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METABOLIC SYNDROME, ADIPONECTIN AND FAT ROS

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The metabolic syndrome, a cluster of insulin resistance, elevated blood pressure, and atherogenic dyslipidemia, is a common basis of atherosclerosis. Accumulation of intra-abdominal visceral fat stands upstream of the metabolic syndrome. Adipose tissue expresses a variety of genes for bioactive secretory proteins conceptualized as adipocytokines. We discovered a novel adipose-specific protein named adiponectin from human fat cDNAs. Adiponectin circulates in the plasma and its serum level is decreased in visceral fat accumulation. Results of experimental and clinical researches have demonstrated that hypoadiponectinemia underlies the pathogenesis of multiple diseases related to visceral fat accumulation, including atherosclerosis, hypertension, cardiac failure, insulin resistance, diabetes, hepatic steatosis, inflammatory bowel disease, and cancers. Recently, we revealed fat-derived reactive oxygen species (fat ROS) as an upstream factor in the development of hypoadiponectinemia and metabolic syndrome. Intervention targeting visceral fat accumulation, hypoadiponectinemia and fat ROS should be the way to therapeutically tackle the metabolic syndrome. ***Biomed Rev 2006; 17: 1-10.***

Key words: Adipocytokines, adiponectin, fat ROS, metabolic syndrome, visceral fat

INTRODUCTION

Metabolic syndrome (MetS) is the pathologic condition coexisting several risk factors for cardiometabolic diseases, including atherosclerosis, hypertension, type 2 diabetes, and dyslipidemia (1). From our and others' clinical works, it is revealed that distribution of body fat more strongly associates with the development of MetS, than absolute amount of body fat, i.e. obesity (2-6). Recently, the diagnostic criteria of MetS is defined globally (1). It includes increase of waist circumference, good indicator for visceral fat accumulation, as a necessary component, and additional 2 or 3 risk factors. There has been a growing evidence that adipose tissue is a huge endocrine organ to produce and secrete a varieties of biological active substances which we conceptualised as adipocytokines (7,8). Dysregulated productions of adipoctytokines in visceral fat obesity lead to the development of MetS. Adiponectin is one of adipocytokines we discovered from human adipose cDNAs (9,10).

Here we summarize the historical movement that led to the concept of MetS and adipocytokines. Especially, we put a high tension on the role of adiponectin both in the pathogenesis and

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the therapy of MetS. We also highlight our recent progress featuring the significance of fat-derived reactive oxygen species (fat ROS) as an upstream factor in the development of hypoadiponectinemia and MetS. Reduction of visceral fat, improvement of hypoadiponectinemia, and decreasing fat ROS should be now being recognized in the prevention and cure of MetS and its associated cardiometabolic diseases.

CONCEPT OF METABOLIC SYNDROME

Multiple risk factor syndrome is a cluster of hyperglycemia, hypertriglyceridemia, increased level of low high-density lipoprotein (HDL) cholesterol and high blood pressure in an individual. Historically, the similar concepts were proposed as "syndrome X" by Reaven (11), "deadly quartet" by Kaplan (12), "insulin resistance syndrome" by De Fronzo (13), and "visceral fat syndrome" by Matsuzawa (14). Syndrome X and insulin resistance syndrome were based on existence of insulin resistance as a common upstream feature. Deadly quartet pointed the significance of abdominal obesity, as well as glucose intolerance, hypertriglyceridemia, and hypertension, whereas visceral fat syndrome, for the first time, put the visceral fat accumulation as a necessary upstream component in the syndrome's pathogenesis similarly to the concept of MetS currently defined.

During the early 1980's, our group introduced computer tomography (CT) scan method to evaluate the amount of adipose tissue associated with obesity (4). The assessment of the total amount of subcutaneous fat by this method did not provide any additional information on obesity-related diseases beyond body mass index (BMI). However, subjects predominantly with intra-abdominal visceral fat excess were revealed to have glucose intolerance, dyslipidaemia and hypertension more frequently, as compared to those with subcutaneous fat accumulation. These results reached the concepts of visceral fat type obesity (VFO) and subcutaneous fat type obesity (SFO) (Fig. 1). Furthermore, approximately 40% of the patients with cardiovascular disease (CVD), irrelevant to obesity or not, was shown to have visceral fat accumulation, accompanied by multiple coronary risk factors (5). Thus, we conceptualized this pathogenetic condition as visceral fat syndrome, almost identical to the MetS currently defined.

CONCEPT OF ADIPOCYTOKINES

To elucidate the missing link between visceral fat accumulation and the development of the clustering of multiple risk factors, our group comprehensively investigated the gene

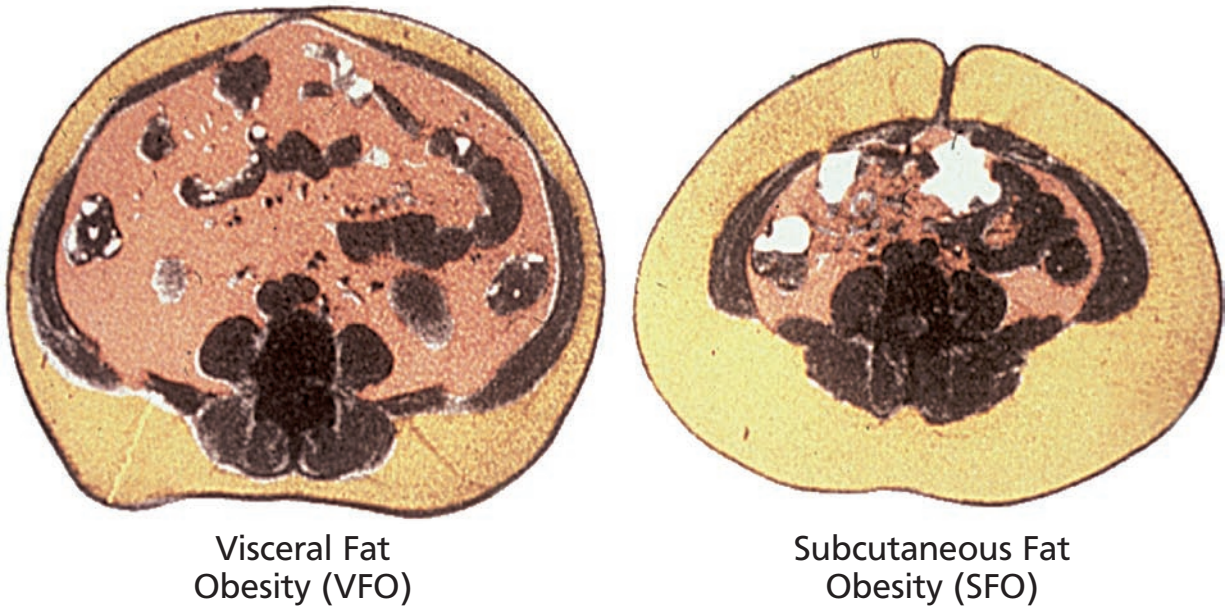

Figure 1. *Visceral fat type obesity (VFO) and subcutaneous fat type obesity (SFO) are assessed by computed tomograpy (CT) at umbilicus level. VFO is more associated with various obesity-related complications, such as insulin resistance, diabetes, dyslipidemia, hypertension, and coronary artery disease, than SFO.*

expression profiles in human adipose tissues, and compared the data with the results obtained from the similar analysis of other human tissues and organs in the projects called Body Mapping (9). The results from Body Mapping revealed that adipose tissues, especially visceral fat, abundantly expressed a variety of genes for secretory proteins. Around 30 % of the genes expressed in human visceral fat tissue and 20 % in human subcutaneous fat tissue coded the genes for secretory proteins. It was shown that adipose tissues expressed the mRNAs for secretory proteins abundantly among the dozens of human tissues and organs analysed. We found plasminogen activator inhibitor type 1 (PAI-1) and heparin binding EGF-like growth factor (HB-EGF) in visceral fat cDNA library. Our subsequent study showed that accumulated visceral adipose tissue overproduces and secretes PAI-1, which in turn raises the risk for thrombotic disorders (7). These were the first examples recognizing adipose tissue-derived secretory factors as direct modulators of vascular biology. We conceptulaized such factors as adipocytokines (7).

Adiponectin

Identification and significance

In Body Mapping, we discovered a novel gene encoding adipocyte-derived secretory protein, lately named adiponectin (10). Adiponectin was expressed exclusively and most abundantly in adipose tissue. The molecule has two domains, a collagen-like fibrous and C1q-like globular domains. Single molecules bind together and form a high-ordered structure (15). We established ELISA system to measure plasma adiponectin levels in human subjects. There were two surprising facts we were faced by in the initial studies: *(i)* plasma adiponecitn levels were extremely high up to 5-20 μg/ml in healthy humans, and *(ii)* plasma adiopnectin levels decreased with the accumulation of body fat, especially visceral fat, while the blood concentrations of other adipocytokines known to date, like leptin and PAI-1, increased in parallel with fat accumulation in body. Following numerous studies have revealed that decreased plasma adiponectin (hypoadiponectinemia) associated with visceral fat accumulation is an upstream pathological condition of many diseases (Fig. 2).

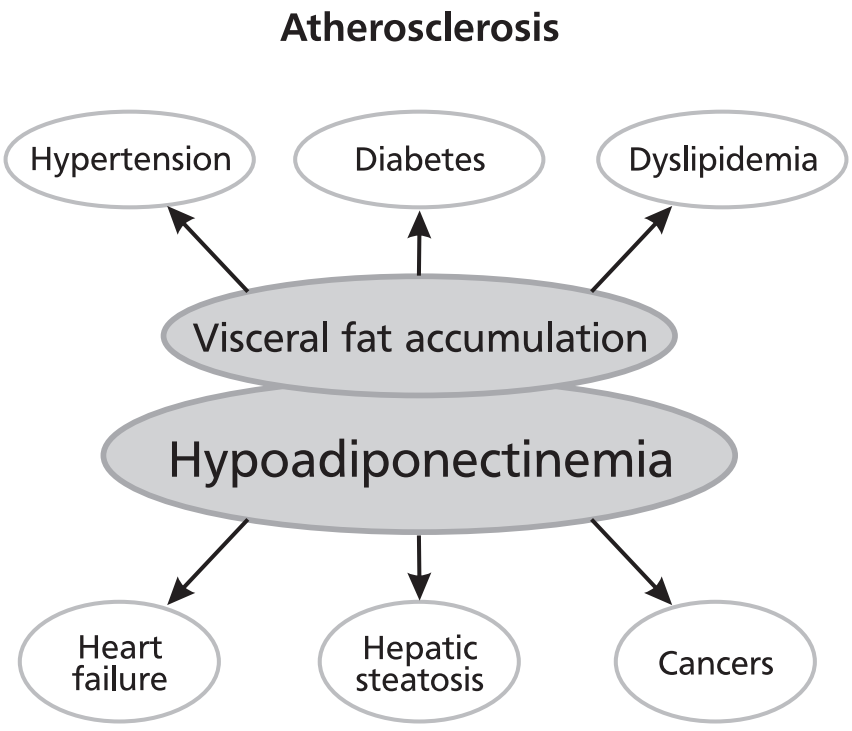

Figure 2. *Hypoadiponectinemia associated with visceral fat accumulation is located at upstream of various lifestyle-related diseases, including diabetes, hypertension, dyslipiedemia, and atherosclerosis. Recent works revealed the association of hypoadiponectinemia with the pathogenesis of heart failure, hepatic steatosis, and various cancers.*

DISEASES FEATURED BY ADIPONECTIN DEFICIT

Atherosclerosis

Our very initial study showed recombinant adiponectin protein bound tightly to collagens I, III, and V, which were known to present in the subendothelial intima (16). Following *in vivo* analysis found that adiponectin adhered subendotherial space in the endothelium-injured arterial walls. Further clinical study showed that plasma adiponectin levels were significantly lower in subjects with CVD, as compared with BMI-matched controls (17). Experimentally, we treated the recombinant adiponectin protein on various types of vascular cells. Administration of adiponectin suppressed *(i)* TNFα-induced expression of adhesion molecules in the vascular endothelial cells by inhibiting nuclear translocation of NF-κB (2,17,18), *(ii)* growth factor-induced proliferation and migration of smooth muscle cells by inhibiting mitogen activated protein (MAP) kinase pathway (3,19), and *(iii)* foam cell transformation of the macrophages by inhibiting the expression of scavenger receptor (20). These data suggested that circulating adiponectin accumulates in the places where the endothelial barrier is damaged, and has protective effects against the series of process for the development of atherosclerosis. To confirm this concept, we generated mice disrupting adiponectin gene. When the vascular walls of adiponectin-knockout (APN-KO) mice were injured by catheter wire, they exhibited severer neointimal thickening (21). Adiponecitn overproduction using adenovirus decreased the size of lipid droplets in the fatty-streak lesion and atherosclerotic plaque in apolipoprotein E-deficient mice (22). These were accompanied by reduced expression of mRNAs of VCAM-1, class A scavenger receptor, and TNF-α in the aorta. These results confirmed the pivotal role of adiponectin deficit in the development of atherosclerosis in animal model.

In human study, subjects with plasma adiponectin levels less than 4 μg/ml reached twice high incidence for CVD, as an independent factor (23). The predictive effect of adiponectin on cardiovascular diseases was initially documented in patients with end-stage renal disease (24). Recent study, with entry of 18,000 US men, also reported that high adiponectin in plasma became a negative indicator for the incidence of myocardial infarction (25). In other study, hypoadiponectinemia was associated with an increased risk of 5-year mortality after ischemic stroke, independently (26). These clinical studies strengthened and confirmed the role of hypoadiponectinemia in atherosclerosis.

Hypertension

Vascular endothelial dysfunction is a common feature of the MetS. Plasma adiponectin levels were significantly correlated with the forearm vasodilator response (27), suggesting that hypoadiponectinemia might impair endothelium dependent vasodilation. Indeed, the acetylcholine, but not sodium nitroprusside, -induced vasorelaxation was impaired in adiponectin-deficient mice (28). Nitric oxide (NO) synthesized by endothelial cells contributes to the vasodilatation. Adiponectin supplement activated AMP kinase and Akt, and subsequently phosphorylated endothelial NO synthase (29) in vascular endothelial cells. Michael Quon *et al* demonstrated that adiponectin enhanced the production of NO using high-sensitive fluorescent method (30). Adiponectin deficiency in mice developed high salt-induced hypertension (28). Furthermore, plasma adiponectin levels were inversely correlated with blood pressures in human subjects regardless with or without insulin resistance (31). Taken together, hypoadiponectinemia may, through disturbing NO production, contribute to the pathogenesis of hypertension in the MetS.

Cardiac failure

Pressure overload by transverse aortic constriction caused severer concentric cardiac hypertrophy in the adiponectin deficient mice (32). The KO mice had smaller left ventricular cavity and died earlier. Supplement of adiponectin using adenovirus reversed the death rate to that of wild type mice. Adiponectin KO mice showed increased extracellular signal-regulated kinase (ERK) and diminished AMP-activated protein kinase (AMPK) signaling in the myocardium. In cultures of cardiac myocytes, adiponectin activated AMPK and inhibited ERK activation. These results suggested that adiponectin should inhibit hypertrophic signaling in the myocardium through activating AMPK. Recent study also demonstrated that adiponectin protected ischemia-reperfusion injury in the heart of mice (33). Parts of these effects were considered mediation via AMPK and COX-2 pathways.

Insulin resistance and diabetes

Plasma levels of adiponectin were lower in the diabetic patients even after BMI-matching, compared to non-diabetic subjects. Plasma adiponectin levels positively correlated with insulin sensitivity determined by glucose clamp in humans and non-human primates (34,35). Pima Indians with high plasma adiponectin at baseline exhibited lower risk of the newly

onset of diabetes (36). When APN-KO mice were fed with high-fat/high sucrose diet, KO mice exhibited higher plasma glucose and insulin levels, showing the characteristics of diet induced-insulin resistance and diabetes (37). These phenotypes were reversed by the adenovirus-mediated supplement of adiponection. Tissue cultured and *in vivo* experiments revealed that adiponectin's ability to enhance insulin action was attributed to its effect on liver and skeletal muscle, mainly through activating AMP-kinase pathway (38,39).

Inflammation

The inflammatory process is common feature of the MetS and involved in the development of CVD. The adipose tissue produces various proinflammatory cytokines such as interleukin-6 (IL-6) and TNF-α, which are secreted into the circulation, and may accelerate the inflammatory process in vascular wall. In vascular endothelial cells, adiponectin suppresses TNF-α signaling pathway and inhibits the expression of adhesion molecules (17,20). In macrophages, adiponectin specifically and markedly suppresses the expression of TNF-α, and almost shut down the secretion of TNF-α from the cells (40). Interestingly, TNF-α also suppresses the expression and the secretion of adiponectin in adipocytes (37). Thus, TNF-α and adiponectin suppress the expression of each other. Recently, an inflammatory marker, high-sensitive CRP (hsCRP), was featured as a novel risk factor for both CVD and type 2 diabetes. Clinically, plasma levels of hsCRP correlated negatively with plasma adiponectin levels. In adiponectin KO mice, adipose expression of TNFα was augmented (37). Similarly, $mRNA^{CRP}$ expression incresed in adipose tissue of adiponectin KO mice (41). In human, $mRNA^{CRP}$ levels in fat correlated negatively with adiponectin mRNA levels (41). These data suggest that reduced expression of adiponectin induces the expression of CRP and TNFα in adipose tissue. In these negative loops between adiponectin and proinflammatory cytokines of CRP and TNF-α, reduction of adiponectin should promote inflammatory process locally in adipose tissue, and remotely in vascular wall, liver, skeletal muscle and other organs, which may underlie systemic low-grade inflammatory activity in MetS.

Plaque rupture is a major event in coronary heart disease. Macrophages take up oxidized LDL and form plaque lesions. Macrophages also produce various metalloproteinases and cause matrix degradation, leading to plaque rupture. Tissue inhibitor of metalloproteinase (TIMP-1) suppresses matrix metalloproteinase, and inhibits matrix degradation, leading to stabilize the plaque. We found that adiponectin induced $mRNA^{TIMP-1}$ expression in macrophages using the gene chip technique (42). We also showed that adiponectin suppressed the expression of scavenger receptor and inhibits plaque formation. Taken together, adiponectin inhibits plaque formation and increases plaque stability, which should contribute to prevention of acute coronary syndromes.

Hepatic steatosis and liver injury

The subjects with MetS often accompany fatty liver. Persistence of fatty liver in obesity sometimes causes hepatic inflammation recently designated non-alcoholic steatohepatitis (NASH). Collagen fibers in liver are synthesized by hepatic stellate cells. We showed that adiponectin significantly suppressed PDGF-induced proliferation and TGF-β-induced collagen production in hepatic stellate cells (43). Adiponectin KO mice showed severer fibrosis induced by carbon tetrachloride compared to wild-type mice through the increase and activation of hepatic stellate cells (43). Such changes were canceled by the adiponecitn supplement by adenovirus. The pathogenesis of NASH and alcoholic liver injury is considered to involve gut-derived lipopolysaccharide (LPS). The complex of LPS and LPS-binding protein (LBP) activates Kupffer cells to secrete TNF-a, which plays an important role in liver injury. On the other hand, IL-10, which is secreted by Kupffer cells, has strong anti-inflammatory effects in the liver, and prevents liver fibrosis. Our recent work showed that recombinant adiponectin suppressed TNF-α production and induced IL-10 production by Kupffer cells in response to LPS stimulation, and adiponectin deficiency in mice enhanced LPS-induced liver injury associated with increased TNF-α and reduced IL-10 production (44). These results suggest hypoadiponectinemia should be one of upstreams of NASH and alcoholic liver injury, and a therapeutic target for these pathologic conditions.

Inflammatory bowel disease

It has been suggested that various immune, genetic, and environmental factors influence both the initiation and progression of inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease. During the last half century, the incidence of IBD has markedly increased in the developed countries, which suggests that changes in lifestyle may contribute to the development of IBD as well as obesity.

We recently investigated the effect of adiponectin on dextran sulfate sodium (DSS)-induced colitis by using APN-KO

mice (45). Adiponectin-KO mice developed much severer colitis compared with WT mice. The mRNA expression levels of chemokines were significantly higher in the colon tissues of DSS-treated APN-KO mice, accompanied by increased cellular infiltration including macrophages. Adenovirus-mediated supplement of adiponectin significantly attenuated the severity of colitis. Recombinant adiponectin inhibited LPS-induced IL-8 production in intestinal epithelial cells. The results suggested that hypoadiponectinemia may be involved in higher incidence of IBD in the developed countries.

Cancer

Obesity and lifestyle such as physical inactivity, overnutrtion, and high fat-low dietary fiber diet are known to be associated with breast, endometrial, prostatic and colon cancers. However, the molecular basis of the link remained unclear. We reported that low serum adiponectin levels were associated with an increased risk and biologically aggressive phenotype of breast cancer in human subjects (46). Mantzoros *et al* (47) also found an inverse relation between serum adiponectin levels and the incident risk for breast cancer in postmenopausal women. A significant inverse association of adiponectin with occurrence of endometrial cancer was reported in a case-control study (48). Another study also showed that body mass index and adiponectin became independent risks for the incidence of endometrial cancer (49). Further, plasma adiponectin levels were lower in patients with prostate cancer, and negatively associated with the histologic grade and stage (50). Association between plasma adiponectin level and colorectal cancer was also studied in 18,225 men by prospective nested case-control study (51). Men with low plasma adiponectin levels had a higher risk of colorectal cancer than men with higher levels. In a mouse tumor model, adiponectin significantly inhibited tumor growth, accompanied by decreased neovascularization and increased tumor cell apoptosis (52). Future studies are necessary to clarify causality and the mechanisms underlying hypoadiponectinemia and several cancers related to obesity.

Hypoadiponectinemia: a common basis of diseases caused by overnutrition

Analysis of genetic hypoadiponectinemia gave us further insight and confidence about the clinical significance of adiponectin. We found several genetic mutations in adiponectin gene (53,54). Among them, we focused on missense mutation with substitution of isoleucine 164 to threonine in the globular domain. Subjects carrying this mutation showed disturbed secretion of this mutant protein (55), had markedly lower plasma adiponectin levels, and revealed to be significantly prone to hypertension, hyperlipidemia, diabetes, and atherosclerosis. This further suggests that hypoadiponectinemia should locate upstream of the MetS.

THERAPEUTIC STRATEGY TO ENHANCE ADIPONECTIN SECRETION AND ACTION

According to the clinical and experimental evidence of adiponectin's multipotential favorable effects on the MetS, it is expected to develop a new therapeutic strategy to tackle this disorder by enhancing adiponectin production and function. For instance, peroxisome proliferator-activated receptor gamma (PPARγ) agonists, thiazolidinediones, have been shown to raise plasma adiponectin levels in human subjects (56). We revealed the existence of PPAR-responsive element (PPRE) in the human adiponectin gene promoter and the detailed transactivation machinery (57). We found the involvement of another transcriptional factor, liver receptor homologue-1 (LRH-1) as a competence factor for full transactivation of human adiponectin gene. Liver receptor homologue-1 enhanced PPARγ-mediated transactivation through LRH-1 responsive element (LRH-RE) close to the PPRE in the adiponectin promoter. The association of LRH-1 with PPARγ was strengthened by PGC-1. To our knowledge, cooperative induction by PPARγ and LRH-1 is known only in adiponectin gene. Thiazolidinediones are known to cause several unfavorable side effects which might be attributed to transactivation of multiple genes having PPRE in those promoters. Pinpoint targeting the cooperative machinery of PPARγ and LRH-1 may lead to the identification of compounds to enhance adiponectin production selectively.

Adiponectin cDNA was initially identified as the most abundantly expressed gene in human adipose tissue. Hence adiponectin is most abundantly synthesized in adipocytes. Previously, we showed that testosterone inhibited adiponectin secretion selectively without altering the mRNA expression of adiponectin and changing the secretion of other adipocytokine like leptin (58). Xu *et al* (59) also demonstrated the suppressive effect of testosterone on the secretion of adiponcetin, especially its high molecular weight form. To elucidate the unique machinery for adiponectin secretion in adipocytes (and also in other cell types) may lead to the discovery of adiponectin secretagogues with novel therapeutic potentials.

FAT ROS: AN EMERGING LINK BETWEEN OBESITY AND HYPOADIPONECTIEMIA

Recently, our group demonstrated that, in obese mice, production of ROS increased selectively in accumulated fat but not in muscle, liver, and aorta (60). The increase of fat ROS was associated with augmented expression of NADPH oxidase and decreased expression of antioxidative enzymes such as Cu, Zn-superoxide dismutase and catalase in adipose tissue (60). We also demonstrated that increased ROS in obesity was causative for various dysregulations of adipocytokines, including decreased production of adiponectin.

In our recent clinical work, visceral fat accumulation was the most potent determinant for systemic ROS in human subjects among various metabolic and body shape parameters (61). The results demonstrated that ROS is more produced in visceral fat accumulation, which may result in the development of hypoadiponectinemia. In the search of the drugs to target fat ROS, we found that angiotensin II receptor blockers (ARBs) have the potent capability to reduce the production of fat ROS via attenuating the expression of NADPH oxidase (62). This suppressive effect of ARBs on fat ROS was accompanied by improvement of adipocytokine dysregulation, like hypoadiponectinemia and also insulin resistance. These results suggested that fat ROS should be a key upstream regulator for the pathogenesis of MetS and shed a new light on the way for effective diagnosis and cure of this syndrome.

CONCLUSION

Metabolic syndrome is formed by the contribution of many factors in a multiplex cascade. To know the factors located more upstream and playing more crucial role is important from the aspect of prevention and cure. Our works and others revealed that visceral fat accumulation and the subsequent hypoadiponectinemia are centered in the whole pathogenesis. Furthermore, our recent works showed that fat ROS should be a missing link between obesity and hypoadiponectinemia (Fig. 3). Moreover, lifestyle intervention and/or drug therapy that target visceral fat accumulation, hypoadiponectinemia and fat ROS should be intensively sought to tackle the MetS being prevailed worldwide.

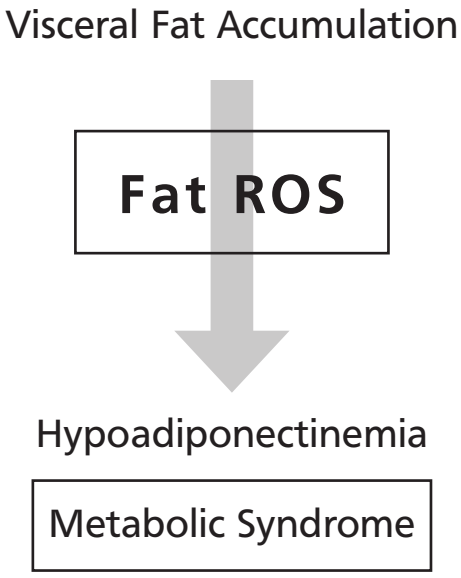

Figure 3. *Fat-derived reactive oxygen species (Fat ROS) is an emerging link between visceral fat accumulation and hypoadiponectinemia. Increased ROS in visceral fat accumulation should lead to dysregulated production of adipocytokines, including hypoadiponectinemia. Dysregulated adipocytokinemia and increased ROS in blood stream should cause the pathogenesis of metabolic syndrome associated with visceral fat accumulation.*

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