO is then consumed by ROS. NO is converted to other RNS; however, it is the predominance of $\cdot O_2^-$ that rapidly reacts with NO to yield $ONOO^-$. Importantly, $ONOO^-$ oxidizes NO synthase, which leads to decreased production of NO. Another notable reaction between ROS and NO is the oxidation of tetrahydrobiopterin (BH_4). In the absence of BH_4, NOS forms $\cdot O_2^-$ instead of NO, a process referred to as *NO uncoupling*, which is another critical source of oxidative stress that promotes endothelial dysfunction observed in the metabolic syndrome.

ROS also regulate various signaling mechanisms, and in excess promote derangements in cell cycle progression, growth, and proliferation, as well as insulin metabolic signaling. Oxidative stress is thought to contribute to the aging process and chronic disease states due to their electrophilic character, which promotes oxidization of cell constituents such as proteins, lipids, and DNA. The paradoxical nature of ROS can be resolved by understanding that only excessive production of these radicals results in damage, whereas their roles as mediators of cell signaling are temporally and spatially controlled. This is illustrated by the fact that ROS are known to oxidize/reduce cysteine residues, a mechanism critical for mitogen-activated protein kinase, protein tyrosine phosphatases, protein tyrosine kinase, transcription factors, and other enzymes that are redox regulated. Thereby, redox-sensitive pathways regulate the cells to activate or inhibit signaling proteins and dynamically alter gene expression according to various agonist/antagonist conditions.

Therapeutic Interventions That Improve the Redox State

There is strong experimental evidence for targeting the RAAS to improve oxidative stress, particularly in the treatment of hypertension.[15,16] The impact of the RAAS has classically been illustrated through the maladaptive effects of Ang II signaling through the AT_1R, as described above. Accordingly, there is sufficient evidence for a beneficial effect inhibition of ACE and blockade of AT_1R on oxidative stress.[10,13,15] Preclinical data from our laboratory and others, in various metabolic rodent models (including transgenic rats that over-express the RAAS), suggest that inhibition of the AT_1R or the mineralocorticoid receptor (MR), and recently with renin inhibition, improves indices of oxidative stress that result in improved cardiovascular remolding and metabolic parameters. On a clinical level, RAAS inhibition through ACE inhibition and Ang II receptor blockers has been studied extensively in patients with hypertension, congestive heart failure, coronary artery disease, and CKD, and is recommended to prevent CVD and nephropathy in patients with type 2 diabetes mellitus.[53,54] Recent data with blockade of the MR have shown promise in patients with heart failure, CKD, and resistant hypertension.[16]

In the context of the metabolic syndrome, some studies suggest a beneficial effect of RAAS blockade on insulin resistance and glucose homeostasis.[55,56] Possible mechanisms responsible for the reduced incidence of diabetes in these trials include improvement in insulin-mediated glucose uptake, enhanced endothelial function, increased NO activation, reduced inflammatory response, and increased bradykinin levels.[13] These effects have largely been described in the context of ACE inhibition and AT_1R blockade. However, the impact of MR antagonism or direct renin inhibition on insulin resistance and glucose homeostasis remains to be fully elucidated.[57,58]

In the context of the metabolic syndrome, some studies suggest a beneficial effect of RAAS blockade on insulin resistance and glucose homeostasis.

From an experimental perspective, in vivo MR antagonism results in decreased NADPH oxidase activity and oxidative stress in concert with improved insulin-dependent glucose uptake, as well as attenuated whole body insulin resistance in skeletal muscle.[57] Nonetheless, there have not been definitive clinical studies to confirm previous experimental and clinical observations suggesting improved insulin sensitivity through MR blockade. The discrepancy may be due to several factors. Most importantly, in the context of the metabolic syndrome, the MR has a high affinity for both aldosterone and 11β-hydroxy glucocorticoids. The presence of much lower levels of this enzyme in nonepithelial tissues allows for glucocorticoids to signal through the MR in cardiovascular and metabolic tissue such as skeletal muscle, liver, and fat. This is of particular importance in individuals with the metabolic syndrome, in whom circulating glucocorticoid concentrations are greater than aldosterone. MR activation by glucocorticoids further potentiates oxidative stress observed in the metabolic syndrome.

Evidence also suggests that statins may have pleiotropic effects beyond their ability to reduce lipids through modulatory effects on oxidant stress. Statins act by blocking 3-hydroxy-3-methylglutaryl coenzyme A reductase and inhibit synthesis of mevalonic acid, a precursor of many nonsteroidal isoprenoid compounds involved in intracellular trafficking of proteins involved in oxidative stress injury (ρ, Ras, Rac, Rab, Ral, and Rap).[59] Statins inhibit the activation of Rac1, critical to activation of NADPH oxidase, by preventing the geranylgeranyl-dependent translocation of Rac1 from the cytosol to the cell membrane, thereby reducing ROS generation.[60-62] Statins have also been shown to block expression of protein subunits of G-proteins ($p22^{phox}$ and NOX2), which determine oxidase activity of NADPH oxidase and expression of GTP-ase (NADPH activator).[62] This then contributes to suppression of prooxidant enzyme systems. Statins have also been shown to improve bioavailable NO and interfere with low-density lipoprotein (LDL) oxidation by several mechanisms, including reducing serum levels of LDL available for oxidation and the expression of scavenger receptor CD36 on monocytes that inhibit uptake of oxidized LDL.[63,64] Regardless of the mechanisms, there is convincing evidence to support that both inhibition of the RAAS and statin therapy in humans ameliorates oxidative stress.

Is There a Role for Chelation Therapy?

There is increasing interest in chelation therapy, especially in the context of targeting poorly liganded iron in the generation of oxidative stress. Iron chelators have been used in various studies that examined therapeutic interventions in the management of coronary disease,[65,66] as well as the investigation of kidney disease.[67] Recently, there have been several reports targeting pharmacologic interventions,[68,69] such as desferrioxamine and the aforementioned statins, on poorly liganded iron and improvements in endothelial function.[66,70] Although there is increasing interest in pharmacologic intervention, there is also a considerable role for dietary nutrients in the chelation of iron and mitigation of the effects of oxidative stress. The best evidence exists with polyphenolic compounds, many of which are widely used as dietary antioxidants.[71,72] This has classically been attributed to a Mediterranean diet, wherein the beneficial effects of the polyphenols are thought to be derived from their antioxidant capabilities.[73-76] Many of the polyphenolic compounds, including flavones, isoflavones, stilbenes, and flavanones, are implicated for their antioxidant effects because they have been shown to chelate iron as well.[77-80] The flavonoid compounds have a catechol moiety that are known iron-binding elements

Although there is increasing interest in pharmacologic intervention, there is also a considerable role for dietary nutrients in the chelation of iron and mitigation of the effects of oxidative stress.

of microbial siderophores.[81] It is no surprise, then, that a considerable number of studies on chronic disease states wherein oxidative stress has been implicated show promise with interventions containing polyphenolic components. However, the uptake of these compounds in the routine management of chronic diseases has been hampered by significant problems in determining absorption, bioavailability, and measures of appropriate markers.

Conclusions

Overfeeding and excess adiposity directly result in insulin resistance. As a response, excesses in insulin potentiate the growth and division of additional adipocytes. These cells—in concert with hepatocytes—produce adipokines and cytokines, which are responsible for systemic inflammation. In the setting of inflammation, poorly liganded or labile iron available within cells through catalysis allows the Haber-Weiss and Fenton equations to generate ROS and the particularly deleterious hydroxyl radical. These processes are fundamental to tissue fibrosis and potentially to instability of atherosclerotic plaque and incident CVD events. Very importantly, chronic CVD and CKD are the important base conditions upon which acute events occur. These events, mediated by tissue hypoxia, generate a much larger quantity of labile iron and allow an explosion of local oxidative stress that results in tissue injury even after the hypoxia has been relieved. Understanding the role of iron and oxidative stress in the setting of obesity and insulin resistance as observed in the metabolic syndrome is critical to advancing diagnostic and therapeutic strategies in the future. ■

References

1. Lakka HM, Laaksonen DE, Lakka TA, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA*. 2002;288:2709-2716.
2. Malik S, Wong ND, Franklin SS, et al. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation*. 2004;110:1245-1250.
3. Park YW, Zhu S, Palaniappan L, et al. The Metabolic syndrome: prevalence and associated risk factor findings in the US Population from the Third National Health and Nutrition Examination Survey, 1988-1994. *Arch Intern Med*. 2003;163:427-436.
4. Isomaa B, Almgren P, Tuomi T, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*. 2001; 24:683-689.
5. Flegal KM, Carroll MD, Kuczmarski RJ, Johnson CL. Overweight and obesity in the United States: prevalence and trends, 1960-1994. *Int J Obes Relat Metab Disord*. 1998;22:39-47.
6. Van Gaal LF, Mertens IL, DeBlock CE. Mechanisms linking obesity with cardiovascular disease. *Nature*. 2006;444:875-880.
7. Bomback AS, Klemmer PJ. Interaction of aldosterone and extracellular volume in the pathogenesis of obesity-associated kidney disease. *Am J Nephrol*. 2009;30:140-146.
8. McFarlane SI, Banerji M, Sowers JR. Insulin resistance and cardiovascular disease. *J Clin Endocrinol Metab*. 2001;86:713-718.
9. Isomaa B. A major health hazard: the metabolic syndrome. *Life Sci*. 2003;73:2395-2411.
10. Sowers JR. Obesity as a cardiovascular risk factor. *Am J Med*. 2003;115(suppl 8A):37S-41S.
11. Reaven GM, Chen YD. Insulin resistance, its consequences, and coronary heart disease. Must we choose one culprit? *Circulation*. 1996; 93:1780-1783.
12. Reaven GM. Insulin resistance, cardiovascular disease, and the metabolic syndrome: how well do the emperor's clothes fit? *Diabetes Care*. 2004;27:1011-1012.
13. Cooper SA, Whaley-Connell A, Habibi J, et al. Renin-angiotensin-aldosterone system and oxidative stress in cardiovascular insulin resistance. *Am J Physiol Heart Circ Physiol*. 2007;293: H2009-H2023.
14. Whaley-Connell A, Pavey BS, McCullough PA, et al; KEEP Investigators. Dysglycemia predicts cardiovascular and kidney disease in the Kidney Early Evaluation Program. *J Clin Hypertens*. 2010;12:51-58.
15. Wei Y, Chen K, Whaley-Connell AT, et al. Skeletal muscle insulin resistance: role of inflammatory cytokines and reactive oxygen species. *Am J Physiol Regul Integr Comp Physiol*. 2008;294: R673-R680.
16. Sowers JR, Whaley-Connell A, Epstein M. The emerging clinical implications of the role of aldosterone in the metabolic syndrome and resistant hypertension. *Ann Intern Med*. 2009; 150:776-783.
17. Lambeth JD. Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. *Free Radic Biol Med*. 2007;43:332-347.
18. Lassègue B, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. *Am J Physiol Regul Integr Comp Physiol*. 2003;285:R277-R297.
19. Rey FE, Cifuentes ME, Kiarash A, et al. Novel competitive inhibitor of NAD(P)H oxidase

Main Points

- Loss of reduction-oxidation (redox) homeostasis and generation of excess free oxygen radicals play an important role in the pathogenesis of diabetes, hypertension, and consequent cardiovascular disease.
- Reactive oxygen species are integral in routine in physiologic mechanisms. However, loss of redox homeostasis contributes to proinflammatory and profibrotic pathways that promote impairments in insulin metabolic signaling, reduced endothelial-mediated vasorelaxation, and associated cardiovascular and renal structural and functional abnormalities.
- Redox control of metabolic function is a dynamic process with reversible pro- and anti-free radical processes. Labile iron is necessary for the catalysis of superoxide anion, hydrogen peroxide, and the generation of the damaging hydroxyl radical. Acute hypoxia and cellular damage in cardiovascular tissue liberate larger amounts of cytosolic and extracellular iron that is poorly liganded; thus, large increases in the generation of oxygen free radicals are possible, causing tissue damage.
- The understanding of iron and the imbalance of redox homeostasis within the vasculature is integral in hypertension and progression of metabolic dysregulation that contributes to insulin resistance, endothelial dysfunction, and cardiovascular and kidney disease.

assembly attenuates vascular O(2)(-) and systolic blood pressure in mice. *Circ Res*. 2001;89:408-414.
20. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev*. 2007;87:245-313.
21. Hall JE, Brands MW, Dixon WN, Smith MJ Jr. Obesity-induced hypertension. Renal function and systemic hemodynamics. *Hypertension*. 1993;22:292-299.
22. DeFronzo RA, Cooke CR, Andres R, et al. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J Clin Invest*. 1975;55:845-855.
23. Rocchini AP, Moorehead C, DeRemer S, et al. Hyperinsulinemia and the aldosterone and pressor responses to angiotensin II. *Hypertension*. 1990;15(6 Pt 2):861-866.
24. Rocchini AP, Marker P, Cervenka T. Time course of insulin resistance associated with feeding dogs a high-fat diet. *Am J Physiol*. 1997;272(1 Pt 1): E147-E154.
25. Cosentino F, Hishikawa K, Katusic ZS, Luscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation*. 1997;96:25-28.
26. Fleming I, Bauersachs J, Fisslthaler B, Busse R. $Ca2^{+}$-independent activation of the endothelial nitric oxide synthase in response to tyrosine phosphatase inhibitors and fluid shear stress. *Circ Res*. 1998;82:686-695.
27. Heitzer T, Brockhoff C, Mayer B, et al. Tetrahydrobiopterin improves endothelium-dependent vasodilation in chronic smokers: evidence for a dysfunctional nitric oxide synthase. *Circ Res*. 2000;86:E36-E41.
28. Mollnau H, Wendt M, Szöcs K, et al. Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. *Circ Res*. 2002; 90:E58-E65.
29. Zhang HH, Huang J, Düvel K, et al. Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. *PLoS ONE*. 2009;4:e6189.
30. Engeli S, Sharma AM. The renin-angiotensin system and natriuretic peptides in obesity-associated hypertension. *J Mol Med*. 2001;79:21-29.
31. Reisin E, Messerli FG, Ventura HO, Frohlich ED. Renal haemodynamic studies in obesity hypertension. *J Hypertens*. 1987;5:397-400.
32. Granger JP, West D, Scott J. Abnormal pressure natriuresis in the dog model of obesity-induced hypertension. *Hypertension*. 1994;23:I8-I11.
33. Schorr U, Blaschke K, Turan S, et al. Relationship between angiotensinogen, leptin and blood pressure levels in young normotensive men. *J Hypertens*. 1998;16:1475-1480.
34. Goodfriend TL, Egan BM, Kelley DE. Aldosterone in obesity. *Endocr Res*. 1998;24:789-796.
35. Goodfriend TL, Egan BM, Kelley DE. Plasma aldosterone, plasma lipoproteins, obesity and insulin resistance in humans. *Prostaglandins Leukot Essent Fatty Acids*. 1999;60:401-405.
36. Licata G, Volpe M, Scaglione R, Rubattu S. Salt-regulating hormones in young normotensive obese subjects. Effects of saline load. *Hypertension*. 1994;23:I20-I24.
37. Cooper R, McFarlane-Anderson N, Bennett FI, et al. ACE, angiotensinogen and obesity: a potential pathway leading to hypertension. *J Hum Hypertens*. 1997;11:107-111.
38. Cooper R, Forrester T, Ogunbiyi O, Muffinda J. Angiotensinogen levels and obesity in four black populations. ICSHIB Investigators. *J Hypertens*. 1998;16:571-575.
39. Engeli S, Negrel R, Sharma AM. Physiology and pathophysiology of the adipose tissue renin-angiotensin system. *Hypertension*. 2000;35: 1270-1277.
40. Hirono Y, Yoshimoto T, Suzuki N, et al. Angiotensin II receptor type 1-mediated vascular oxidative stress and proinflammatory gene expression in aldosterone-induced hypertension: the possible role of local renin-angiotensin system. *Endocrinology*. 2007;148:1688-1696.
41. Schiffrin EL. Effects of aldosterone on the vasculature. *Hypertension*. 2006;47:312-318.
42. Williams JS, Williams GH. 50th anniversary of aldosterone. *J Clin Endocrinol Metab*. 2003;88: 2364-2372.
43. Brown NJ. Aldosterone and vascular inflammation. *Hypertension*. 2008;51:161-167.
44. de Boer RA, Voors AA, Muntendam P, et al. Galectin-3: a novel mediator of heart failure development and progression. *Eur J Heart Fail*. 2009;11:811-817.
45. Wei Y, Whaley-Connell AT, Habibi J, et al. Mineralocorticoid receptor antagonism attenuates vascular apoptosis and injury via rescuing protein kinase B activation. *Hypertension*. 2009;53:158-165.
46. Stas S, Whaley-Connell A, Habibi J, et al. Mineralocorticoid receptor blockade attenuates chronic overexpression of the renin-angiotensin–II-aldosterone system stimulation of reduced NADPH oxidase and cardiac remodeling. *Endocrinology*. 2007;148:3773-3780.
47. Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. *Diabetes Care*. 2008;31(Suppl 2): S170-S180.
48. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res*. 2000;86:494-501.
49. Li JM, Mullen AM, Yun S, et al. Essential role of the NADPH oxidase subunit p47(phox) in endothelial cell superoxide production in response to phorbol ester and tumor necrosis factor-alpha. *Circ Res*. 2002;90:143-150.
50. Kell DB. Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. *BMC Med Genomics*. 2009;2:2.
51. Bolignano D, Coppolino G, Lacquaniti A, Buemi M. From kidney to cardiovascular diseases: NGAL as a biomarker beyond the confines of nephrology. *Eur J Clin Invest*. 2010;40: 273-276.
52. Bolignano D, Donato V, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. *Am J Kidney Dis*. 2008;52:595-605.
53. Cohn JN. Reducing cardiovascular risk by blockade of the renin-angiotensin-aldosterone system. *Adv Ther*. 2007;24:1290-1304.
54. American Diabetes Association. Standards of medical care in diabetes—2008. *Diabetes Care*. 2008;31(suppl 1):S12-S54.
55. Bosch J, Yusuf S, Gerstein HC, et al; DREAM Trial Investigators. Effect of ramipril on the incidence of diabetes. *N Engl J Med*. 2006;355: 1551-1562.
56. McMurray JJ, Holman RR, Haffner SM, et al; NAVIGATOR Study Group. Effect of valsartan on the incidence of diabetes and cardiovascular events. *N Engl J Med*. 2010;362:1477-1490.
57. Lastra G, Habibi J, Whaley-Connell A, et al. Direct renin inhibition improves systemic insulin resistance and skeletal muscle glucose transport in a transgenic rodent model of tissue renin overexpression. *Endocrinology*. 2009;150: 2561-2568.
58. Habibi J, Whaley-Connell A, Hayden MR, et al. Renin inhibition attenuates insulin resistance, oxidative stress, and pancreatic remodeling in the transgenic Ren2 rat. *Endocrinology*. 2008; 149:5643-5653.
59. Dusi S, Domini M, Rossi F. Mechanism of NADPH oxidase activation in human neutrophils: p67phox is required for translocation of rac1 but not rac2 from cytosol to the membranes. *Biochem J*. 1995;308:991-994.
60. Whaley-Connell AT, Morris EM, Rehmer N, et al. Albumin activation of NAD(P)H oxidase activity is mediated via Rac1 in proximal tubule cells. *Am J Nephrol*. 2007;27:15-23.
61. Habibi J, Whaley-Connell A, Qazi MA, et al. Rosuvastatin, a 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor, decreases cardiac oxidative stress and remodeling in Ren2 transgenic rats. *Endocrinology*. 2007;148:2181-2188.
62. Wolfrum S, Jensen KS, Liao JK. Endothelium dependent effects of statins. *Arterioscler Thromb Vasc Biol*. 2003;23:729-736.
63. Campese VM, Nadim MK, Epstein M. Are 3 hydroxy3-methyglutaryl CoA reductase inhibitors renoprotective? *J Am Soc Nephrol*. 2005;16:S11-S17.
64. Mason PR, Walter MF, Jacob RF. Effects of HMG-CoA reductase inhibitors on endothelial function. *Circulation*. 2004;109:II-34-II-41.
65. Horwitz LD, Sherman NA, Kong Y, et al. Lipophilic siderophores of Mycobacterium tuberculosis prevent cardiac reperfusion injury. *Proc Natl Acad Sci U S A*. 1998;95:5263-5268.
66. Duffy SJ, Biegelsen ES, Holbrook M, et al. Iron chelation improves endothelial function in patients with coronary artery disease. *Circulation*. 2001;103:2799-2804.
67. De Vries B, Walter SJ, Von Bonsdorff L, et al. Reduction of circulating redox-active iron by apotransferrin protects against renal ischemia-reperfusion injury. *Transplantation*. 2004;77: 669-675.
68. Hovorka S, Schöneich C. Oxidative degradation of pharmaceuticals: theory, mechanisms and inhibition. *J Pharm Sci*. 2001;90:253-269.
69. England K, Driscoll CO, Cotter TG. ROS and protein oxidation in early stages of cytotoxic drug induced apoptosis. *Free Radic Res*. 2006; 40:1124-1137.
70. Dombrecht EJ, De Tollenaere CB, Aerts K, et al. Antioxidant effect of bisphosphonates and simvastatin on chondrocyte lipid peroxidation. *Biochem Biophys Res Commun*. 2006;348:459-464.
71. Shahidi F, Wanasundara PK. Phenolic antioxidants. *Crit Rev Food Sci Nutr*. 1992;32:67-103.
72. Wright JS, Johnson ER, DiLabio GA. Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. *J Am Chem Soc*. 2001;123:1173-1183.
73. Ness AR, Powles JW. Fruit and vegetables, and cardiovascular disease: a review. *Int J Epidemiol*. 1997;26:1-13.
74. Krauss RM, Eckel RH, Howard B, et al. AHA Dietary Guidelines: revision 2000: a statement for

healthcare professionals from the Nutrition Committee of the American Heart Association. *Circulation*. 2000;102:2284-2299.
75. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu Rev Nutr*. 2001; 21:381-406.
76. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? *Free Radic Biol Med*. 2006;41: 1727-1746.
77. Cook NC, Samman S. Flavonoids—chemistry metabolism, cardioprotective effects, and dietary sources. *J Nutr Biochem*. 1996;7:66-76.
78. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev*. 1998;56:317-333.
79. Fraga CG, Oteiza PI. Iron toxicity and antioxidant nutrients. *Toxicology*. 2002;180:23-32.
80. Arts ICW, Hollman PCH. Polyphenols and disease risk in epidemiologic studies. *Am J Clin Nutr*. 2005;81(1 Suppl):317S-325S.
81. Nijveldt RJ, van Nood E, van Hoorn DE, et al. Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr*. 2001;74:418-425.