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FORUM REVIEW ARTICLE

Oxidative Stress as Pathogenesis of Cardiovascular Risk Associated with Metabolic Syndrome

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Abstract

Metabolic syndrome (MetS) is characterized by accumulation of visceral fat associated with the clustering of metabolic and pathophysiological cardiovascular risk factors: impaired glucose tolerance, dyslipidemia, and hypertension. Although the definition of MetS is different among countries, visceral obesity is an indispensable component of MetS. A growing body of evidence suggests that increased oxidative stress to adipocytes is central to the pathogenesis of cardiovascular disease in MetS. Increased oxidative stress to adipocytes causes dysregulated expression of inflammation-related adipocytokines in MetS, which contributes to obesity-associated vasculopathy and cardiovascular risk primarily through endothelial dysfunction. The purpose of present review is to unravel the mechanistic link between oxidative stress and cardiovascular risk in MetS, focusing on insulin resistance, hypertension, and atherosclerosis. Then, therapeutic opportunities translated from the bench to bedside will be provided to develop novel strategies to cardiovascular risk factors in MetS. *Antioxid. Redox Signal.* 15, 1911–1926.

Introduction

Metabolic syndrome (MetS) is characterized by accumulation of visceral fat associated with the clustering of metabolic and pathophysiological cardiovascular risk factors: impaired glucose tolerance (IGT), dyslipidemia, and hypertension (HTN) (47). Although the definition of MetS is different among countries, visceral obesity is an indispensable component of MetS. The prevalence of MetS is rapidly increasing worldwide not only in industrialized countries but also in developing countries associated with an increase in food intake. MetS has a strong impact on the global incidence of the life-threatening cardiovascular disease such as stroke and myocardial infarction (2, 53). Although the MetS is multifactorial in origin, IGT, dyslipidemia, and HTN are caused by the same underlying mechanism—endothelial dysfunction primarily mediated by oxidative stress.

It is now apparent that visceral adipose tissue is an endocrine organ that secretes many bioactive molecules, known as adipocytokines (20, 134, 152). The production of adipocytokines is of particular interest, because their local secretion by perivascular adipose depots may provide a new mechanistic link between obesity and its associated cardiovascular complications. Increased oxidative stress to adipocytes causes dysregulated expression of inflammation-related adipocytokines in MetS. Increasing evidence supports the central role of adipose tissue in the development of systemic inflammatory state, which contributes to obesity-associated vasculopathy and cardiovascular risk (12, 76, 87). These adipocytokines are generally divided into pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin-6, monocyte chemoattractant protein-1, plasminogen activator inhibitor-1, and anti-inflammatory cytokines such as adiponectin. Imbalance between pro-inflammatory cytokines and anti-inflammatory cytokines is responsible for oxidative stress especially to endothelial cells and underlies the pathogenesis of the obesity-associated insulin resistance, IGT, type-2 diabetes mellitus (T2DM), HTN, dyslipidemia, and vascular disease. Although obstructive sleep apnea syndrome represents another important cause of oxidative stress in MetS (67, 77), this topic will not be discussed in this review because unlike the increase in visceral adipose tissue, which is involved in a definition of MetS, not all the individuals with MetS are associated with obstructive sleep apnea syndrome.

The purpose of the present review is to overview the mechanistic link between oxidative stress and cardiovascular risk in MetS based on the evidence obtained from animal experiments and clinical trials. This review specifically focusses on insulin resistance and atherosclerosis, which are intimately related to oxidative stress to endothelial cells. Then, therapeutic opportunities translated from the bench to bedside will be provided to develop novel strategies for preventing cardiovascular risk associated with MetS.

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Mechanisms Underlying Cardiovascular Risk in MetS

Central role of oxidative stress in visceral adipose tissue

A growing body of evidence suggests that increased oxidative stress in white adipose tissue is central to the pathogenesis of cardiovascular disease in MetS. Although the molecular mechanism of oxidative stress to adipocytes remains unclear and appears to be multifactorial, the development of adipocyte hypertrophy and hypoxia has been implicated in oxidative stress (54). Reactive oxygen species (ROS) production increases in parallel with fat accumulation in adipocytes and increased levels of fatty acid stimulate ROS production in adipocytes through the activation of NADPH oxidase and decreased expression of antioxidative enzymes (49). Exposure of adipocytes to oxidative stress decreases anti-inflammatory adiponectin (57, 133) and increases pro-inflammatory adipocytokines (23, 49, 121). Involvement of a local renin-angiotensin aldosterone system (RAAS) has also been proposed as a potential mediator of oxidative stress to adipocytes (22). Irrespective of the mechanism of ROS production, oxidative stress in the visceral adipose tissue is an upstream event that mediates systemic inflammation and oxidative stress in the remote tissue through dysregulation of adipocytokine production. Systemic inflammation then causes a variety of metabolic and cardiovascular disorders through oxidative stress to endothelial cells (Fig. 1).

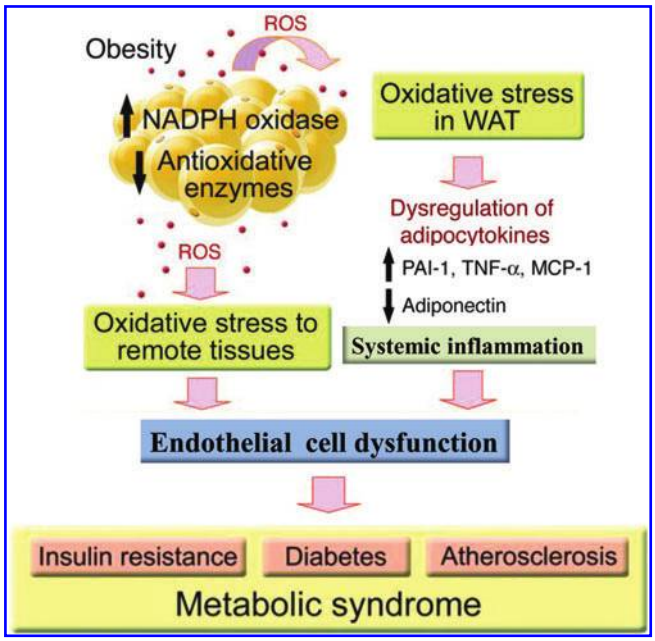

FIG. 1. Mechanism of obesity-induced cardiovascular risk. In white adipose tissue (WAT) reactive oxygen species (ROS) production increases in parallel with fat accumulation in adipocytes through the activation of NADPH oxidase and decreased expression of antioxidative enzymes. Oxidative stress in WAT causes dysregulation of adipocytokines; increased generation of pro-inflammatory cytokines such as plasminogen activator inhibitor (PAI)-1, tumor necrosis factor-α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1); and decreased generation of anti-inflammatory cytokines such as adiponectin. Dysregulation of adipocytokines causes oxidative stress to remote tissues and systemic inflammation responsible for endothelial cell dysfunction, which is central to the pathogenesis of insulin resistance, diabetes, and atherosclerosis in metabolic syndrome (MetS). This illustration is adapted from Furukawa *et al.* (49).

Oxidative stress and insulin resistance

Oxidative stress to endothelial cells and subsequent decrease in glucose uptake and utilization by major energy-consuming organs such as the liver and skeletal muscle are responsible for insulin resistance. In MetS, endothelial cells are directly exposed to ROS through high levels of circulating pro-inflammatory cytokines generated in the visceral adipose tissue and low levels of adiponectin. Moreover, endothelial cell generation of ROS is increased by activation of NADPH oxidase through the action of local RAAS (31, 73). Indeed, RAAS-associated signaling by way of the angiotensin (Ang) II type-1 receptors and mineral corticoid receptors triggers tissue activation of the NADPH oxidase and increased production of ROS in endothelial cells (132). This vicious cycle of ROS generation in endothelial cells is an important mechanism of transition from insulin resistance and IGT to T2DM in MetS.

Oxidative stress to endothelial cells decreases bioavailability of nitric oxide (NO) and causes loss of blood flow regulation in response to increased oxygen demand and energy utilization. Reduced bioavailability of NO results from decreased synthesis by uncoupling of endothelial NO synthase (eNOS) through ROS-induced oxidation and depletion of the eNOS cofactor, tetrahydrobiopterin (BH4) (11, 125), in combination with enhanced consumption in tissues by high levels of superoxide generating peroxynitrite. This molecule is highly toxic and causes endothelial cell death (36) that further reduces endothelial cell generation of NO. eNOS-derived NO also plays a crucial role in angiogenesis by upregulating vascular endothelial growth factors and increasing mobilization of endothelial progenitor cells from the bone marrow (41, 82). Thus, ROS-induced endothelial dysfunction impairs blood flow regulation and reduces expansion of the capillary network, with attenuation of microcirculatory blood flow in metabolically active tissues contributing to the impairment of insulin-stimulated glucose and lipid metabolism.

Another critical effect of ROS on the glucose uptake mechanism is the activation of serine/threonine kinase cascades such as c-Jun N-terminal kinase and nuclear factor-kappaB, and others that in turn phosphorylate multiple targets, including the insulin receptor and the insulin receptor substrate (IRS) proteins (14, 43, 104). Increased serine phosphorylation of IRS reduces its ability to undergo tyrosine phosphorylation and may accelerate the degradation of IRS-1 (7), leading to the disruption of signaling pathways for glucose uptake by glucose transporter-4 (GLUT4) through IRS-1 and phosphatidylinositol 3-kinase (PI3K)/Akt. GLUT4 causes impairment of insulin-stimulated skeletal muscle glycogen synthesis, which appears to underlie the mechanism of insulin resistance (19).

Impaired glucose uptake by adipocytes thorough the IRS-1, PI3K/Akt, and GLUT4 axis may cause an additional adverse effect on insulin resistance. Adipose tissue has been proposed to act as a glucose sensor (138). Adipocytes, therefore, detect the absence of glucose uptake by GLUT4 and, in response, secrete adipocytokines such as retinol-binding protein 4 to restrict glucose uptake in the skeletal muscle and increase

glucose output by the liver by blocking insulin signaling (95), thereby increasing the blood glucose level. It was found that the expression of GLUT4 is reduced in adipocytes, but not in skeletal muscle, of animals and humans with obesity and T2DM (127). Thus, oxidative stress-induced downregulation of GLUT4 in adipocytes is a representative mechanism of insulin resistance and T2DM in MetS. The putative model of insulin resistance and T2DM in MetS is illustrated in Figure 2.

Oxidative stress and HTN

Endothelial dysfunction contributes to HTN, one of the diagnostic criteria of MetS. Reduced bioavailability of NO appears to be a key process through which endothelial dysfunction is manifested in HTN. Accumulating evidence suggests that NO plays a major role in regulating blood pressure and that impaired NO bioactivity is an important mechanism of HTN (78, 125, 141, 145). Mice with disruption of the gene for eNOS have elevated blood pressure levels compared with control animals (128, 135), suggesting a genetic component to the link between impaired NO bioactivity and HTN. Although the contribution of NO may vary between different models of HTN, a unifying characteristic of these models is the presence of oxidative stress that participates in the maintenance of elevated arterial pressure and seems to be a common denominator underlying endothelial dysfunction in various forms of experimental HTN. In the presence of oxidative stress, eNOS acts as a double-edged sword. Superoxide produced by inflammatory cells or endothelial cells stimulated with pro-inflammatory adipocytokines react with NO, thereby stimulating the production of peroxynitrite. Peroxynitrite in turn causes uncoupling of eNOS, therefore switching an antiatherosclerotic NO-producing enzyme to an enzyme that may accelerate the atherosclerotic process by producing superoxide (69, 94). Besides circulating inflammatory cells and pro-inflammatory adipocytokines, there are a variety of sources of ROS in the vascular tissue. ROS-producing enzymes involved in increased oxidative stress within the vascular tissue include NADPH oxidase, xanthine oxidase, and mitochondrial superoxide-producing enzymes. Of these, local RAAS-mediated NADPH oxidase activation is of prime importance in endothelial cell generation of ROS, which contributes to endothelial dysfunction and HTN (122).

Oxidative stress-induced uncoupling of NOS is not confined to eNOS. Oxidative stress on endothelial cells increases expression of inducible NOS (iNOS). Unlike eNOS, iNOS is constitutively active and generates robust NO. Because oxidative stress depletes BH4 and uncouples iNOS, it is possible that iNOS uncoupling exaggerates oxidative stress and creates a vicious cycle of endothelial dysfunction and HTN. The potential role of NOS uncoupling in HTN and the therapeutic opportunity that targets NOS uncoupling will be discussed later.

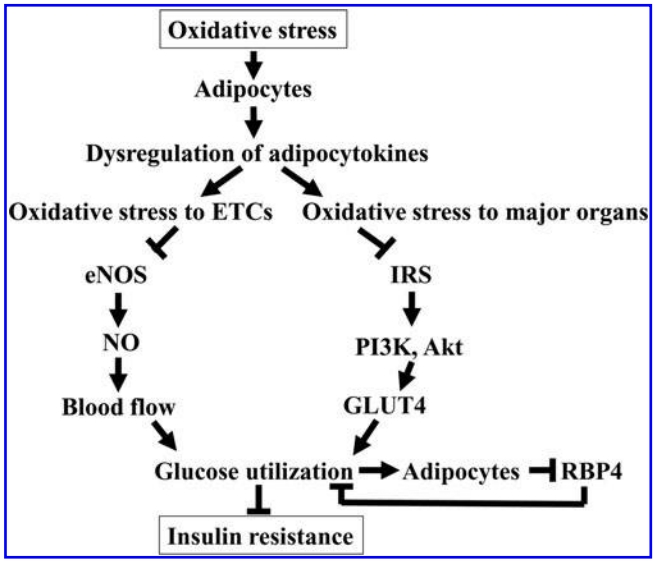

FIG. 2. Schematic drawing of the role of oxidative stress to adipocytes in insulin resistance. Oxidative stress to adipocytes causes dysregulation of adipocytokines that mediate systemic inflammation and oxidative stress to endothelial cells (ETCs) and major energy-consuming organs such as liver and skeletal muscles. Oxidative stress to ETCs promotes uncoupling of endothelial nitric oxide synthase (eNOS) and reduces bioavailability of NO that impairs blood flow in metabolically active tissues, leading to the impairment of insulin-stimulated glucose utilization. Oxidative stress to major energy-consuming organs, on the other hand, increases serine phosphorylation and degradation of insulin receptor substrate (IRS) and disrupts signaling pathways for glucose uptake by glucose transporter-4 (GLUT4) through the IRS-1 and phosphatidylinositol 3-kinase/Akt signaling pathway. Impaired glucose uptake by adipocytes thorough GLUT4 causes secretion of retinol-binding protein 4 (RBP4) to restrict glucose uptake in skeletal muscle and increase glucose output by the liver, thereby reducing glucose utilization and contributing to insulin resistance.

Oxidative stress and atherosclerosis

Atherosclerosis is one of the representative manifestations of vascular pathology in MetS. Development of insulin resistance, HTN, and dyslipidemia culminates in atherosclerosis. A growing body of evidence indicates that pro-inflammatory cytokines generated in the visceral adipose tissues are associated with atherosclerosis. A sequence of events that are participated in the development of atheromatous plaque is illustrated in Figure 3.

One of the triggers of atheromatous plaque formation is endothelial generation of ROS (Fig. 3A). Another initial participant in atheromatous lesion-prone sites includes the intimal influx and accumulation of low-density lipoprotein (LDL), which is further enhanced in the presence of triglyceride. LDL is oxidized by ROS, and oxidized LDL is taken up by macrophages *via* their scavenger receptors CD36 to form foam cells (26, 115, 143). Monocyte-macrophage recruitment to the intima is likely to be regulated not only by a multiplicity of adhesion molecules, integrins, and selectins, but also by chemokines such as monocyte chemoattractant protein-1, which is constitutively synthesized and secreted by endothelial cells and smooth muscle cells (SMCs) migrated from the media and adventitia (16, 153). Transcriptional upregulation of these molecules is enhanced by ROS, which are derived from endothelial cells, activated macrophages, and SMCs. On the other hand, such ROS are also pivotal in the oxidation of LDL, creating a self-perpetuating cycle in foam cell accumulation and atherosclerotic plaque formation. At the same time, SMCs migrate from the media to the intimal

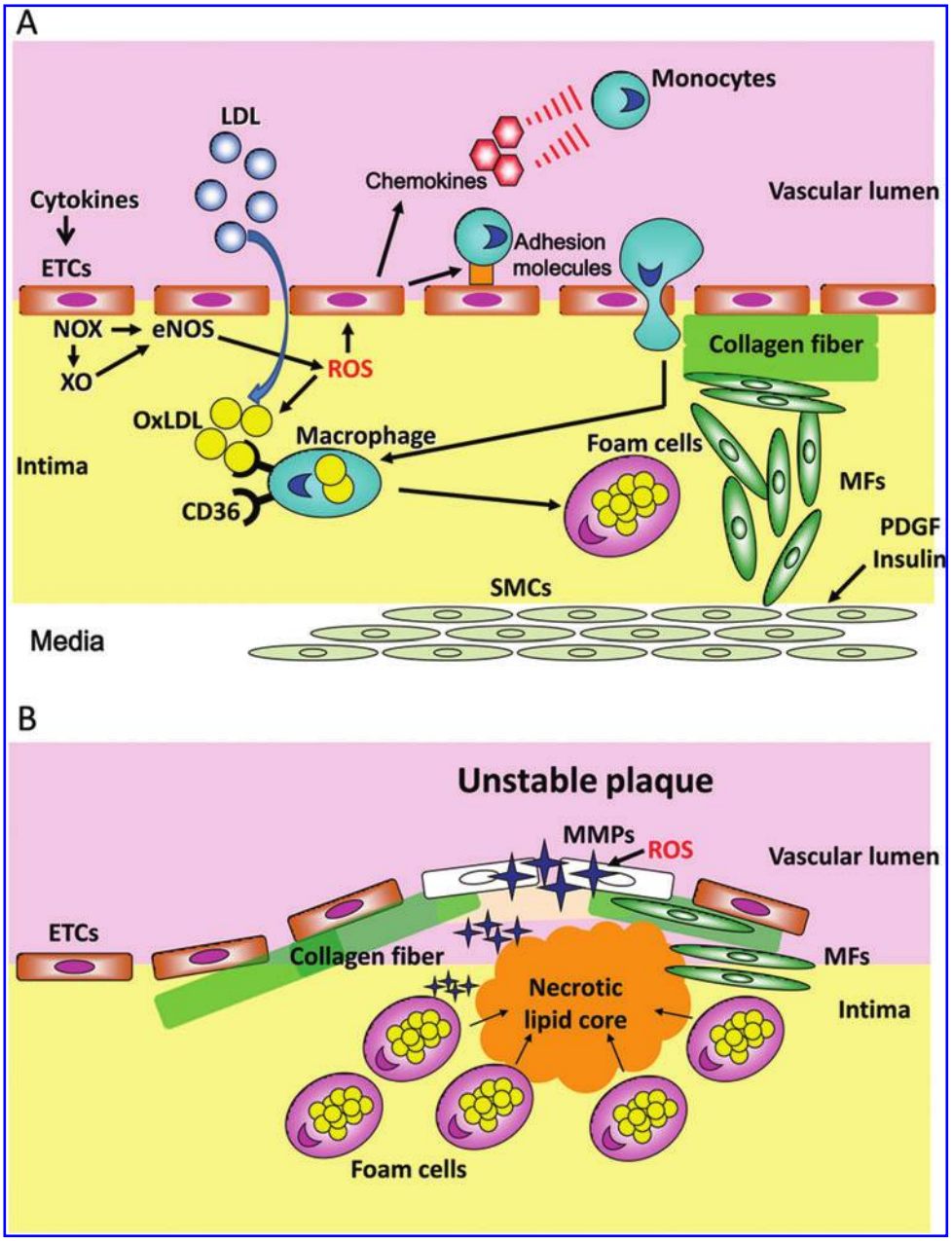

FIG. 3. A sequence of events participated in the development of atheromatous plaque. (A) An early phase of atheromatous plaque formation. Pro-inflammatory cytokines trigger endothelial cell (ETC) generation of ROS by activating NADPH oxidase (NOX) and xanthine oxidase (XO). These ROS promote eNOS uncoupling and accentuate ROS generation. The intimal influx and accumulation of low-density lipoprotein (LDL) represents another trigger of atheromatous plaque formation. LDL is oxidized by ROS, and oxidized LDL (OxLDL) is taken up by macrophages *via* their scavenger receptors CD36 to form foam cells. ROS stimulate monocyte-macrophage recruitment to the intima by enhanced expression of adhesion molecules and chemokines. At the same time smooth muscle cells (SMCs) migrate from the media to the intimal endothelial layer, differentiated into myofibroblasts (MFs), and proliferate under the regulation of a number of mitogens, including platelet-derived growth factor (PDGF) and insulin. Proliferating MFs synthesize collagen and promote thickening of the intima. **(B)** A late stage of atheromatous plaque development. A robust increase in inflammation and oxidative stress causes apoptosis of foam cells, leading to the formation of the necrotic lipid core. The necrotic lipid core is covered with a thin fibrous cup creating unstable plaque as a result of degradation of collagen fiber by metalloproteinases (MMPs) in the presence of ROS.

endothelial layer. They are differentiated into myofibroblasts and proliferate under the regulation of a number of mitogens, including platelet-derived growth factor (37, 97). Insulin also acts as a growth factor and enhances intimal myofibroblast growth when serine residues of IRS-1 are phosphorylated by oxidative stress (65, 149). In addition, collagen synthesis by proliferating myofibroblasts is substantial for the thickening of the intima.

In the late stage of plaque development (Fig. 3B), a robust increase in inflammation and oxidative stress causes apoptosis of foam cells and is responsible for the formation of the necrotic lipid core (64, 84). The necrotic lipid core is covered with a fibrous cup enriched with collagen fiber. However, the fibrous cap is degraded by redox-sensitive activation of matrix metalloproteinases in the presence of ROS (79, 151). The thin fibrous cup is prone to be ruptured in response to increased shear stress caused by elevated intraluminal pressure and luminal narrowing of the coronary artery (92, 157). Therefore, such an advanced atheromatous plaque consisted of necrotic lipid core covered with a thin fibrous cap is termed unstable plaque.

Therapeutic Opportunities for Cardiovascular Risk Factors in MetS

Therapeutic approaches to MetS comprises lifestyle modification in conjunction with drug treatment of the MetS-associated complications. Healthier eating and regular

exercise greatly reduce waistline and body mass index, lower blood pressure, and improve lipid profile. Lifestyle modification has been shown to prevent T2DM development. Nevertheless, appropriate treatment of cardiovascular risk factors in MetS often requires pharmacologic intervention against IGT or T2DM with insulin-sensitizing agents, such as thiazolidinediones (TDZs) and metformin, whereas statins and fibrates or angiotensin-converting enzyme (ACE) inhibitors and Ang II type-1 receptor blockers (ARBs) are the first-line lipid-modifying or anti-HTN drugs. These pharmacological interventions inhibit oxidative stress, but unlike general antioxidants of which efficacy to prevent cardiovascular risk factors is still controversial, they prevent only harmful ROS generation, leaving beneficial ROS. Thus, these drugs are designated as a class of preventive antioxidants enabling a causal therapy against oxidative stress through site-specific inhibition of ROS and preservation of redox signaling necessary for cardiovascular protection (107). Further, preventive antioxidants appear to increase eNOS-derived NO, which prevents insulin resistance, HTN, and atherosclerosis (98). Discussed in this section are therapeutic opportunities for cardiovascular risk factors in MetS focusing on the strategy to inhibit oxidative stress and inflammation in the visceral adipose tissue and preserve endothelial functions NO generation that are central to prevent cardiovascular risk factors in MetS.

Caloric restriction and adiponectin

A large body of experimental and epidemiological evidence has established an association between visceral obesity and MetS. Caloric restriction (CR) primary affects energy stores in visceral adipose tissue (32). Indeed, a substantial improvement in all aspects of MetS with only a moderate degree of weight loss by CR has been observed in a large number of randomized, controlled studies and can also be obtained in severe obesity, despite the fact that the patients remain obese (33). The reasons for this apparent dissociation between weight loss and metabolic improvement are not yet clearly understood, but may involve the relationship between visceral fat and metabolic alterations. The results of some studies suggest that the favorable metabolic changes observed in obese patients with CR and weight loss may be directly attributable to a reduction in visceral fat (59). Moreover, visceral adipose tissue is a pivotal organ in aging process and in the determination of life span. There is growing evidence that the effect of reduced adipose tissue mass on life span could be due to the prevention of obesity-related metabolic disorders, including T2DM and atherosclerosis (15).

The mechanism underlying improvements of the aspect of MetS and prevention of aging by CR has been extensively investigated. Aging is associated with increased visceral fat, and recent studies suggested that visceral fat could influence longevity (88, 96, 163). It has recently been proposed that silent information regulator 2 (SIR2) ortholog, sirtuin 1 (SIRT1), the mammalian ortholog of the life-extending yeast gene *SIR2* are involved in the molecular mechanisms linking lifespan to adipose tissue. SIRT1 represses peroxisome proliferator-activated receptor (PPAR)-γ transactivation and inhibits lipid accumulation in adipocytes (113). The favorable effect of adipose tissue reduction on lifespan could be due to increased production of anti-inflammatory adipocytokines and decreased production of pro-inflammatory adipocytokines as described before. Although many model organisms have consistently demonstrated positive responses to CR, it remains to be shown whether CR will extend lifespan in humans. The first results from a long-term, randomized, controlled CR study in nonhuman primates showing statistically significant benefits on longevity have now been reported (27). Additionally, positive results from short-term, randomized, controlled CR studies in humans are suggestive of potential health and longevity gains (61). However, the current environment of excess caloric consumption and high incidence of overweight/obesity illustrate the improbable nature of the long-term adoption of a CR lifestyle by a significant proportion of the human population. Thus, the search for substances that can reproduce the beneficial physiologic responses of CR without a requisite calorie intake reduction, termed CR mimetics, has gained momentum.

The molecular mechanism underlying the efficacy of reduction of visceral fat mass by CR to reduce cardiovascular risk factors may be related to increased generation of adiponectin in the visceral adipocytes (158). It has been demonstrated that CR in rats significantly increases the level of circulating adiponectin, a distinctive marker of differentiated adipocytes (167). PPAR-γ is a member in the nuclear receptor superfamily that mediates part of the regulatory effects of dietary fatty acids on gene expression and may be a molecular link between CR and increased generation of adiponectin. CR for 2 and 25 months, significantly increased the expression of PPAR-γ, C/EBPβ, and Cdk-4, and partially attenuated age-related decline in C/EBPα expression relative to rats fed *ad libitum* (166). As a result, adiponectin was upregulated at both mRNA and protein levels, resulting in activation of target genes involved in fatty acid oxidation and fatty acid synthesis. Moreover, CR significantly decreased the ratio of C/EBPβ isoforms LAP/LIP, suggesting the suppression of gene transcription associated with terminal differentiation while facilitating preadipocytes proliferation. Morphometric analysis revealed a greater number of small adipocytes in CR relative to *ad libitum* feeding. Immunostaining confirmed that small adipocytes were more strongly positive for adiponectin than the large ones. Overall, these results suggest that CR increased the expression of adipogenic factors and maintained the differentiated state of adipocytes, which is critically important for adiponectin biosynthesis. On the other hand, adiponectin is a CR mimetic. It has been demonstrated that mice with transgenic expression of human adiponectin that had persistent hyperadiponectinemia exhibited significantly decreased weight gain associated with less fat accumulation and smaller adipocytes in both visceral and subcutaneous adipose tissues (106). Macrophage infiltration in adipose tissue was markedly suppressed in the transgenic mice. In the hyperadiponectinemic mice, daily food intake was not altered, but oxygen consumption was significantly greater, suggesting increased energy expenditure. Moreover, high-calorie diet–induced premature death was almost completely prevented in the hyperadiponectinemic mice in association with attenuated oxidative DNA damage. The transgenic mice also showed longer life span on a conventional low-fat chow.

Adiponectin circulates mainly as a low-molecular-weight (180 kDa) hexamer and a high-molecular-weight ($\sim$360 kDa) multimer. Adiponectin multimers exert differential biologic effects, with the high-molecular-weight multimer associated with favorable metabolic effects, that is, greater insulin

sensitivity, reduced visceral adipose mass, reduced plasma triglycerides, and increased high-density lipoprotein (HDL)-cholesterol (75), and adiponectin knockout mice manifest insulin resistance, IGT, and dyslipidemia (168). Adiponectin, thus, influences atherosclerosis by affecting the balance of atherogenic and antiatherogenic lipoproteins in plasma, and by modulating cellular processes involved in foam cell formation. The metabolic effects of adiponectin are mediated thorough adiponectin receptor-1 and adiponectin receptor-2 (68). These adiponectin receptors are linked to AMP-activated protein kinase (AMPK) and PPAR-α, respectively. AMPK is a cellular energy sensor that contributes to the regulation of energy balance and caloric intake (21, 150). The activity of AMPK is determined by cellular AMP/ATP. AMPK can phophorylate several enzymes involved in anabolism to prevent further ATP consumption, and induces some catabolic enzymes to increase ATP generation. Further, AMPK stimulates glucose utilization in the skeletal muscle and inhibits gluconeogenesis in the liver (159). On the other hand, PPAR-α participates in fatty acid oxidation, thereby increasing energy consumption.

Adiponectin is known to enhance ischemic tolerance in the heart. It has been demonstrated that short-term CR increases adiponectin levels and exerts a cardioprotective effect against ischemia/reperfusion injury in the wild-type mouse but not adiponectin antisense transgenic mouse heart (131), suggesting that adiponectin is an obligatory mediator of CR-induced ischemic tolerance in the heart. This cardioprotective effect of adiponectin is mediated by AMPK-mediated signaling. Prolonged CR also confers ischemic tolerance, but this effect is independent of AMPK and mediated by a NO-dependent increase in nuclear Sirt1 (130), which is responsible for a NAD-dependent deacetylase and prevention of apoptosis in cardiac myocytes (3). Thus, CR increases ischemic tolerance *via* adiponectin- and Sirt1-dependent mechanisms.

Cardioprotection by adiponectin is at least in part mediated by an antioxidative/nitrosative effect. The recent study has demonstrated that adiponectin reduces oxidative/nitrosative stress by inhibiting NADPH oxidase and iNOS expression and ameliorates ischemia/reperfusion injury in mice (139), and this action is AMPK independent (154). A recent study (86) using a rat model of nonalcoholic steatohepatitis suggests that this antioxidative effect may be mediated by adiponectin receptor-2.

Adiponectin also acts as an anti-inflammatory molecule through a receptor-independent mechanism. The serum concentration of adiponectin exceeds a micromolar level that is extremely higher than estimated its receptor density. Thus, receptor-independent mechanism has been implicated in the anti-inflammatory action of adiponectin. This anti-inflammatory effect may play a crucial role in preventing the development of atherosclerosis and vulnerable plaque. Plaque necrosis arises from a combination of foam cell apoptosis and defective clearance of these dead cells, a process called efferocytosis (137). Defective efferocytosis contributes to necrotic core and the vulnerable plaque formation within advanced atheroma that is thought to promote plaque disruption and, ultimately, acute atherothrombotic vascular disease (144). Molecular-genetic causation studies in mouse models of advanced atherosclerosis have provided evidence that several molecules known to be involved in efferocytosis, including complement C1q, play important roles in the clearance of apoptotic cells in advanced plaques (144). Molecular structure of adiponectin is akin to complement C1q, and adiponectin binds to a number of target molecules, including damaged endothelium and the surface of apoptotic cells (110). Thus, adiponectin may play a crucial role in efferocytosis and prevention of vulnerable plaque formation. Although a recent study showed lack of association between adiponectin levels and atherosclerosis (99), this attractive hypothesis needs to be explored. The potential roles of adiponectin in prevention of cardiovascular risk are illustrated in Figure 4.

Exercise

Several nonpharmacological interventions can prevent endothelial dysfunction or improve impaired endothelium-dependent vasodilation. Probably, the most effective nonpharmacological measure for the management of MetS is represented by aerobic physical activity, which can reduce production of oxidative stress associated with increasing age. It has been demonstrated that exercise alone is an effective nonpharmacological treatment strategy for insulin resistance, MetS, and cardiovascular disease risk factors in older obese adults (160). In addition, several randomized, controlled studies have shown that aerobic types of exercise are protec-

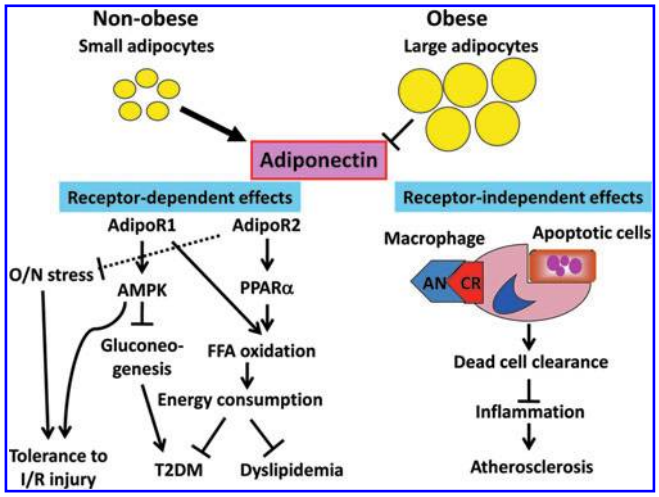

FIG. 4. Potential roles of adiponectin in the prevention of cardiovascular risk factors. Small adipocytes in the visceral adipose tissue in nonobese subjects increase generation of adiponectin through adiponectin receptor-1 (AdipoR1) and adiponectin receptor-2 (AdipoR2)-dependent and -independent mechanisms. AdipoR1 improves insulin resistance and inhibits the development of type-2 diabetes mellitus (T2DM) by inhibiting gluconeogenesis in the liver and stimulating glucose utilization in the skeletal muscle through the action of AMP-kinase (AMPK). The AdipoR1-AMPK signaling also confers tolerance to ischemia/reperfusion (I/R) injury. AdipoR2 increases free fatty acid (FFA) oxidation and energy consumption though the activation of peroxisome proliferator activated receptor-α (PPAR-α), thereby preventing T2DM and dyslipidemia. AdipoR2 may also be involved in tolerance to I/R injury by inhibiting oxidative/nitrosative (O/N) stress (*dotted line*). A receptor-independent action of adiponectin (AN) is involved in an antiatherosclerotic effect through dead cell clearance and inhibition of inflammation by acting as a complement for macrophages to eliminate apoptotic cells from the atheromatous plaque.

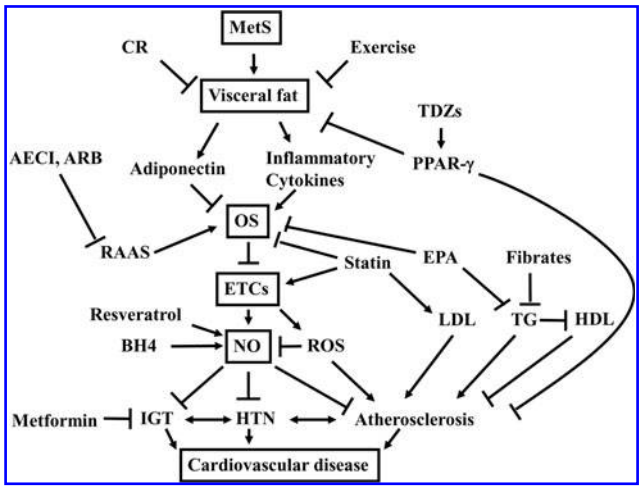

FIG. 5. Schematic drawing of therapeutic opportunities for prevention of cardiovascular disease in MetS. MetS is characterized by the cluster of cardiovascular risk factors: impaired glucose tolerance (IGT), hypertension (HTN), and dyslipidemia that culminate in cardiovascular disease. Reduction of visceral fat by caloric restriction (CR) and exercise increases adiponectin and decreases inflammatory cytokines, thereby inhibiting systemic inflammation and oxidative stress (OS) to endothelial cells (ETCs). Inhibition of the renin-angiotensin-aldosterone system (RAAS) by employing an angiotensin converting inhibitor (ACEI) or angiotensin II type-1 receptor blocker (ARB) is more direct approach to inhibit OS to ETCs. Antidyslipidemic agents, statin and eicosapentaenoic acid (EPA), exert ETC protection independent of their effects on LDL and triglyceride (TG) levels. Fibrates, on the other hand, decrease TG and increase high-density lipoprotein (HDL), thereby preventing atherosclerosis. Although OS to ETCs promotes further generation of ROS that inhibit bioavailability of NO by generating peroxynitrite or causing uncoupling of NO synthase, ETC-derived NO synthesis can be increased by resveratrol and an NO synthase cofactor tetrahydrobiopterin (BH4). Antidiabetic agents such as metformin and thiazolidinediones (TDZs) that act through an increase in insulin sensitivity improve not only IGT but also hyperinsulinemia responsible for atherosclerosis and cardiovascular disease.

tive against age-related increases in visceral adiposity in growing children and adolescents (72). Moreover, physical activity can improve endothelial dysfunction even in patients with cardiovascular risk factors such as essential HTN. It is worth noting that most of nonpharmacological measures for prevention of cardiovascular risk act by preventing or reducing inflammation and oxidative stress. Current evidence supports that aerobic exercise, alone or combined with CR, improves symptoms of MetS, possibly by altering systemic levels of inflammatory adipocytokines (162). A number of studies show that increased physical activity leads to lower circulating levels of pro-inflammatory cytokines and higher levels of adiponectin. The mechanism underlying reduced oxidative stress in visceral white adipose tissue by exercise may be related to the decreased expression of NADPH oxidase in addition to an enhanced antioxidant defense system, and the prevention of dysregulated production of inflammation-related adipocytokines (121), suggesting that exercise is a fundamental approach to protect against cardiovascular risk in MetS. However, limited data show that exercise training does not influence serum adiponectin levels (6, 83, 101). Conversely, exercise training may influence pro-inflammatory cytokine production (18, 46, 148). Future studies are needed to investigate the cellular mechanisms by which exercise training affects inflammation and whether alterations in inflammation are one mechanism by which exercise improves components of MetS in at-risk individuals.

Antagonists against RAAS

Any anti-HTN therapies have been shown to reduce the risk of total major cardiovascular events. Recently, the relevance of the type of anti-HTN therapy used to treat HTN patients in facilitating the development of T2DM has been demonstrated in different trials. The recognition of the risk present in HTN patients with MetS for developing T2DM reinforces the need to consider the ideal anti-HTN therapy, either mono or combination, in these patients. The available evidence showing that an ACE inhibitor or an ARB is the most suitable therapy to be started in these patients, alone or in combination, due to their capacity to prevent or retard the development of T2DM (48, 124).

Adipocytes are a suggested source of components of the RAAS, with regulation of their production related to obesity-HTN (22). Ang II has been demonstrated to promote oxidative stress *via* overexpression of NADPH oxidase in adypocytes (50, 66). It has been demonstrated that blockade of Ang II type-1 receptors reduces oxidative stress in adipose tissue and ameliorates adipocytokine dysregulation (74). Therefore, ACE inhibitors and ARBs represent promising tools for inhibiting oxidative stress in adipocytes, thereby preventing the production of pro-inflammatory adipokines responsible for systemic inflammation and oxidative stress in MetS.

Insulin sensitizers

There are a number of pharmacological tools for treatment of T2DM. Ever since insulin was discovered in the early 20th century, it had been an only drug in patients with insulin-dependent DM and T2DM for many years. Then, sulfonyl urea became available in the mid 20th century, and it had been a first choice of drugs in patients with T2DM. However, recognition of deleterious cardiovascular effects of hyperinsulinemia in patients with T2DM has shifted paradigm of T2DM treatment from increasing blood insulin level to insulin sensitivity. Insulin acts as not only blood glucose-lowering hormone but also acts as a growth factor under oxidative stress that may be involved in atherosclerosis. Ruige and associates (119) have demonstrated that hyperinsulinemia is an independent risk factor of coronary artery disease. In addition, insulin activates the PI3K-Akt axis that is known to play a role in the control of aging (9, 109), thereby possibly restricting life span. On the contrary, pharmacological interventions that increase insulin sensitivity reduce cardiovascular complications and are, therefore, expected to promote longevity. A PPAR-γ activator TZD and metformin are quite promising tools to substantially improve the cluster of cardiovascular risk factors in patients with MetS complicated with T2DM, whereas α-glucosidase inhibitors may also be effective to prevent hyperinsulinemia by inhibiting postprandial hyperglycemia. A newly emerged antidiabetic drug dipeptidyl peptidase-IV (DPP-4) inhibitors increase glucose-dependent stimulation of insulin secretion, and unlike

sulfonyl urea it do not cause hypoglycemia or inhibit ATP-sensitive potassium channels that are thought to be crucial in cytoprotection in both pancreatic islet β-cells and cardiomyocytes (39, 114). Although DPP-4 inhibitors are promising antidiabetic drugs, the cardiovascular effect of the DPP-4 inhibitors in patients with T2DM remains to be investigated, because they induce postprandial hyperinsulinemia. Focussed here are two representative insulin sensitizer, TZDs and metformin, because these antidiabetic drugs have an established underlying mechanism for beneficial cardiovascular effects and have shown strong clinical evidence of reduced cardiovascular risk in patients with T2DM.

The beneficial effect of TDZ is attributed to activation of PPAR-γ. A flurry of human and animal studies has shed a light on the mechanisms how TZDs act, and which of their physiological effects are dependent on PPAR-γ. It is now evident that TDZ stimulates PPAR-γ in adipocytes in the visceral adipose tissue and increases the generation of adiponectin (24, 126). Further, new roles for PPAR-γ signaling beyond the metabolic effects through adiponectin have been discovered in inflammation, bone morphogenesis, endothelial function, cancer, longevity, and atherosclerosis (59). All of the major cells in the vasculature express PPAR-γ, including endothelial cells, vascular SMCs, and monocytes/macrophages (40, 55). PPAR-γ is present in intimal macrophages in human atheromas (5). Recent experimental studies provide evidence that PPAR-γ may function to protect the vasculature from injury (81, 103). Cell culture studies have shown that TZD inhibits both the proliferation and migration of vascular SMCs (105). TZDs block vascular SMC growth by inducing cell cycle arrest in G1 through an inhibition of retinoblastoma protein phosphorylation (80). Migration of monocytes and vascular SMCs is also inhibited by TZDs, possibly through decreased matrix metalloproteinase production (108). Activation of PPAR-γ by TZDs in macrophages induces ATP binding cassette transporter A1 expression to promote reverse cholesterol transport (102). These effects of PPAR-γ culminate in protection of endothelial cells. Thus, TZD activation of PPAR-γ may protect against atherosclerosis both by normalizing pro-atherogenic metabolic abnormalities of the insulin resistance/diabetes milieu and through an inhibition of vascular SMC growth and movement. Consistent with this hypothesis is the fact that in a large, placebo-controlled, outcome study in secondary prevention, PROactive study, the use of pioglitazone in addition to an existing optimized macrovascular risk management resulted in a significant reduction of macrovascular endpoints within a short observation period that was comparable to the effect of statins and ACE inhibitors in other trials (38). In addition, the efficacy of TDZs in preventing atherosclerosis in patients with T2DM has been confirmed by subsequent clinical trials (51). These results underline the value of TDZs for managing the increased cardiovascular risk in MetS complicated with T2DM.

Metformin is widely used as a hypoglycemic reagent for T2DM. The reduction of hepatic gluconeogenesis is thought to be a key effect of metformin, and its molecular mechanism is attributed to the reduction of glucose-6-phosphatase activity, as well as suppression of mRNA expression levels of multiple genes linked to the metabolic pathways involved in glucose and lipid metabolism in the liver (60). However, metformin exerts cardiovascular protection independent of the blood glucose-lowering efficacy. It has been demonstrated that metformin improves endothelial functions in Otsuka Long-Evans Tokushima fatty rat mesenteric arteries by suppressing vasoconstrictor prostanoids and by reducing oxidative stress (85). Metformin confers cardioprotection against ischemia/reprfusion injury through a PI3K-mediated inhibition of mitochondrial permeability transition pore opening (13). In addition, metformin attenuated oxidative stress-induced cardiomyocyte apoptosis and prevented the progression of heart failure in dogs, and this cardioprotective effect was dependent on the activation of AMPK (123). Consistent with the beneficial cardiovascular effect in animals, the UK Prospective Diabetes Study (144a) showed that metformin decreases macrovascular morbidity and mortality independent of glycemic control in patients with T2DM. A subsequent randomized, placebo-controlled trial has demonstrated that metformin can reduce inflammatory markers and improve endothelial function (35). The potential vascular protective effects of metformin may complement other strategies within such a framework. Thus, metformin treatment may represent a relevant element of an integrated pharmacotherapy to prevent not only T2DM but also cardiovascular disease in MetS.

Antidyslipidemic agents

Because atherosclerosis is facilitated by dyslipidemia and oxidative stress in patients with T2DM, numerous studies have investigated relative contribution of dyslipidemia and oxidative stress to atherogenesis in diabetic animals. It has been demonstrated that antioxidants vitamin E and probucol and a 3-hydroxy-3-methylglutaryl Co-A reductase inhibitor lovastatin significantly reduced plasma triglyceride in the diabetic hamsters fed the atherogenic diet (42). In this study, vitamin E treatment increased total cholesterol, and probucol reduced HDL-cholesterol without affecting total cholesterol, whereas lovastatin reduced total cholesterol and selectively decreased non-HDL-cholestrerol, and significantly reduced fatty streak lesion formation in the aortic arch. Although vitamin E and probucol were effective in reducing several indices of oxidative stress, including plasma lipid peroxides, cholesterol oxidation products, and *in vitro* LDL oxidation, they had no effect on fatty streak lesion formation. These results indicate that the LDL in diabetic animals is more susceptible to oxidation than in nondiabetic hamsters and that not only vitamin E and probucol but also lovastatin provide antioxidant protection. It appears that in this combined model of T2DM and hypercholesterolemia, lovastatin prevented progression of fatty streak lesion formation by reducing total cholesterol and non-HDL-cholesterol and inhibiting oxidative stress.

The pleiotropic effects of statins that prevent atherogenesis have been extensively investigated. Emerging evidence suggests that these cholesterol-independent effects are predominantly due to their ability to inhibit isoprenoid synthesis, particularly geranylgeranylpyrophosphate and farnesylpyrophosphate, which are important post-translational lipid attachments of the Rho GTPases and activation of its downstream target, Rho-kinase (ROCK) (117). Inhibition of ROCK by statins may also be associated with inhibition of oxidative stress mediated by activation of NADPH oxidase. It has been shown that rosuvastatin attenuated the Ang II-mediated upregulation of NAPDH oxidase subunits as well as nuclear factor-kappaB associated with downregulation of

Ang II type-1 receptors and the lectin-like oxidized LDL receptor LOX-1, leading to the reduction of oxidized LDL (70). In this regard, while increased ROCK activity is associated with endothelial dysfunction, cerebral ischemia and coronary vasospasms in MetS, the inhibition of ROCK by statins leads to upregulation of eNOS, decreased vascular inflammation, and reduced atherosclerotic plaque formation (165).

In MetS, increased triglyceride in conjunction with elevated LDL plays a crucial role in atherogenesis. Such combined dyslipidemia often requires multiple antidyslipidemic agents. Fibrates effectively reduce fasting and postprandial hypertriglycemia, shift the distribution of LDL particles toward less dense particles, and increase HDL-cholesterol. The finding of triglyceride-rich lipoproteins in human atheroma has provided substantial pathophysiologic evidence for a direct role of triglyceride in atherogenesis (25, 58). Thus, fibrates represent particularly important tools to manage dyslipidemia in MetS complicated with T2DM. Indeed, compelling evidence from meta-analysis of a number of clinical studies on a large aggregate of patients has established an increased level of triglycerides as an independent risk factor for atherosclerotic coronary heart disease (8, 52, 100). However, combination of statins and fibrates is often contraindicated by increased incidence of myopathy. On the other hand, niacin, fibrates, and bile acid sequestrants are effective in combination with statins in lowering LDL, triglycerides, and total cholesterol levels and increasing HDL. Niacin-statin therapy reduces atherosclerotic progression and coronary events (10, 30, 34, 156). ω-3 polyunsaturated fatty acids, which are abundant in fish oil, are another promising tool for combination therapy for dyslipidemic patients. The Japan Eicosapentaenoic Acid (EPA) Lipid Intervention Study demonstrated that EPA prevented major coronary events, including sudden cardiac death, fatal and nonfatal myocardial infarction, and other nonfatal events, including unstable angina pectoris, angioplasty, stenting, or coronary artery bypass grafting in hypercholesterolemic patients (161). The beneficial effect of EPA significantly correlated with the reduction of triglyceride and the increase in HDL (120), although cardiovascular protection by EPA may also be attributed to the anti-inflammatory effect and inhibition of platelet function (93, 112). Taken all together, both triglyceride and HDL levels correlate with cardiovascular risk and should be considered secondary targets of therapy. Combination therapy can be safe and effective and can be constructed to affect all lipoprotein parameters. However, studies still are needed showing definite evidence on differential therapy in lipid lowering based on prospective, controlled trials with endpoints of macro- and microangiopathy in MetS complicated with T2DM and dyslipidemia.

Other potential pharmacological tools

Epidemiological studies suggest that the consumption of wine, particularly of red wine, reduces the incidence of mortality and morbidity from coronary heart disease. This has given rise to what is now popularly termed the "French paradox" (28). The cardioprotective effect has been attributed to antioxidants present in the polyphenol fraction of red wine. Grapes contain a variety of antioxidants, including resveratrol, catechin, epicatechin, and proanthocyanidins. Of these, resveratrol is present mainly in grape skin, whereas proanthocyanidin is present in the seeds. Emerging evidence indicates that resveratrol confers protection against ischemia/reperfusion injury through its antioxidant activity and upregulation of NO production (56, 63, 116). Moreover, resveratrol modulates vascular cell function, inhibits LDL oxidation, and suppresses platelet aggregation (17). Miatello and associates (89) demonstrated that chronic administration of resveratrol prevented atherosclerosis in rats, and raised the hypothesis that the increase in eNOS activity may contribute to the protective properties of resveratrol against cardiovascular disease. Results from other laboratories support the unifying hypothesis that the improvements in risk factors by resveratrol are mediated by eNOS (111, 118, 164). These results suggest that an adequate supplementation of resveratrol might help to prevent or delay the occurrence of atherogenic cardiovascular disease associated with insulin-resistant states in MetS. In addition, recent data provide interesting insights into the effect of resveratrol on the lifespan of simple eukaryotes such as yeast and flies by activating the longevity genes and has been suggested as a CR mimetic (33, 62, 136, 155), implicating the potential of resveratrol as an antiaging agent in treating age-related human diseases. This attractive property of resveratrol against atherosclerosis and aging should be studied in human especially in patients with MetS. However, the phenolic compound possesses a low bioavailability and rapid clearance from the plasma (29). Thus, bioavailability, metabolism, and tissue distribution of resveratrol in humans need to be clearly established to develop better biological effects.

Another potential pharmacological tool for the management of cardiovascular risk factors in MetS is BH4. BH4 is a cofactor of eNOS, iNOS, and neuronal NOS and necessary for NO biosynthesis. Lack of BH4 is associated with uncoupling of NOS, leading to the generation of more superoxide and less NO that shifts the nitroso-redox balance and may have adverse consequences on cardiovascular function. This transformation of NOS especially eNOS from a protective enzyme to a contributor to oxidative stress has been observed in several *in vitro* models, in animal models of cardiovascular disease, and in patients with cardiovascular risk factors (45, 90, 91). BH4 is highly sensitive to oxidation by ROS and peroxynitrite and is converted to dihydrobiopterin (BH2). Oxidative stress imposed on endothelial cells causes depletion of BH4 and eNOS uncoupling. In many cases, supplementation with BH4 under pathological conditions with oxidative stress has been shown to correct eNOS dysfunction in animal models and patients (45, 90, 91). However, true mechanistic relationship between endothelial BH4 levels and eNOS regulation *in vivo* by administration of BH4 remains controversial. High extracellular BH4 concentrations may result in nonspecific antioxidant effects that indirectly increase NO bioactivity by ROS scavenging rather than by modulation of eNOS activity. Further, the effects of supplementation with BH4 or biopterin analogs on NO bioactivity are unpredictable in vascular disease states in which oxidative stress is increased (140, 146). Indeed, it remains unclear whether adequate eNOS cofactor function *in vivo* is related to absolute BH4 levels in the endothelial cell, or whether the relative balance between reduced BH4 and oxidized BH2 may be more important (147). Intracellular BH4 levels are regulated by the activity of the *de novo* biosynthetic pathway and the salvage pathway. In the *de novo* biosynthetic pathway, guanosine triphosphate cyclohydrolase (GTPCH)-1 catalyzes GTP to dihydroneopterin triphosphate. BH4 is generated by further steps catalyzed by

6-pyruvoyltetrahydropterin synthase and sepiapterin reductase (142). GTPCH-1 appears to be the rate-limiting enzyme in BH4 biosynthesis; transgenic overexpression of GTPCH-1 is sufficient to augment BH4 levels in endothelial cells and preserve NO-mediated endothelial function in diabetic mice (4). In the salvage pathway, BH4 is synthesized from BH2 by sepiapterin reductase and dihydrofolate reductase. Exogenous BH4 is labile in physiological solution. It has been reported that *in vivo* half-life of BH4 is 3.3–5.1 h in the plasma of healthy adult humans (44). Because not all oxidized BH4 is converted to BH2, which is further degraded to dihydroxanthopterin and excreted to urine (129), BH2 availability for the salvage pathway may be limited under oxidative stress even with BH4 supplementation. Thus, sepiapterin may serve as an effective substrate for BH4 *via* the salvage pathway. Folic acid and vitamin C are also able to restore eNOS functionality, most probably by enhancing BH4 levels through mechanisms yet to be clarified (129). The therapeutic efficacy of BH4 has been examined in patients with HTN, peripheral arterial disease, and coronary artery disease, and these studies consistently demonstrated the beneficial effect of BH4 on endothelial dysfunction (71). However, a phase-2 clinical trial sponsored by the U.S. pharmaceutical company BioMarin failed to observe an ameliorative effect of oral administration of BH4 in patients with poorly controlled HTN. Further studies are needed to address whether BH4 or its analogs truly exert salutary effects on endothelial dysfunction induced by a variety of vascular disease. Therapeutic opportunities for prevention of cardiovascular disease in MetS are illustrated in Figure 5.

Concluding Remarks

Abdominal obesity is a cause of all the morbidity of MetS. Oxidative stress develops in hypertrophied adipocytes, which increase the synthesis of pro-inflammatory cytokines, while decreasing anti-inflammatory cytokines. Dysregulation of such adipocytokines is responsible for systemic inflammation and oxidative stress and contributes to the pathogenesis of the obesity-associated morbidity in MetS. Decrease in abdominal obesity by lifestyle interventions is fundamental approach to MetS. However, CR and exercise are often difficult in patients with MetS. Thus, alternative strategies are required to prevent cardiovascular risk in MetS. Accumulating basic research evidence indicates that endothelial cells are primarily affected by inflammation and become a source of further oxidative stress in the vascular wall and surrounding cells, leading to IGT, HTN, and atherosclerosis. Thus, the endothelium is recognized as a major therapeutic target in the prevention and treatment of vascular disease in patients with MetS. The purpose of improving endothelial function is to restore normal biosynthesis of NO and the reduction of excessive generation of ROS. Currently available pharmacological tools such as ACE inhibitors, ARBs, TDZs, metformin, and statins are effective in preventing cardiovascular risk in MetS through reduction of inflammation and oxidative stress either in the visceral adipose tissue or endothelial cells. Further studies are needed to develop more effective strategy to manage cardiovascular risk in MetS.

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Abbreviations Used

ACE = angiotensin-converting enzyme
ACEI = angiotensin-converting inhibitor
AMPK = AMP-activated protein kinase
Ang II = angiotensin II
ARBs = angiotensin II type-1 receptor blockers
BH2 = dihydrobiopterin
BH4 = tetrahydrobiopterin
CR = caloric restriction
DPP-4 = dipeptidyl peptidase-IV
eNOS = endothelial nitric oxide synthase
EPA = eicosapentaenoic acid
GLUT4 = glucose transporter-4
GTPCH = guanosine triphosphate cyclohydrolase
HDL = high-density lipoprotein
HTN = hypertension
IGT = impaired glucose tolerance
iNOS = inducible nitric oxide synthase
IRS = insulin receptor substrate
LDL = low-density lipoprotein
MCP-1 = monocyte chemoattractant protein-1
MetS = metabolic syndrome
NO = nitric oxide
OxLDL = oxidized LDL
PAI-1 = plasminogen activator inhibitor-1
PI3K = phosphatidylinositol 3-kinase
PPAR = peroxisome proliferator activated receptor
RAAS = renin-angiotensin aldosterone system
ROCK = Rho-kinase
ROS = reactive oxygen species
SMCs = smooth muscle cells
T2DM = type-2 diabetes mellitus
TDZs = thiazolidinediones
TNF-α = tumor necrosis factor-α

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