Send Orders for Reprints to reprints@benthamscience.net

412 *Current Vascular Pharmacology,* **2014**, *12*, 412-426

Endothelial Dysfunction, Obesity and Insulin Resistance

Dolores Prieto*, Cristina Contreras and Ana Sánchez

Department of Physiology, Faculty of Pharmacy, Universidad Complutense de Madrid, 28040-Madrid, Spain

Abstract: Obesity is a metabolic disorder of increasing prevalence worldwide and a risk factor for the development of insulin resistance (IR), metabolic syndrome and type 2 diabetes. Obesity is related to endothelial dysfunction through indirect mechanisms such as IR and the associated risk factors, and through direct mechanisms including the production of proinflammatory adipokines and elevated levels of free fatty acids (FFAs) by adipose tissue. Both clinical and experimental studies using genetic and diet-induced animal models of obesity have consistently shown impaired metabolic, agonist-or flow-induced vasodilatations correlated with the amount of visceral adipose tissue and improved by dietary interventions and exercise. Compromised bioavailability of NO due to oxidative stress emerges as a main cause of endothelial dysfunction in obesity. Inflamed adipose tissue due to hypoxia, and in particular perivascular adipose tissue (PVAT), secrete larger amounts of reactive oxygen species (ROS) and adipokines that deteriorate NO signaling pathways. Abnormal production and activity of the vasoconstrictor/proatherogenic peptide endothelin-1 (ET-1) is also a hallmark of the obesity-associated endothelial dysfunction. Obesity, and in particular visceral obesity, is one of the main causes of IR, and the pathogenic factors that induce endothelial dysfunction in the earlier stages of obesity will further deteriorate the insulin signaling pathways in endothelial cells thus leading to blunted vasodilatation and abnormal capillary recruitment and substrate delivery by insulin to the target tissues. The present review is an attempt to summarize the current knowledge and the latest novel findings on the pathogenic mechanisms underlying endothelial dysfunction in obesity, in particular the local contribution of oxidative stress and inflammatory response from PVAT, and its role in the obesity-associated cardiovascular and metabolic complications.

Keywords: Endothelial dysfunction, endothelin-1, inflammation, nitric oxide, insulin resistance, obesity, oxidative stress, perivascular adipose tissue.

1. INTRODUCTION

Obesity is a metabolic disorder of increasing prevalence worldwide and a key factor for the development of insulin resistance (IR), metabolic syndrome and type 2 diabetes mellitus, all of which are risk factors for cardiovascular disease. The mechanisms underlying the pathophysiology of obesity include changes in insulin sensitivity, dyslipidemia, vascular dysfunction, and most importantly, inflammation. Enlarged adipose tissue secretes higher levels of proinflammatory cytokines and non-esterified free fatty acids (FFAs) that lead to low-grade inflammation and vascular dysfunction, and promote IR not only in skeletal muscle and liver, but also in other tissues including endothelial cells [1,2].

Healthy endothelium is a paracrine, autocrine and endocrine organ that plays a key role in homeostasis by actively secreting various vasoactive and trophic molecules that affect vasomotion, endothelial and vascular smooth muscle (VSM) cell growth and proliferation, endothelial-leukocyte interactions, platelet adhesion, coagulation, inflammation and permeability [3,4]. By sensing blood flow-induced shear stress or in response to chemical signals, endothelial cells can acutely regulate vascular tone through the release of vasoactive and trophic factors including vasodilators such as nitric oxide (NO), cyclooxygenase-derived prostacyclin (PGI_2) and various endothelium-derived hyperpolarizing factors (EDHFs). Some of these vasodilators like NO and prostacyclin have also inhibitory actions on cell growth, coagulation and inflammation [3,4,5]. On the other hand, endothelial cells are also able to synthesize vasoconstrictors including prostanoids like thromboxane A_2 (TXA_2), reactive oxygen species (ROS) and potent vasoconstrictor peptides such as endothelin 1 (ET-1) that can also have proliferative actions besides inducing contraction [4]. Endothelial cells play a key role in the immune reaction by acutely regulating leucocytes recruitment or by induction of leucocyte adhesion molecules, inflammatory activation of the endothelium occurring in response to inflammatory cytokines and also to ROS generated in the inflammatory process or by disturbed metabolic conditions [3].

Despite the endothelium can adapt to various stimuli including mechanical, oxidative and metabolic stresses, inflammation and hypoxia, endothelial dysfunction is an early pathogenic event in vascular dysfunction and represents a maladapted endothelial phenotype consisting of impaired vasodilatation, angiogenesis and barrier function along with elevated expression of pro-inflammatory and pro-thrombotic factors [4,6].

2. ENDOTHELIAL DYSFUNCTION IN OBESITY

Overweight and obesity are associated with impaired endothelium-dependent vasodilation, a hallmark of endothe-

*Address correspondence to this author at the Department of Physiology, Faculty of Pharmacy, Universidad Complutense, 28040-Madrid, Spain; Tel:/Fax: 34913942267; E-mail: dprieto@farm.ucm.es

1875-6212/14 $58.00+.00

lial dysfunction first reported in clinical studies showing blunted increases in leg blood flow in response to administration of endothelium-dependent agonists in obese patients with augmented body mass index (BMI) [7]. Impairment of vasodilator responses induced by either agonists or flow was further demonstrated in obese individuals [8-13], correlated with the volume of visceral adipose tissue [8,14-18] and improved by weight lost, dietary interventions and exercise [10,19-22].

Obesity is associated with endothelial dysfunction through *indirect mechanisms*, such as IR and the association with risk factors (including diabetes mellitus, hypertension and dyslipidemia) [6], and through *direct mechanisms* including among others the production of adipokines and pro-inflammatory cytokines and the elevated levels of FFAs which in turn induce oxidative stress leading to reduced nitric oxide (NO) availability [23-26]. Thus, obesity may be a primary cause of coronary and systemic endothelial dysfunction before the establishment of IR, as demonstrated in animal models of diet-induced obesity [25,27-28] and also in clinical studies showing blunted limb flow-induced vasodilatation [8,9,29-31] and reduced increases in coronary blood flow in response to acetylcholine [32,33] independently of IR. Endothelial dysfunction is therefore an early manifestation of obesity that might independently increase cardiovascular risk. Compromised endothelial responses to physiological challenges in obesity reflect, as under other vascular risk conditions, a loss of balance between endothelial vasodilator/antiatherogenic and vasoconstrictor/proatherogenic factors.

2.1. Endothelial-dependent Vasodilatation in Obesity

Endothelium is a key mediator of tissue perfusion in response to increased blood flow and metabolic demand and also to chemical stimuli like acetylcholine and autacoids. Reduced endothelium-dependent vasodilator responses to infused agonists and flow [7,9,11,16,17] and blunted muscle perfusion in response to exercise [30] have been observed in obese individuals. Likewise, impaired vasodilator responses evoked by agonists [25,28,34-39], flow [40], functional reactive hyperemia [35,41-44] and hypoxia [35,44-46] have been confirmed in arteries from several experimental models of genetic and diet-induced obesity.

2.1.1. Nitric Oxide

Compromised bioavailability and abnormal NO signaling are key pathogenic factors in the obesity-associated endothelial dysfunction and have been involved in the reduced vasodilator responses to agonists [28,34,36-39,47], blunted functional hyperemia [48] and reduced relaxations to hypoxia [46] of systemic arteries from obese animals. Endothelial dysfunction based on NO deficiency can be ascribed to several potential mechanisms including reduced expression/activity of the eNOS enzyme, eNOS uncoupling due to substrate or cofactors deficiency, and enhanced scavenging of NO by increased superoxide production. Although eNOS protein levels and activity have eventually been reported to be reduced in arterioles from obese animals [37,49,50], most studies have shown unchanged [39,51] or upregulated levels of eNOS in cerebral [52], coronary [53,54] and systemic arteries [38,39,55] from experimental models of obesity, which was ascribed to compensatory mechanisms that go along with an enhanced production of superoxide anions and oxidative stress.

Endothelial NO formation can be stimulated by vasodilators such as acetylcholine or bradykinin acting on G_q protein-coupled receptors through Ca^{2+}-calmodulin-dependent activation of eNOS, but also by posttraslational modification of eNOS through phosphorylation at Ser1177 by serine kinases including PKB/Akt kinase, AMP-activated kinase (AMPK), protein kinase A (PKA), protein kinase G (PKG) and calmodulin-dependent kinase II (CaMKII) [56]. eNOS phophorylation by Akt kinase is stimulated by insulin and other agents including vascular endothelial growth factor (VEGF), β-adrenoceptors, statins, and also by mechanical factors such as laminar shear stress and flow increase [56]. Interestingly, adipokines can modulate this pathway and NO production and while leptin stimulates eNOS Ser1177 phosphorylation by Akt kinase activation [57,58], adiponectin modulates Ser1177 phosphorylation through AMPK to enhance NO production [56,59,60]. Conversely, resistin, interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) decrease eNOS Ser1177 phosphorylation, resulting in diminished eNOS activity and less NO generation [56].

Impairment of the endothelial PI3K/Akt pathway and eNOS phosphorylation activated by insulin and its role in the pathogenesis of vascular IR in obesity will be discussed later in this chapter. However, obesity is also associated with resistance to eNOS phosphorylation and reduced NO-dependent relaxations induced by flow, and by other factors such as insulin like growth factor (IGF-I) [61] and by acetylcholine through activation of Janus kinase 2 (JAK2), tyrosine phosphorylation of insulin receptor substrate 1 (IRS)-1 and downstream activation of phosphatidyl inositol (PI) 3-kinase (PI3K) and phosphorylation of Akt and eNOS [62], as shown in aorta from animals fed a high-fat (HF) diet. Recent studies have reported blunted acetylcholine and flow-induced vasodilatation associated to reduced heart phosphorylated eNOS in pressurized coronary arterioles from mice fed a HFD [63]. Moreover, perivascular fat (PVAT) has been demonstrated to down-regulate AMPK phosphorylation of eNOS thus impairing NO-mediated vasodilatation in obese animals [64]. Abnormal eNOS phosphorylation and NO production in obesity may also affect the vasodilator effects of β-adrenoceptor agonists, as reported in obese pre-menopausal women [65] and overweight/obese individuals [12], where increased abdominal adiposity was an independent predictor of this endothelial dysfunction [65]. Reduced cardiac output and depressor responses to isoproterenol resulting in impaired ability to regulate blood pressure after stress have been demonstrated to be due to abnormal β-adrenoceptor signaling in both cardiac and vascular myocytes from obese Zucker rats (OZR) [66], and blunted NO-mediated relaxations elicited by the β-adrenoceptor agonist isoproterenol have been reported in mesenteric [66] and penile small arteries [67] from the same strain. Defective eNOS phosphorylation and NO production in obesity can be triggered by lipid mediators and adipokines, as it will be discussed below.

2.1.2. Prostanoids

Cyclooxygenase (COX) enzymes metabolize arachidonic acid (AA) into both vasodilator (PGI_2, PGE_2) and vasocon-

strictor (TXA_2, PGH_2) prostanoids. In healthy blood vessels, most prostanoids are formed by the constitutive endothelial COX-1, while COX-2 is an inducible isoform usually up-regulated by inflammatory, mitogenic and physical stimuli [68]. An imbalance between the production of vasodilator and vasoconstrictor prostanoids may also contribute to endothelial dysfunction and abnormal VSM reactivity in obese humans [9] and in arteries from experimental models of obesity and metabolic syndrome [69-73]. Thus, impaired functional hyperemia and blunted blood flow increase during exercise were due to impaired PGI_2-mediated vasodilation in skeletal muscle arterioles from OZR [41,43]. Reduced release of vasodilator prostaglandins from both COX-1 and COX-2 pathways is involved in the endothelial dysfunction and blunted acetylcholine-induced relaxations of penile arteries from OZR [73]. Furthermore, up-regulation of COX-2 and imbalanced production of COX-2 products with reduced PGI_2/TXA_2 ratio and impaired acetylcholine relaxations have been found in mesenteric arterioles from a diet-induced model of obesity where COX-2 blockade restored endothelial function [55]. Interestingly, up-regulation of endothelial COX-2 was coupled to enhanced production of vasodilator prostaglandins thus protecting coronary arteries in OZR [74].

Obesity augments COX-dependent vasoconstriction, as first reported in clinical studies showing the enhancing effect of cyclooxygenase blockade with indomethacin on the blunted acetylcholine-induced forearm vasodilatation in obese subjects [9]. Further experimental studies confirmed that augmented AA-mediated contractions were due to increased COX-1 expression and activity in aorta from rats fed a HF-diet [70], and that increased COX-1-dependent TP receptor-mediated vasoconstriction induced by acetylcholine along with a marked increase in TP receptor gene expression were involved in the endothelial dysfunction of carotid artery from diet-induced obese mice [69]. Augmented TXA_2 production and TP receptor activity have likewise been involved in the blunted hypoxic vasodilatation [71] and impaired functional hyperaemia [43], respectively, of skeletal muscle arterioles from OZR.

2.1.3. EDHF

EDHF plays a key role along with NO and prostacyclin in the endothelium-dependent relaxations mainly in small arteries by increasing potassium conductance and promoting propagation of hyperpolarizing in the underlying smooth muscle. Both blunted and preserved EDHF-mediated vasodilator responses have been reported in obesity. In viscerally obese patients, bradykinin-induced increases in forearm blood flow irrespective of NOS and COX inhibition were impaired and inhibited by ouabain and potassium channel blockers [75] and blunted flow-evoked vasodilation due to defective EDHF-component has recently been reported in penile small arteries from genetically obese rats [40]. Consistent with the latter reports, acetylcholine-induced EDHF-mediated responses were also found to be impaired in small mesenteric arteries of OZR [76] and HF diet-fed rats [77], at least partly related to altered connexin 40-associated gap junctions and inward rectifying potassium (K_{IR}) channel conductance, respectively.

On the other hand, EDHF has also been suggested to compensate for the reduced NO bioavailability in obesity based on studies demonstrating preserved or augmented endothelial agonist-and flow-induced relaxations [78-82]. Thus, the contribution of EDHF to endothelium-dependent vasodilatation was enhanced or unaltered in small and large arteries from LDLR-/- mice fed a HF diet [78] suggesting no link between serum cholesterol levels and changes in EDHF-mediated responses, and in contrast to high glucose exposure that resulted in endothelial dysfunction with impairment of both NO and EDHF components [78]. Augmented EDHF-relaxant responses and maintained vasodilator function were also found in branches of saphenous artery [81] and in coronary microvessels from rats fed a HF diet [83]. Obesity-induced impairment of agonist- and flow-mediated vasodilation of coronary arterioles was dramatically improved with a low-carbohydrate diet most likely through the production of an EDHF independent of NO [79]. This EDHF was further shown to be H_2O_2 and suggested to compensate for the reduced bioavailability of NO, since both acetylcholine- and flow-induced vasodilatation were blocked by the nonspecific potassium channel antagonist tetraethylammonium and by catalase in coronary arterioles from obese but not lean animals [80].

2.2. Endothelial-dependent Vasoconstriction in Obesity

Endothelial dysfunction in obesity has also been characterized by augmented levels and activity of vasoconstrictor and proatherogenic endothelial factors, in particular of the potent peptide ET-1. Plasma circulating levels of ET-1 were elevated in obese patients with metabolic syndrome [84] and administration of selective ET_A receptor antagonists produced significant vasodilatation in obese subjects [85], ET_A-mediated augmented endogenous vasoconstrictor activity being correlated with BMI [86] and of special relevance in peripheral blood vessels. Furthermore, under conditions of ET_A receptor blockade, inhibition of NO synthesis induced greater vasoconstriction in obese but not type 2 diabetic individuals, thus unmasking an augmented NO synthesis capacity and suggesting that impaired NO bioavailability by endogenous ET-1 may contribute to endothelial dysfunction in obesity [87]. Experimental studies have confirmed the involvement of ET-1 through ET_A receptors in the blunted endothelial dependent relaxations of arteries from diet-induced obese mice [69,88]. Augmented ET-1-evoked vasoconstriction mainly associated to enhanced expression of ET_A [89,90] and eventually to ET_B [90] VSM receptors has been reported in some vascular beds from genetic and diet-induced models of obesity, while up-regulation of endothelial ET_B receptors coupled to enhanced production of NO and vasodilatation has been found to counterbalance ET-1 vasoconstriction in coronary arteries from OZR [91]. Up-regulation of ET-1 precursor has been found associated to enhanced NADPH activity, oxidative stress and expression of the nuclear transcription factor NF-kB in endothelial cells from obese individuals suggesting that ET-1 may induce endothelial dysfunction through ROS production [92]. Recent studies have implicated ET-1 in the hypoadiponectinemia in obesity and demonstrated that augmented levels of ET-1 were inversely correlated with adiponectin plasma levels in obese children and exposure of adipocytes to ET-1 or serum from obese individuals decreased adiponectin expression, an effect mediated by ET_A and ET_B receptors through

Table 1. Studies on the mechanisms of endothelial dysfunction in obesity involving prostanoids and EDHF

Study	Dysfunction	Mechanism	Vascular bed/model	Reference
PROSTANOIDS				
Clinical	↓ACh	↑COX-vasoconstriction ↑ROS	ForearmBF/Obese individuals	Perticone *et al*., 2001 [9]
Animal	↓HypoxD	↓ PGI_2 ↑ROS	Skeletal muscle/OZR	Frisbee, 2001 [45]
Animal	↓ACh	↑COX-1 ↑TP expression/activity	Carotide/HFD mice	Traupe *et al*., 2002 [69]
Animal	↓Hyperemia	↓ PGI_2 ↑TP activity	Skeletal muscle/OZR	Xiang *et al*., 2006 [43]
Animal	↑AA-contraction	↑COX-1 ↑AA-contraction	Aorta/HFD rat	Smith & Dorrance, 2006 [70]
Animal	↓HypoxD	↔ PGI_2 ↑TXA_2↑ROS	Skeletal muscle/OZR	Goodwill *et al*., 2008 [71]
Animal	↓Hyperemia	↓ PGI_2 ↔ TXA_2↑ROS, ↑nitrotyrosine PGIS	Femoral/OZR	Hodnett *et al*., 2009 [129]
Animal	↓ACh	↓ PGI_2 derived from COX-1 and COX-2	Penile arteries /OZR	Sánchez *et al*., 2010 [73]
Animal	↔ ACh	↑PGI_2 ↑COX-2	Coronary/OZR	Sánchez *et al*., 2010 [74]
Animal	↓ACh	↓ PGI_2/TXA_2 ↑COX-2↑ROS	Mesenteric/MSG rat	Lobato *et al*., 2011 [55]
EDHF				
Clinical	↓BK	↓EDHF	ForearmBF/Obese individuals	Kreutzenberg *et al.*, 2003 [75]
Animal	↓ACh	↓EDHF ↓connexin 40	Mesenteric/OZR	Young *et al*., 2008 [76]
Animal	↓ACh	↓EDHF ↓K_{IR}	Mesenteric/HFD	Haddock *et al*., 2011 [77]
Animal	↓FMD	↓EDHF	Penile/OZR	Schorring *et al*., 2012 [40]
Animal	↓ACh ↓FMD	↑EDHF↑K^+ activity	Coronary/OZR	Focardi *et al*., 2007 [79]
Animal	↔ ACh	↑EDHF	Aorta/mesenteric /HFD mice	Ellis *et al*., 2008 [78]
Animal	↔ ACh	↑EDHF	Coronary/HFD rat	Feher *et al*., 2010 [83]
Animal	↔ ACh	↑EDHF	Saphenous artery/HFD rat	Chadha *et al*., 2010 [81]
Animal	↓ACh ↓FMD	↑EDHF↑H_2O_2↑catalase↑SOD	Coronary/OZR	Focardi *et al*., 2013 [80]

ACh: acetylcholine vasodilatation; BF: blood flow; BK: bradykinin vasodilatation; COX: cyclooxygenase; EDHF:endothelium-derived hyperpolarizing factor; FMD: flow-mediated dilatation; HFD: high fat diet-induced obesity; HypoxD: hypoxic dilatation; K_{IR}: Inward rectifying K^+ channel; PGI_2 prostacyclin; PGIS:prostacyclin synthase; ROS: Reactive oxygen species; SOD: superoxide dismutase; TXA_2: Thromboxane A_2; TP: TXA_2:/prostagandin H_2 receptor.

activation of the mitogen activated protein kinase (MAPK) pathway [93].

The renin-AII-aldosterone system (RAAS) is activated in obesity and has been associated to the development of hypertension. Augmented AII-stimulated forearm vasoconstriction is found in viscerally obese normotensive men [94] and both renal angiotensin converting enzyme (ACE) activity [95] and AII-induced vasoconstriction in the renal [96] and coronary circulation [97] were enhanced in diet-induced and genetic models of obesity. Adipocytes synthesize and secrete almost all the RAAS components, including angiotensinogen, ACE, AT-1 and AT-2 receptors and aldosterone [98,99], AII secretion being higher in white PVAT from resistance arteries [98]. HF diet up-regulates expression of AT-1 receptors [89] and PVAT-secreted Ang II has been shown to induce vasoconstriction by promoting NADPH-derived ROS production and to trigger adipose tissue inflammation through the release of chemokines and through enhanced expression of adhesion molecules via AT-1 receptor activation of c-Jun N-terminal kinase (JNK) and MAPK pathways [99,100].

3. MECHANISMS UNDERLYING ENDOTHELIAL DYSFUNCTION IN OBESITY: INFLAMMATION AND OXIDATIVE STRESS

Several excellent reviews have recently dealt with the pathophysiological mechanisms underlying endothelial dysfunction in obesity and agreed in the essential role of inflammation and oxidative stress [6,82,101-110]. Adipocytes undergo considerable hyperthrophy in obesity and expanded -*'inflamed'*- adipose tissue changes to a proinflammatory phenotype marked by the increased ROS production [111] and by the excess release of FFAs and abnormal profile of adipokine secretion, with elevated circulating and tissue levels of leptin, resitin and proinflammatory cytokines such as IL-6 and TNFα, monocyte chemoattractant protein-1 (MCP-1) and plasminogen activator inhibitor type 1 (PAI-1), and reduced expression and levels of anti-inflammatory and cardiometabolic protective adipokines such as adiponectin and fatty acid binding protein 4 (FABP-4) [103,106,108,112]. Of relevance is the key role of PVAT in the local inflammatory response of the vascular wall and in the pathogenesis of obesity-associated

Table 2. Studies on the mechanisms of endothelial dysfunction in obesity involving ET-1

Study	Dysfunction	Mechanism	Vascular bed/model	Reference
ENDOTHELIN-1				
Clinical	↑vasoconstriction	↑ET_A receptor activity	LegBF/Obese individuals	Mather *et al*., 2002 [85]
Animal	↓ACh vasodilatation	↑ET_A receptor activity	Carotid/HFD mice	Traupe *et al*., 2002 [69]
Clinical	↑vasoconstricción	↑ET_A receptor activity ↓NO	LegBF/Obese individuals	Mather *et al*., 2004 [87]
Clinical	↑vasoconstricción	↑ET_A receptor activity	ForearmBF/Obese patiens	Cardillo *et al*., 2004 [86]
Animal	↔vasoconstriction	↑ET_B receptors activity ↑NO	Coronary/OZR	Katakam *et al*., 2006 [91]
Animal	↑ACh vasoconstricción	↑ET_A expression	Aorta/ HFD mice	Mundy *et al*., 2007 [89]
Clinical	↑vasoconstricción	↑ET_A receptor activity	LegBF/Obese individuals	Yoon *et al*., 2008
Animal	↓ACh vasodilatation	↓NO ↑$ONOO^-$ ↑NADPH oxidase ↑ET-1	Aorta/HFHSD rat	Bourgoin *et al*., 2008 [88]
Animal	↑ET-1 vasoconstriction	↑ET_A ↑ET_B VSM receptor expression	Penile/OZR	Contreras *et al*., 2013 [90]

ACh: acetylcholine; BF: blood flow; ET-1: endothelin 1; HFD: high fat diet-induced obesity; HFHSD high fat high sucrose diet-induced obesity; NO: nitric oxide; ONOO-: peroxynitrite; OZR: obese Zucker rat; VSM: vascular smooth muscle.

endothelial and vascular dysfunction [103,106,112,113] (reviewed in this monograph by Van der Voorde *et al*).

Hypoxia due to the lack of parallel increased blood supply to match augmented size of hypertrophied adipocytes is an essential factor underlying the proinflammatory changes in adipose tissue in obesity [106,114-116], as depicted by the lower partial O_2 pressures measured in visceral adipose tissue from obese humans and experimental animal models [114,115]. Hypoxia, which triggers infiltration of macrophages and other immune cells such as CD4+ and CD8+T cells [117], blunts the protective anticontractile properties of PVAT in obese individuals through increased release of inflammatory cytokines, enhanced oxidative stress and reduced adiponectin release and NO-mediated adiponectin vasodilator effects [113,118].

3.1. Oxidative Stress

Despite several factors can compromise NO synthesis, which usually protects the vessel wall from molecular events that lead to atherosclerosis, a reduction in the amount of bioavailable NO due to oxidative stress is considered a main cause of endothelial dysfunction in obesity. NO produced by eNOS may be rapidly inactivated by reaction with superoxide anions (O_2^-) to form peroxynitrite anion ($OONO^-$) [119], a powerful oxidative and highly toxic radical that causes oxidative damage to DNA, proteins and lipids, eNOS uncoupling, augmented apoptosis and tissue injury and inflammation [120,121]. Enhanced peroxynitrite formation, as depicted from the high nitrotyrosine content in the arterial wall, has been demonstrated in arteries from obese animals [25,88,122,123] and augmented ROS production has consistently been shown to induce endothelial dysfunction and to impair NO-mediated agonist- [25,28,34,47,50,52,55,122, 124-126] and hypoxia- [46] induced vasodilatation in arteries from both genetic and diet-induced models of obesity. Caloric restriction reversed ROS-induced endothelial dysfunction [125] and ROS scavengers restored NO-mediated relaxations [28,34,36,46,47,127,128].

Oxidative stress also contributes to the impaired prostanoid-mediated arterial relaxations in obesity, as shown by the beneficial effects of ROS scavengers in the blunted hypoxic vasodilator responses involving prostacyclin [71] and confirmed by the attenuated production of PGI_2 associated to the increased nitration of tyrosine residues of the prostacyclin synthase in skeletal muscle arterioles from OZR [71,129]. Peroxynitrite is able to inactivate PGI_2 synthase through tyrosine nitration and increased mitochondria fatty acid oxidation with the subsequent augmented superoxide have been shown to enhance tyrosine nitration and inactivation of PGI_2 synthase in the aortic endothelium of OZR [130].

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a major source of vascular ROS generation in obesity. Expression of NADPH oxidase- regulatory subunit p47-phox and of the redox sensitive transcriptional factor NFκ-B was enhanced in endothelial cells and correlated with BMI, waist circumference and total body fat in obese individuals [92]. Augmented NADPH activity measured by serine phosphorylation of the p47-phox subunit in aorta [131] or by enhanced expression of NADPH oxidase subunits p22- and p40-phox in coronary arteries [37], Nox-4 in cerebral arteries [52] and Nox-1 subunit in aorta from OZR [106], Nox-1 in coronary arterioles from HFD fed mice [63], and p47- and p67-phox in coronary endothelium of pig fed a HF diet [25] was associated to enhanced vascular ROS production and endothelial dysfunction. Accordingly, treatment with the NADPH oxidase inhibitor apocynin diminished superoxide production and restored endothelium-dependent relaxations in arteries from obese animals [47,52,53,63,122,123,125, 126,131,132]. Xanthine oxidase- derived endothelial ROS production, which is associated to endothelial dysfunction in hypercholesterolemia [133], has also been involved in vascular oxidative stress in obesity, and enzyme inhibitors markedly reduced superoxide generation [126] and restored endothelium-dependent relaxations [28] in arteries from obese animals fed a HF diet.

Augmented ROS production in response to high levels of lipids and cytokines activates the oxidative stress sensitive

nuclear transcription factor NF-kB that directy up-regulates NADPH oxidase in endothelial cells of obese individuals [134] and regulates the expression of genes encoding adhesion molecules, COX-2 and pro-inflammatory cytokines TNFα, IL-6 and C reactive protein (CRP), which in turn may activate NADPH oxidase and ROS generation thus further impairing endothelial function [50,134,135]. Vascular activity of NF-kB associated to increased oxidative stress and endothelial dysfunction and to reduced expression of the NF-kB inhibitor IkBα, has been found in obese individuals [134] and HF diet-fed animals [50], and pharmacological inhibition of NF-kB activation with salicylate reduced oxidative stress in endothelial cells and restored flow-mediated dilatation in obese subjects [134].

Augmented NADPH and xanthine oxidase-derived ROS can lead to eNOS uncoupling which further contributes to vascular oxidative stress and endothelial dysfunction. Peroxynitrite causes eNOS uncoupling by reducing cellular transport of the eNOS substrate L-arginine, by oxidizing eNOS precursor BH_4 and by triggering proteasome-dependent degradation of the rate-limiting enzyme in BH_4 synthesis GTP-cyclohydrolase, by regulating the zinc-thiolate center of eNOS and by increasing AMPK-dependent Ser1179 eNOS phosphorylation that augments electron flow through the enzyme and its activity [136-138]. Uncoupled eNOS generates superoxide instead of NO that continues to alter cell signalling processes in endothelial and VSM cells. eNOS uncoupling has been demonstrated to underlie endothelial dysfunction in arteries from mice fed a HF diet where relaxations to acetylcholine were improved by inhibition of NADPH oxidase, xanthine oxidase or by BH_4 precursors [55,122,125], and enhanced ROS production was associated to increased eNOS phosphorylation but decreased dimer: monomer ratio of eNOS, indicative of enzyme uncoupling [122].

Despite systemic oxidative stress was found to be markedly increased, correlated with BMI and waist circumference and suggested to accumulate in fat in obese humans [139,140] and animal models of obesity [131], experimental studies have revealed no changes in systemic oxidative stress associated to abnormal endothelial function, suggesting that local vascular oxidative stress is a major determinant of endothelial dysfunction in the earlier stages of obesity [25]. Accordingly, the relevant contribution of oxidative stress from PVAT to obesity-associated endothelial dysfunction has recently been unveiled. Enhanced NADPH activity and elevated ROS were associated with PVAT hypertrophy and inflammatory cell infiltration, increased vascular expression of adhesion molecules, chemoatractant protein MCP-1, inflammatory cytokines and decreased levels of adiponectin and superoxide dismutase (SOD) in PVAT from both large and small arteries of genetically obese mice [122]. Enhanced ROS production from this inflamed PVAT was furthermore associated with eNOS uncoupling [122] and with reduction of the anticontractile properties of PVAT [141]. Impaired adiponectin NO-mediated vasodilator properties of PVAT due to hypoxia, inflammatory damage and oxidative stress have also been found in arteries from obese patients where the loss of PVAT anticontractile function was rescued by free radical scavengers, SOD and catalase [113]. Recent studies have confirmed that mRNA expression of Ncf2, a gene that encodes the p67-phox subunit of NADPH oxidase is augmented along with increased expression of proinflammatory adipokines, MCP-1 and leptin in PVAT of diet-induced obese mice and associated to enhanced formation of NADPH-derived superoxide and augmented vasoconstriction due to the reduction of anticontractile properties of PVAT [126]. The vasorelaxant effect of PVAT-derived hydrogen peroxide (H_2O_2) was changed into vasoconstriction in arteries from obese patients [113] and mice [126] through oxidative stress, and increased amounts of H_2O_2 produced by PVAT from obese animals were suggested to contribute to augmented ROS production through the ability of peroxide to activate NADPH oxidase and further generate superoxide [120,142].

3.2. Lipotoxicity

Atherogenic dyslipidemia and overproduction of FFAs-induced ROS is another mechanism underlying the association between adipose tissue and endothelial dysfunction in obesity. Pathophysiological concentrations of FFAs impair endothelial function and blunt agonist- and flow-induced vasodilatation [143]. FFAs not only damage insulin signaling pathways causing IR [2,6,144-146], but also produce endothelial dysfunction by inducing oxidative stress in part through activation of NADPH oxidase [131,143,147], by interfering with eNOS expression and activity through ROS-induced abnormal Ca^{2+} signaling and blunted Ca^{2+}-calmodulin-dependent eNOS activation [148,149] and by promoting vascular cell proliferation and inflammation [149,150]. The mechanisms underlying FFAs-induced endothelial dysfunction and eNOS inhibition involve activation of PKC and kinase IKβ (IKKβ) that regulates activation of the transcriptional patnhway IκB-α/NF-κB [145,147,151].

Endothelial dysfunction and abnormal blood pressure following HF feeding has been ascribed to FFAs–induced impairment of eNOS phosphorylation [152]. Intermediate lipid metabolites resulting from FFAs accumulation have been involved in defective eNOS phosphorylation, blunted NO vascular production and endothelial dysfunction in obesity. Thus, activation of protein kinase C (PKC)β in endothelial cells and vascular tissue by increased levels of diacylglycerol (DAG) inhibits Akt stimulation by insulin and VEGF thus causing endothelial dysfunction in obese rats fed a HF diet [153]. The sphingolipide ceramide derived from FFAs metabolism precipitates endothelial dysfunction by preventing phosphorylation of a pool of Akt that colocalizes with eNOS via Hsp90, thereby compromising full eNOS phosphorylation and NO production in HF diet-fed obese animals [154]. Lysophosphatidylcholine (LPC), a highly atherogenic product of lipid metabolism which increases endothelial permeability and impair endothelium-dependent and independent vasodilatation [155] has been found to be elevated and associated to endothelial dysfunction in experimental obesity [25].

3.3. Adipokines

Obesity and HF feeding up-regulate expression and induce a substantial release of cytokines such as TNFα, MCP-1, IL-6 and IL-8 from adipose tissue and PAVT [112]. TNFα levels are increased in the adipose tissue and plasma of obese individuals [156] and in addition to interfere with insulin

signaling in endothelial cells [146,157] and to up-regulate endothelial cell adhesion molecules [101], TNFα downregulates expression of eNOS [158], inhibits PI3K-mediated flow induced phosphorylation of eNOS [157], directly activates NADPH oxidase increasing oxidative stress and stimulates lipolysis resulting in FFAs release [56], thus causing endothelial dysfunction in humans [159] and blunted acetylcholine vasodilation in obese animals [37]. Recent studies demonstrate that increased release of TNFα in visceral fat from obese humans is associated to blunted NO-mediated endothelial relaxation due to enhanced NADPH-derived ROS production in the small arteries from fat, which suggests that impaired blood flow in adipose tissue might further contribute to hypoxia and inflammation [26]. IL-6 levels augment proportionally to adiposity and alter endothelial function through CRP-mediated NOS inhibition, AII-stimulated ROS production, enhanced expression of adhesion molecules and thrombus formation [101,105]. Furthermore, IL-6 enhances stability of the negative eNOS regulator caveolin-1 thus reducing NOS activity and endothelial NO production [160].

Leptin deficiency is associated with severe obesity and IR and obesity is considered a leptin-resistant state with high circulating levels of leptin [25,63,161]. Acute exposure to leptin stimulates NO production in endothelial cells through Akt-dependent eNOS phosphorylation [58,162], potentiates insulin-induced NO production [57] and evokes endothelium-dependent vasodilatation [24]. Leptin also stimulates ET-1 release from endothelial cells [163]. Hyperleptinemia is associated with reduced plasma NO metabolites and coronary endothelial dysfunction, i.e. *"leptin resistance"*, in experimental models of genetic [24] and diet-induced [25,63,164] obesity. Recent studies have demonstrated that leptin expression in PVAT is up-regulated by HF feeding and associated with blunted endothelium-dependent relaxations and exacerbated coronary endothelial dysfunction in obese animals [112,126,165]. Hyperleptinemia reduces intracellular L-arginine and augments superoxide and peroxynitrite formation in endothelial cells resulting in eNOS uncoupling and endothelial dysfunction in obesity [166]. Furthermore, leptin has pro-inflammatory effects and stimulates MCP-1 production through induction of mitochondrial ROS, plasma leptin levels being correlated with those of inflammatory markers [110,167].

Resistin levels are increased in obesity [168] and this adypokine may also contribute to endothelial dysfunction by stimulating production of pro-inflammatory TNFα, MCP-1 and Il-6 through NF-kB, by inducing ET-1 release, by enhancing expression of adhesion molecules [101,105,110], by promoting proliferation and migration of endothelial cells [169] and by augmenting oxidative stress through increased NADPH oxidase activity [170] and through p38 MAPK and JNK-dependent impairment of the mitochondrial respiratory chain [171]. Resistin directly induces eNOS down-regulation and reduces NO production through overproduction of ROS [171] and blunts both endothelium-dependent and endothelium-independent relaxations and also insulin signaling and eNOS phosphorylation in endothelial cells [155,172].

Adiponectin derived from adipose tissue is a regulator of endothelial function that has protective cardiovascular effects in part through stimulation of NO production in endothelial cells by AMPK-dependent phosphorylation of eNOS [59,173] and inhibition of FFAs-induced accumulation of ROS [174]. Furthermore, adiponectin inhibits inflammatory activation of the endothelium and monocyte adhesion [175]. Plasma adiponectin levels are reduced in obesity and associated cardiometabolic disorders [176,177], and a reduced expression of adiponectin along with blunted NO-mediated protective vascular effects of adiponectin due to hypoxia and inflammation has been found in PVAT from obese individuals and animals associated with endothelial dysfunction [112,113,122,126].

In addition to leptin, adiponeptin, TNF-α and IL-6, more recently identified adipokines that promote inflammation include IL-18, angiopoietin-like protein 2 (ANGPTL2), CC-chemokine ligand 2 (CCL2), CXC-chemokine resistin [105], and some like adipocyte fatty acid binding protein (A-FABP) and lipocalin 2 that promote lipotoxicity in endothelial cells and cause endothelial dysfunction in obesity, and that have been discussed in more detail in recent extensive reviews [103,109,110].

4. OBESITY, IR AND ENDOTHELIAL DYSFUNCTION

Metabolic impairments in obesity often deteriorate in overt diabetes. Obesity, and in particular visceral obesity, is one of the main causes of IR, and endothelial dysfunction in obese individuals and animal models of obesity is influenced by the IR state *per se* independently of dysglycemia [9], and mediated by the reduced insulin-stimulated NO release [7,146,178]. During obesity induced by HF feeding, inflammation and IR develop in the vasculature well before these responses are detected in muscle, liver or adipose tissue, suggesting that the vasculature is more susceptible than other tissues to the deleterious effects of nutrient overload [1].

4.1. Normal Insulin Signaling

Insulin regulates glucose homeostasis by promoting glucose uptake by skeletal muscle and adipose tissue facilitating translocation of the glucose transporter 4 (GLUT-4) to the cell membrane and further activating downstream pathways of glucose metabolism, and also by regulating nutrient delivery to target tissues through actions on the microvasculature [146,179]. Upon binding to its receptor, insulin concomitantly activates two intracellular signaling pathways, the PI3K/Akt pathway after phosphorylation of the insulin receptors substrate (IRS), responsible for the hormone anabolic actions and for eNOS phosphorylation and NO production in the vascular endothelium, and the proatherogenic Ras-Raf-MAPK pathway involved in gene transcription, cell growth and differentiation, promoting the production of vasoconstrictor ET-1 [146]. By stimulating endothelial NO-mediated vasodilatation and increased blood flow and also by inducing capillary recruitment and flow redistribution, insulin further enhances glucose up-take in skeletal muscle [180,181]. Under physiological conditions, ET-1 actions produced by stimulation of the endothelium by insulin including expression of PAI-1 and cell adhesion molecules ICAM-1, VCAM-1 and E-selectin in endothelial cells [182], are probably offset by NO production induced by the hormone [146,183].

4.2. Vascular IR

IR is classically defined as a diminished sensitivity to metabolic actions of insulin and reduced glucose uptake by different tissues, although blunted sensitivity to the vascular actions of the hormone also contributes to the phenotype of the insulin resistant states [146,178,179]. IR is a typical feature of metabolic disorders like type 2 diabetes, obesity, glucose intolerance and dyslipidemias, but it is also present in cardiovascular diseases such as hypertension, coronary artery disease and atherosclerosis, all of which are characterized by endothelial dysfunction [146,184]. Decreased sensitivity of resistance vessels to insulin-induced vasodilatation was first reported in obese individuals by Laasko *et al.* [185] and suggested to contribute to IR by impairing insulin hemodynamic effects on blood blow and substrate delivery. Impaired endothelium-dependent vasodilatation and capillary recruitment by insulin was later shown in obese individuals and type 2 diabetes patients [7,11,21,186], and also in animal models of obesity associated IR, where the vascular NO-mediated responses evoked by insulin were blunted [49,53,67,88, 152,178]. Furthermore, recent *in vivo* evidence demonstrates that insulin signaling mediating transendothelial transport of insulin, which facilitates insulin delivery to and glucose uptake by skeletal muscle [187], is impaired in experimental models of both genetic and diet-induced obesity [188], consistent with the reduced delivery of insulin to insulin-sensitive targets in obese individuals [189] and HF diet-induced IR animals [190]. This extends the contribution of vascular IR to metabolic IR in obesity.

An essential feature of metabolic and vascular IR in obesity is the selective alteration of the PI3K/Akt pathway, while the MAPK/ET-1 pathway remains intact or is heightened [49,146,179,188,191-193]. Since metabolic IR is accompanied by compensatory hyperinsulinemia to maintain glycaemia, to vascular level hyperinsulinemia should induce an imbalance between decreased PI3K insulin-dependent and non-affected MAPK insulin-dependent actions, favoring prohypertensive vascular MAPK-mediated actions of insulin increasing vasoconstriction, expression of adhesion molecules and mytogenic actions [49,67,85,87,179,182]. Thus, in obese hypertensive patients, increased BMI is associated with enhanced ET-dependent vasoconstrictor activity, suggesting that this abnormality plays a role in the pathophysiology of obesity-related hypertension [85].

To molecular level, alterations at any level of the PI3K/Akt signaling pathway have been reported to impair endothelial effects of insulin. Insulin receptor knockout mice (IRKO) exhibit metabolic IR along with impaired insulin-stimulated phosphorylation of eNOS resulting in blunted basal and insulin-induced production of endothelial NO [194]. In arteries from IR humans and rodent models of genetic and diet-induced obesity, defective insulin pathway and impaired vascular actions of insulin have been found associated to blunted insulin-induced tyrosine phosphorylation of the IRS-1 and IRS-2 [191] or reduced expression/activity of IRS-2 [188], downstream reduced insulin-stimulated phosphorylation of Akt [1,153,191,195] and/or eNOS [1,152, 153,195,196] and decreased expression of eNOS [1,152,153, 195,196]. We have found preserved insulin-mediated vasorelaxation along with up-regulation of the Akt/eNOS pathway and impairment of the MAPK cascade in coronary arteries from OZR, which suggests that coronary arteries are initially protected from vascular IR in this genetic model of obesity-associated IR [54], and in contrast to that observed for skeletal muscle arterioles from the same animals [49].

Lipotoxicity as a Common Cause of IR and Endothelial Dysfunction: Inflammation and Oxidative Stress

Several factors have been involved in the injury of the insulin pathways that contribute to the development of IR. As discussed above, inflamed adipose tissue in obesity is a highly active endocrine organ secreting larger amounts of ROS, FFAs and inflammatory cytokines that have been shown to impair insulin signaling in endothelial cells [179,184,197]. Thus, constant exposure of the vasculature to abnormal higher levels of circulating FFAs in obesity may simultaneously cause IR in metabolic tissues and endothelial dysfunction in vascular tissues [144,146,198]. FFAs elevation impaired both glucose up-take and basal and insulin-mediated NO production, vasodilatation and capillary recruitment in human skeletal muscle [143,144]. Elevated levels of intracellular lipid intermediates such as DAG and ceramides resulting from FFAs metabolism, have been demonstrated to interfere insulin signaling in endothelial cells causing IR through the same mechanisms that directly cause endothelial dysfunction such as oxidative stress and inflammation [143,197]. Inflammatory cytokines like TNFα blunt insulin induced IRS-1 tyrosine, PKB Ser473 and eNOS Ser1179 phosphorylation and NO production in endothelial cells [157] and insulin-evoked vasodilation and glucose uptake in humans [159]. Exposure of endothelial cells to high concentrations of palmitate and high plasmatic levels of FFAs in HF-diet obese animals inhibit insulin-mediated tyrosine phosphorylation of IRS-1 and serine phosphorylation of Akt/eNOS and NO production [145,196], this inhibition being mediated by inflammatory mediators such as IKβ kinase (IKKβ), a regulator of the transcriptional factor NF-kB [145], and Toll-like-receptor (TLR)-4, a mediator of innate immunity [196]. Recent studies demonstrate the involvement of inflammatory mediators from PVAT in obesity-associated vascular IR [199]. Thus, PVAT regulates insulin-induced vasoreactivity in skeletal muscle arterioles through secretion of vasorelaxant adiponectin and the subsequent activation of the AMPKα2 pathway, this effect being blunted by JNK from PVAT in obese mice [199].

Augmented levels of lipid metabolites such as DAG enhance PKC activity thus impairing insulin-induced Akt phosphroylation and NO production in aorta from OZR, insulin responses being normalized by selective inhibitors of PKCβ [153]. Increased PKCθ activity has also been shown to underlie endothelial IR induced by FFAs and to mediate insulin-induced vasoconstriction due to blunted Akt phosphorylation and enhanced ERK-MAPK phosphorylation upon exposure to high concentrations of palmitate [151]. Conversely, we have recently demonstrated that supraphysiological concentrations of FFAs did not alter either endothelium-dependent relaxations or Akt/eNOS serine phosphorylation by insulin, but reduced both basal and insulin-stimulated ERK-MAPK activity in coronary arteries from healthy animals, which suggests that coronary arteries are

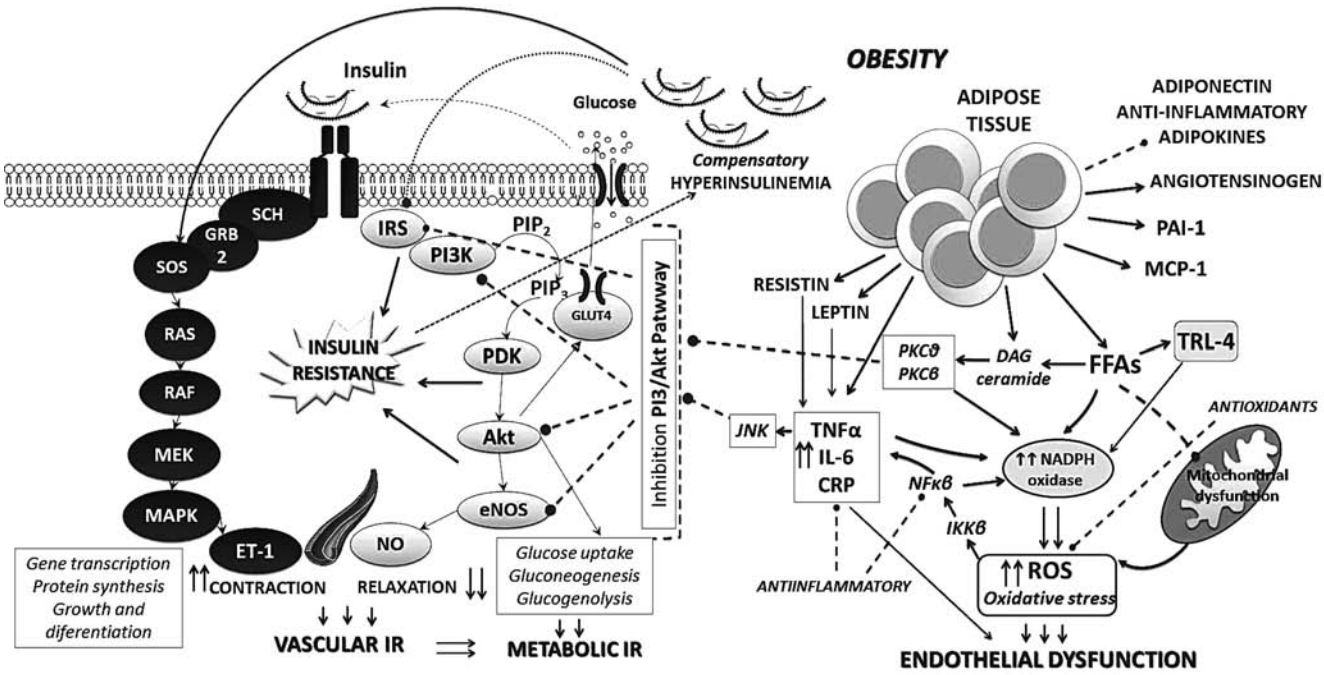

Fig. (1). Vascular insulin resistance (IR) in obesity. In vascular endothelial cells, insulin activates two signaling pathway, the PI3K/Akt-dependent pathway releasing vasodilator NO and the MAPK-dependent proathrogenic pathway inducing ET-1 formation. Inflamed adipose tissue in obesity releases larger amounts of free faty acids (FFAs), intermediate lipid metabolites (diacylglycerol, DAG and ceramide) and inflammatory cytokines, and inhibits production of anti-inflammatory adipokines like adiponectin. FFAs and lipid intermediates activate kinases like PKCβ, PKCθ and IKKβ that phosphorylate and blunt the insulin IRS/PI3K/Akt/eNOS pathway and stimulate ROS production. Inflammation increases reactive oxygen species (ROS) by NADPH oxidase induction, and oxidative stress in turn induces inflammatoy cytokines production. FFAs induce mitochondrial dysfunction releasing large amount of ROS, resulting in greater amount of inflammatory markers, which not only inhibits insulin vasodilator pathway inducing IR but also stimulates the vasoconstrictor proatherogenic MAPK pathway. IR triggers compensatory hyperinsulinemia which further accentuates the imbalance between vasodilator and vasoconstrictor actions of insulin. CRP: Reactive protein C; Kinases Ikβ: IKK β; Nuclear factor kappa B (NF-kB); plasminogen activator inhibitor-1 (PAI-1); phosphoinositide-dependent kinase 1 (PDK); Toll-like-receptor (TLR).

initially protected from FFAs-induced endothelial IR in obese animals [54].

As discussed above, one potential mechanism linking high levels of FFAs/lipid intermediates and inflammation is oxidative stress that can be generated by both mitochondrial electron transport and by cytosolic NADPH oxidases. Lipid infusion increases FFAs levels along with the production of ROS and inflammatory markers, impairing flow-induced vasodilatation in humans [143]. FFAs can stimulate NADPH oxidase activity to produce ROS through the concomitant production of DAG and PKC activation in both VSM and endothelial cells [147]. Nox4-derived superoxide production has been involved in the palmitate-stimulated TLR4 activation to NF-κβ activity, IL-6 and ICAM expression in endothelial cells, and HF feeding increased Nox4 vascular expression in wild but not in TLRA(-/-) mice [200]. Importantly, ROS and insulin are recognized as two key players in the pathogenesis of vascular dysfunction in IR states and insulin activation of NADPH oxidase and ROS production were shown to be augmented along with decreased NO bioavailability and blunted insulin-induced vasodilatation in coronary arteries from insulin resistant obese Zucker rats [53]. Furthermore, recent studies demonstrate that enhanced ROS production in response to insulin in cerebral arteries from hyperinsulinemic OZR impairs the synthesis of the NOS cofactor BH_4 and of GTP-CH enzyme inducing NOS uncoupling and leading to a vicious cycle with further generation of ROS, blunted insulin-induced Akt and eNOS phosphorylation and enhanced PKC and ERK-MAPK activation which causes insulin impaired vasodilatation and endothelial dysfunction [201]. Restoration of insulin sensitivity by genetic deletion of the insulin-desensitizing enzyme of the insulin signaling pathway, protein tyrosine phosphatase (PTP) 1B, restored endothelial function and lowered levels of oxidative stress through decreased expression of Nox 1 and its molecular regulators Noxo1 and Noxa1 in obese leptin resistant db/db mice [202].

5. CONCLUDING REMARKS

Endothelial dysfunction is an early manifestation of vascular dysfunction in obesity which precedes development of IR and cardiometabolic complications. The present review was an attempt to summarize the large body of experimental evidence providing insight into the key role of lipotoxicity, inflammation and oxidative stress to the compromised bioavailability of NO which emerges as a main cause of endothelial dysfunction in obesity. Of relevance is the local contribution of inflamed PVAT along with visceral adipose tissue to the high levels of vascular oxidative stress and larger secretion of inflammatory adipokines that deteriorate

NO pathways leading to endothelial dysfunction. Obesity is a main cause of IR, and the pathogenic factors that induce endothelial dysfunction in the earlier stages of obesity further deteriorate insulin signaling in endothelial cells causing blunted nutritive blood flow and substrate delivery to the target tissues and thus contributing to metabolic IR.

Various clinical and experimental studies have proved the beneficial effects of exercise, weight loss and dietary interventions in restoring endothelial function in obesity, which suggests that effective educational programs should be combined with therapies aimed to reduce oxidative stress [134] and inflammation in order to prevent obesity-associated cardiovascular and metabolic complications. In this regard, drugs like insulin sensitizers, statins and inhibitors of RAAS, currently prescribed in other cardiovascular disorders, have been reported to improve endothelial function in obesity in part by restoring NO synthesis and reducing levels ROS and of inflammatory markers [82,124,203-205] (reviewed in this monograph by Comerma-Steffensen *et al*). Since altered crosstalk between adipose tissue and arteries plays a key role in the pathogenesis of obesity and IR, pharmacological manipulation of adipose tissue neovascularization by angiogenic modulators in order to regulate adipose tissue expansion represents a novel therapeutical option [206].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Authors´ research was funded by grants SAF2009-10448 from the Spanish Ministerio de Ciencia e Innovación (Spain) and SAF2012-31631 from MINECO (Spain).

LIST OF ABREVIATIONS

AII	=	Angiotensin II
ACE	=	Angiotensin converting enzyme
AMPK	=	AMP-activated kinase
BMI	=	Body mass index
CaMKII	=	Calmodulin-dependent kinase II
COX	=	Cyclooxygenase
DAG	=	Diacylglycerol
ET-1	=	Endothelin 1
FFAs	=	Free fatty acids
HF	=	High-fat
IKKβ	=	IKβ kinase
IGF-I	=	Insulin like growth factor
IRS	=	Insulin receptor substrate
IL-6	=	Interleukine 6
JNK	=	c-Jun N-terminal kinase
MAPK	=	Mitogen activated protein kinase
MPC-1	=	Monocyte chemotactic protein-1
NADPH	=	Nicotinamide adenine dinucleotide phosphate
Nox	=	NADPH oxidase
NF-kB	=	Nuclear factor kappa B
OZR	=	Obese Zucker rats
PVAT	=	Perivascular fat
PI	=	Phosphatidyl inositol
PI3K	=	Phosphatidyl inositol 3-kinase
PGI_2	=	Prostacyclin
PKA	=	Protein kinase A
PKC	=	Protein kinase C
PKG	=	Protein kinase G
ROS	=	Reactive oxygen species
TLR	=	Toll-like-receptor
TNFα	=	Tumor necrosis factor-α
VEGF	=	Vascular endothelial growth factor
VSM	=	Vascular smooth muscle

REFERENCES

[1] Kim F, Pham M, Maloney E, *et al*. Vascular inflammation, insulin resistance, and reduced nitric oxide production precede the onset of peripheral insulin resistance. Arterioscler Thromb Vasc Biol 2008; 28(11): 1982-8.

[2] Rask-Madsen C, Kahn CR.Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. Arterioscler Thromb Vasc Biol 2012; 32(9): 2052-9.

[3] Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. Nat Rev Immunol 2007; 7(10): 803-15.

[4] Vanhoutte PM, Shimokawa H, Tang EH, Feletou M. Endothelial dysfunction and vascular disease. Acta Physiol 2009; 196: 193-222.

[5] Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol 2012; 10(1): 4-18.

[6] Bakker W, Eringa EC, Sipkema P, van Hinsbergh VW. Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. Cell Tissue Res 2009; 335(1): 165-89.

[7] Steinberg HO, Chaker H, Leaming R, *et al*. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest 1996; 97: 2601-10.

[8] Brook RD, Bard RL, Rubenfire M, Ridker PM, Rajagopalan S. Usefulness of visceral obesity (waist/hip ratio) in predicting vascular endothelial function in healthy overweight adults. Am J Cardiol 2001; 88(11): 1264-9.

[9] Perticone F, Ceravolo R, Candigliota M, *et al*. Obesity and body fat distribution induce endothelial dysfunction by oxidative stress: protective effect of vitamin C. Diabetes 2001; 50(1): 159-65.

[10] Sciacqua A, Candigliota M, Ceravolo R, *et al*. Weight loss in combination with physical activity improves endothelial dysfunction in human obesity. Diabetes Care 2003; 26(6): 1673-8.

[11] Van Guilder GP, Hoetzer GL, Dengel DR, Stauffer BL, DeSouza CA. Impaired endothelium-dependent vasodilation in normotensive and normoglycemic obese adult humans. J Cardiovasc Pharmacol 2006; 47(2): 310-3.

[12] Van Guilder GP, Stauffer BL, Greiner JJ, Desouza CA. Impaired endothelium-dependent vasodilation in overweight and obese adult humans is not limited to muscarinic receptor agonists. Am J Physiol Heart Circ Physiol 2008; 294(4): H1685-92.

[13] Han KA, Patel Y, Lteif AA, Chisholm R, Mather KJ. Contributions of dysglycaemia, obesity, and insulin resistance to impaired endothelium-dependent vasodilation in humans. Diabetes Metab Res Rev 2011; 27(4): 354-61.

[14] Hashimoto M, Akishita M, Eto M, *et al*. The impairment of flow-mediated vasodilatation in obese men with visceral fat accumulation. Int J Obes Relat Metab Disord 1998; 22(5): 477-84.

[15] Arcaro G, Zamboni M, Rossi L, *et al*. Body fat distribution predicts the degree of endothelial dysfunction in uncomplicated obesity. Int J Obes Relat Metab Disord 1999; 23: 936-42.

[16] Parikh NI, Keyes MJ, Larson MG, *et al*. Visceral and subcutaneous adiposity and brachial artery vasodilator function. Obesity (Silver Spring) 2009; 17(11): 2054-9.

[17] Sturm W, Sandhofer A, Engl J, *et al*. Influence of visceral obesity and liver fat on vascular structure and function in obese subjects. Obesity 2009; 17(9): 1783-8.

[18] Romero-Corral A, Sert-Kuniyoshi FH, Sierra-Johnson J, *et al*. Modest visceral fat gain causes endothelial dysfunction in healthy humans. J Am Coll Cardiol 2010; 56(8): 662-6.

[19] Ziccardi P, Nappo F, Giugliano G, *et al*. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. Circulation 2002; 105(7): 804-9.

[20] Woo KS, Chook P, Yu CW, *et al*. Effects of diet and exercise on obesity-related vascular dysfunction in children. Circulation 2004; 109(16): 1981-6.

[21] De Filippis E, Cusi K, Ocampo G, *et al*. Exercise-induced improvement in vasodilatory function accompanies increased insulin sensitivity in obesity and type 2 diabetes mellitus. J Clin Endocrinol Metab 2006; 91(12): 4903-10.

[22] Pierce GL, Beske SD, Lawson BR, *et al*. Weight loss alone improves conduit and resistance artery endothelial function in young and older overweight/obese adults. Hypertension 2008; 52(1): 72-9.

[23] De Kreutzenberg SV, Crepaldi C, Marchetto S, *et al*. Plasma free fatty acids and endothelium-dependent vasodilation: effect of chain-length and cyclooxygenase inhibition. J Clin Endocrinol Metab 2000; 85(2): 793-8.

[24] Knudson JD, Dincer UD, Zhang C, *et al*. Leptin receptors are expressed in coronary arteries, and hyperleptinemia causes significant coronary endothelial dysfunction.Am J Physiol Heart Circ Physiol 2005; 289(1): H48-56.

[25] Galili O, Versari D, Sattler KJ, *et al*. Early experimental obesity is associated with coronary endothelial dysfunction and oxidative stress. Am J Physiol Heart Circ Physiol 2007; 292(2): H904-11.

[26] Virdis A, Santini F, Colucci R, *et al*. Vascular generation of tumor necrosis factor-α reduces nitric oxide availability in small arteries from visceral fat of obese patients. J Am Coll Cardiol 2011; 58(3): 238-47.

[27] Naderali EK, Brown MJ, Pickavance LC, Wilding JP, Doyle PJ, Williams G. Dietary obesity in the rat induces endothelial dysfunction without causing insulin resistance: a possible role for triacylglycerols. Clin Sci (Lond) 2001; 101(5): 499-506.

[28] Erdei N, Tóth A, Pásztor ET, *et al*. High-fat diet-induced reduction in nitric oxide-dependent arteriolar dilation in rats: role of xanthine oxidase-derived superoxide anion. Am J Physiol Heart Circ Physiol 2006; 291(5): H2107-15.

[29] Meyer AA, Kundt G, Steiner M, Schuff-Werner P, Kienast W. Impaired flow-mediated vasodilation, carotid artery intima-media thickening, and elevated endothelial plasma markers in obese children: the impact of cardiovascular risk factors. Pediatrics 2006; 117(5): 1560-7.

[30] Karpoff L, Vinet A, Schuster I, *et al*. Abnormal vascular reactivity at rest and exercise in obese boys. Eur J Clin Invest 2009; 39(2): 94-102.

[31] Lind L, Siegbahn A, Ingelsson E, Sundström J, Arnlöv J. A detailed cardiovascular characterization of obesity without the metabolic syndrome. Arterioscler Thromb Vasc Biol 2011; 31(8): e27-34.

[32] Al Suwaidi J, Higano ST, Holmes DR Jr, Lennon R, Lerman A. Obesity is independently associated with coronary endothelial dysfunction in patients with normal or mildly diseased coronary arteries. J Am Coll Cardiol 2001; 37: 1523-8.

[33] Martin JW, Briesmiester K, Bargardi A, Muzik O, Mosca L,nDuvernoy CS. Weight changes and obesity predict impaired resting and endothelium-dependent myocardial blood flow in postmenopausal women. Clin Cardiol 2005; 28: 13-8.

[34] Frisbee JC, Stepp DW. Impaired NO-dependent dilation of skeletal muscle arterioles in hypertensive diabetic obese Zucker rats. Am J Physiol Heart Circ Physiol 2001; 281: H1304-11.

[35] Phillips SA, Sylvester FA, Frisbee JC. Oxidant stress and constrictor reactivity impair cerebral artery dilation in obese Zucker rats. Am J Physiol Regul Integr Comp Physiol 2005; 288: R522-R530.

[36] Oltman CL, Richou LL, Davidson EP, Coppey LJ, Lund DD, Yorek MA. Progression of coronary and mesenteric vascular dysfunction in Zucker obese and Zucker diabetic fatty rats. Am J Physiol Heart Circ Physiol 2006; 291: H170-8.

[37] Picchi A, Gao X, Belmadani S, Potter BJ, Focardi M, Chilian WM, Zhang C. Tumor necrosis factor-alpha induces endothelial dysfunction in the prediabetic metabolic syndrome. Circ Res 2006; 99: 69-77.

[38] Bouvet C, Belin de Chantemèle E, Guihot AL, *et al*. Flow-induced remodeling in resistance arteries from obese Zucker rats is associated with endothelial dysfunction. Hypertension 2007; 50(1): 248-54.

[39] Villalba N, Martínez P, Bríones AM, *et al*. Differential structural and functional changes in penile and coronary arteries from obese Zucker rats. Am J Physiol Heart Circ Physiol 2009; 297(2): H696-707.

[40] Schjørring O, Kun A, Flyvbjerg A, Kirkeby HJ, Jensen JB, Simonsen U. Flow-evoked vasodilation is blunted in penile arteries from Zucker diabetic fatty rats. J Sex Med 2012; 9(7): 1789-800.

[41] Frisbee JC. Impaired skeletal muscle perfusion in obese Zucker rats. Am J Physiol Regul Integr Comp Physiol 2003; 285(5): R1124-34.

[42] Frisbee JC, Delp MD. Vascular function in the metabolic syndrome and the effects on skeletal muscle perfusion: lessons from the obese Zucker rat. Essays Biochem 2006; 42: 145-61.

[43] Xiang L, Naik JS, Hodnett BL, Hester RL. Altered arachidonic acid metabolism impairs functional vasodilation in metabolic syndrome. Am J Physiol Regul Integr Comp Physiol 2006; 290: R134-R138.

[44] Frisbee JC, Hollander JM, Brock RW, Yu HG, Boegehold MA. Integration of skeletal muscle resistance arteriolar reactivity for perfusion responses in the metabolic syndrome. Am J Physiol Regul Integr Comp Physiol 2009; 296(6): R1771-82.

[45] Frisbee JC. Impaired dilation of skeletal muscle microvessels to reduced oxygen tension in diabetic obese Zucker rats. Am J Physiol Heart Circ Physiol 2001; 281(4): H1568-74.

[46] Prieto D, Kaminski PM, Bagi Z, Ahmad M, Wolin MS. Hypoxic relaxation of penile arteries: involvement of endothelial nitric oxide and modulation by reactive oxygen species. Am J Physiol Heart Circ Physiol 2010; 299(3): H915-24.

[47] Agouni A, Lagrue-Lak-Hal AH, Mostefai HA, *et al*. Red wine polyphenols prevent metabolic and cardiovascular alterations associated with obesity in Zucker fatty rats (Fa/Fa). PLoS One 2009; 4 (5): e5557.

[48] Frisbee JC. Reduced nitric oxide bioavailability contributes to skeletal muscle microvessel rarefaction in the metabolic syndrome. Am J Physiol Regul Integr Comp Physiol 2005; 289(2): R307-16.

[49] Eringa EC, Stehouwer CD, Roos MH, Westerhof N, Sipkema P. Selective resistance to vasoactive effects of insulin in muscle resistance arteries of obese Zucker (fa/fa) rats. Am J Physiol Endocrinol Metab 2007; 293(5): E1134-9.

[50] Kobayasi R, Akamine EH, Davel AP, Rodrigues MA, Carvalho CR, Rossoni LV. Oxidative stress and inflammatory mediators contribute to endothelial dysfunction in high-fat diet-induced obesity in mice. J Hypertens 2010; 28(10): 2111-9.

[51] Fulton D, Harris MB, Kemp BE, Venema RC, Marrero MB, Stepp DW. Insulin resistance does not diminish eNOS expression, phosphorylation, or binding to HSP-90.Am J Physiol Heart Circ Physiol 2004; 287(6): H2384-93.

[52] Erdös B, Snipes JA, Miller AW, Busija DW. Cerebrovascular dysfunction in Zucker obese rats is mediated by oxidative stress and protein kinase C. 2004; Diabetes 53: 1352-59.

[53] Katakam PV, Tulbert CD, Snipes JA, *et al*. Impaired insulin-induced vasodilation in small coronary arteries of Zucker obese rats is mediated by reactive oxygen species. Am J Physiol Heart Circ Physiol 2005; 288: H854-60.

[54] Contreras C, Sánchez A, García-Sacristán A, Martínez MC, Andriantsitohaina R, Prieto D. Preserved insulin vasorelaxation and up-regulation of the Akt/eNOS pathway in coronary arteries from insulin resistant obese Zucker rats. Atherosclerosis 2011; 217(2): 331-9.

[55] Lobato NS, Filgueira FP, Akamine EH, *et al*. Obesity induced by neonatal treatment with monosodium glutamate impairs microvas-

cular reactivity in adult rats: role of NO and prostanoids. Nutr Metab Cardiovasc Dis 2011; 21(10): 808-16.

[56] Huang PL. eNOS, metabolic syndrome and cardiovascular disease. Trends Endocrinol Metab 2009; 20(6): 295-302.

[57] Vecchione C, Maffei A, Colella S, *et al.* Leptin effect on endothelial nitric oxide is mediated through Akt-endothelial nitric oxide synthase phosphorylation pathway. Diabetes 2002; 51: 168-173.

[58] Blanquicett C, Graves A, Kleinhenz DJ, Hart CM. Attenuation of signaling and nitric oxide production following prolonged leptin exposure in human aortic endothelial cells. J Investig Med 2007; 55(7): 368-77.

[59] Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. J Biol Chem 2003; 278(45): 45021-6.

[60] Deng G, Long Y, Yu YR, Li MR. Adiponectin directly improves endothelial dysfunction in obese rats through the AMPK-eNOS Pathway. Int J Obes (Lond) 2010; 34(1): 165-71.

[61] Imrie H, Abbas A, Viswambharan H, *et al*. Vascular insulin-like growth factor-I resistance and diet-induced obesity. Endocrinology 2009; 150(10): 4575-82.

[62] Zecchin HG, Priviero FB, Souza CT, *et al.* Defective insulin and acetylcholine induction of endothelial cell-nitric oxide synthase through insulin receptor substrate/Akt signaling pathway in aorta of obese rats. Diabetes 2007; 56(4): 1014-24.

[63] Park Y, Booth FW, Lee S, Laye MJ, Zhang C. Physical activity opposes coronary vascular dysfunction induced during high fat feeding in mice. J Physiol 2012; 590 (Pt 17): 4255-68.

[64] Ma L, Ma S, He H, *et al.* Perivascular fat-mediated vascular dysfunction and remodeling through the AMPK/mTOR pathway in high-fat diet-induced obese rats. Hypertens Res 2010; 33(5): 446-53.

[65] Suh HS, Park YW, Kang JH, Lee SH, Lee HS, Shim KW. Vascular endothelial dysfunction tested by blunted response to endothelium-dependent vasodilation by salbutamol and its related factors in uncomplicated pre-menopausal obese women. Int J Obes (Lond) 2005; 29(2): 217-22.

[66] D'Angelo G, Mintz JD, Tidwell JE, Schreihofer AM, Pollock DM, Stepp DW. Exaggerated cardiovascular stress responses and impaired beta-adrenergic-mediated pressor recovery in obese Zucker rats. Hypertension 2006; 48(6): 1109-15.

[67] Contreras C, Sánchez A, Martínez P, *et al*. Insulin resistance in penile arteries from a rat model of metabolic syndrome. Br J Pharmacol 2010; 161(2): 350-64.

[68] Warner TD, Mitchell JA. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. FASEB J 2004; 18(7): 790-804.

[69] Traupe T, Lang M, Goettsch W, *et al.* Obesity increases prostanoid-mediated vasoconstriction and vascular thromboxane receptor gene expression. J Hypertens 2002; 20(11): 2239-45.

[70] Smith AD, Dorrance AM. Arachidonic acid induces augmented vasoconstriction via cyclooxygenase 1 in the aorta from rats fed a high-fat diet. Prostaglandins Leukot Essent Fatty Acids 2006; 75(1): 43-9.

[71] Goodwill AG, James ME, Frisbee JC. Increased vascular thromboxane generation impairs dilation of skeletal muscle arterioles of obese Zucker rats with reduced oxygen tension. Am J Physiol Heart Circ Physiol 2008; 295(4): H1522-28.

[72] Xiang L, Dearman J, Abram SR, Carter C, Hester RL. Insulin resistance and impaired functional vasodilation in obese Zucker rats. Am J Physiol Heart Circ Physiol 2008; 294(4): H1658-H1666.

[73] Sánchez A, Contreras C, Villalba N, *et al.* Altered arachidonic acid metabolism via COX-1 and COX-2 contributes to the endothelial dysfunction of penile arteries from obese Zucker rats. Br J Pharmacol 2010; 159(3): 604-16.

[74] Sánchez A, Contreras C, Martínez P, *et al.* Enhanced cyclooxygenase 2-mediated vasorelaxation in coronary arteries from insulin-resistant obese Zucker rats. Atherosclerosis 2010; 213(2): 392-9.

[75] De Kreutzenberg SV, Kiwanuka E, Tiengo A, Avogaro A. Visceral obesity is characterized by impaired nitric oxide-independent vasodilation. Eur Heart J 2003; 24(13): 1210-5.

[76] Young EJ, Hill MA, Wiehler WB, Triggle CR, Reid JJ. Reduced EDHF responses and connexin activity in mesenteric arteries from the insulin-resistant obese Zucker rat. Diabetologia 2008; 51(5): 872-81.

[77] Haddock RE, Grayson TH, Morris MJ, Howitt L, Chadha PS, Sandow SL. Diet-induced obesity impairs endothelium-derived hyperpolarization via altered potassium channel signaling mechanisms. PLoS One 2011 21; 6(1): e16423.

[78] Ellis A, Cheng ZJ, Li Y, *et al.* Effects of a Western diet versus high glucose on endothelium-dependent relaxation in murine micro- and macro-vasculature. Eur J Pharmacol 2008; 601: 111-17.

[79] Focardi M, Dick GM, Picchi A, Zhang C, Chilian WM. Restoration of coronary endothelial function in obese Zucker rats by a low-carbohydrate diet. Am J Physiol Heart Circ Physiol 2007; 292(5): H2093-9.

[80] Focardi M, Dick GM, Picchi A, Zhang C, Chilian WM. Restoration of coronary endothelial function in obese Zucker rats by a low-carbohydrate diet. Am J Physiol Heart Circ Physiol 2007; 292(5): H2093-9.

[81] Chadha PS, Haddock RE, Howitt L, *et al*. Obesity up-regulates intermediate conductance calcium-activated potassium channels and myoendothelial gap junctions to maintain endothelial vasodilator function. J Pharmacol Exp Ther 2010; 335(2): 284-93.

[82] Bagi Z, Feher A, Cassuto J. Microvascular responsiveness in obesity: implications for therapeutic intervention. Br J Pharmacol 2012; 165(3): 544-60.

[83] Feher A, Rutkai I, Beleznai T, *et al.* Caveolin-1 limits the contribution of BK(Ca) channel to EDHF-mediated arteriolar dilation: implications in diet-induced obesity. Cardiovasc Res 2010; 87(4): 732-9.

[84] Ferri C, Bellini C, Desideri G, *et al*. Circulating endothelin-1 levels in obese patients with the metabolic syndrome. Exp Clin Endocrinol Diabetes 1997; 105 (Suppl 2): 38-40.

[85] Mather KJ, Mirzamohammadi B, Lteif A, Steinberg HO, Baron AD. Endothelin contributes to basal vascular tone and endothelial dysfunction in human obesity and type 2 diabetes. Diabetes 2002; 51(12): 3517-23.

[86] Cardillo C, Campia U, Iantorno M, Panza JA. Enhanced vascular activity of endogenous endothelin-1 in obese hypertensive patients. Hypertension 2004; 43(1): 36-40.

[87] Mather KJ, Lteif A, Steinberg HO, Baron AD. Interactions between endothelin and nitric oxide in the regulation of vascular tone in obesity and diabetes. Diabetes 2004; 53(8): 2060-6.

[88] Bourgoin F, Bachelard H, Badeau M, *et al.* Endothelial and vascular dysfunctions and insulin resistance in rats fed a high-fat, high-sucrose diet. Am J Physiol Heart Circ Physiol 2008; 295(3): H1044-55.

[89] Mundy AL, Haas E, Bhattacharya I, *et al.* Fat intake modifies vascular responsiveness and receptor expression of vasoconstrictors: implications for diet-induced obesity. Cardiovasc Res 2007; 73(2): 368-75.

[90] Contreras C, Sánchez A, Martínez P, *et al.* Impaired Endothelin Calcium Signaling Coupled to Endothelin type B receptors in penile arteries from insulin-resistant obese Zucker rats. J Sex Med 2013; 10(9): 2141-53.

[91] Katakam PV, Snipes JA, Tulbert CD, Mayanagi K, Miller AW, Busija, DW. Impaired endothelin-induced vasoconstriction in coronary arteries of Zucker obese rats is associated with uncoupling of [Ca2+]i signaling. Am J Physiol 2006; 290 (557): R145-153.

[92] Silver AE, Beske SD, Christou DD, *et al.* Overweight and obese humans demonstrate increased vascular endothelial NAD(P)H oxidase-p47(phox) expression and evidence of endothelial oxidative stress. Circulation 2007; 115(5): 627-37.

[93] Nacci C, Leo V, De Benedictis L, *et al.* Elevated Endothelin-1 (ET-1) Levels May Contribute to Hypoadiponectinemia in Childhood Obesity. J Clin Endocrinol Metab 2013; 98(4): E683-93.

[94] Nielsen S, Halliwill JR, Joyner MJ, Jensen MD. Vascular response to angiotensin II in upper body obesity. Hypertension 2004; 44(4): 435-41.

[95] Barton M, Carmona R, Morawietz H, *et al.* Obesity is associated with tissue-specific activation of renal angiotensin-converting enzyme *in vivo*: evidence for a regulatory role of endothelin. Hypertension 2000; 35(1 Pt 2): 329-36.

[96] Stepp DW, Boesen EI, Sullivan JC, Mintz JD, Hair CD, Pollock DM. Obesity augments vasoconstrictor reactivity to angiotensin II in the renal circulation of the Zucker rat. Am J Physiol Heart Circ Physiol 2007; 293(4): H2537-42.

[97] Zhang C, Knudson JD, Setty S, *et al*. Coronary arteriolar vasoconstriction to angiotensin II is augmented in prediabetic metabolic syndrome via activation of AT1 receptors. Am J Physiol Heart Circ Physiol 2005; 288(5): H2154-62

[98] Gálvez-Prieto B, Bolbrinker J, Stucchi P, *et al*. Comparative expression analysis of the renin-angiotensin system components between white and brown perivascular adipose tissue. J Endocrinol 2008; 197(1): 55-64.

[99] Briones AM, Nguyen Dinh Cat A, Callera GE, *et al*. Adipocytes produce aldosterone through calcineurin-dependent signaling pathways: implications in diabetes mellitus-associated obesity and vascular dysfunction. Hypertension 2012; 59(5): 1069-78.

[100] Nguyen Dinh Cat A, Briones AM, Callera GE, *et al*. Adipocyte-derived factors regulate vascular smooth muscle cells through mineralocorticoid and glucocorticoid receptors. Hypertension 2011; 58(3): 479-88.

[101] Singer G, Granger DN. Inflammatory responses underlying the microvascular dysfunction associated with obesity and insulin resistance. Microcirculation 2007; 14(4-5): 375-87.

[102] Stapleton PA, James ME, Goodwill AG, Frisbee JC. Obesity and vascular dysfunction. Pathophysiology 2008; 15(2): 79-89

[103] Ouwens DM, Sell H, Greulich S, Eckel J. The role of epicardial and perivascular adipose tissue in the pathophysiology of cardiovascular disease. J Cell Mol Med 2010; 14(9): 2223-34.

[104] Rajsheker S, Manka D, Blomkalns AL, Chatterjee TK, Stoll LL, Weintraub NL. Crosstalk between perivascular adipose tissue and blood vessels. Curr Opin Pharmacol 2010; 10(2): 191-6.

[105] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011; 11(2): 85-97.

[106] Aghamohammadzadeh R, Withers S, Lynch F, Greenstein A, Malik R, Heagerty A. Perivascular adipose tissue from human systemic and coronary vessels: the emergence of a new pharmacotherapeutic target. Br J Pharmacol 2012; 165: 670-82.

[107] Goodwill AG, Frisbee JC. Oxidant stress and skeletal muscle microvasculopathy in the metabolic syndrome. Vasc Pharmacol 2012; 57(5-6): 150-9.

[108] Payne GA, Kohr MC, Tune JD. Epicardial perivascular adipose tissue as a therapeutic target in obesity-related coronary artery disease. Br J Pharmacol 2012; 165(3): 659-69.

[109] Gu P, Xu A. Interplay between adipose tissue and blood vessels in obesity and vascular dysfunction. Rev Endocr Metab Disord 2013; 14(1): 49-58.

[110] Mattu HS, Randeva HS. Role of adipokines in cardiovascular disease. J Endocrinol 2013; 216(1): T17-36.

[111] Salgado-Somoza A, Teijeira-Fernandez E, Fernandez AL, Gonzalez-Juanatey JR, Eiras S. Proteomic analysis of epicardial and subcutaneous adipose tissue reveals differences in proteins involved in oxidative stress. Am J Physiol Heart Circ Physiol 2010; 299: H202-9.

[112] Chatterjee TK, Stoll LL, Denning GM, *et al*. Proinflammatory phenotype of perivascular adipocytes: influence of high-fat feeding. Circ Res 2009; 104: 541-49.

[113] Greenstein AS, Khavandi K, Withers SB, *et al*. Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. Circulation 2009; 119(12): 1661-70.

[114] Hosogai N, Fukuhara A, Oshima K, *et al*. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes 2007; 56: 901-911.

[115] Pasarica M, Sereda OR, Redman LM, *et al*. Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. Diabetes 2009; 58: 718-725.

[116] Trayhurn P. Hypoxia and adipose tissue function and dysfunction in obesity. Physiol Rev 2013; 93(1): 1-21.

[117] Nishimura S, Manabe I, Nagasaki M, *et al*. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 2009; 15(8): 914-20.

[118] Chen B, Lam KS, Wang Y, *et al*. Hypoxia dysregulates the production of adiponectin and plasminogen activator inhibitor-1 independent of reactive oxygen species in adipocytes. Biochem Biophys Res Commun 2006; 341: 549-56.

[119] Moncada S, Higgs EA. Nitric oxide and the vascular endothelium. Handb Exp Pharmacol 2006; 176(Pt 1): 213-54.

[120] Wolin MS. Interactions of oxidants with vascular signaling systems. Arterioscler Thromb Vasc Biol 2000; 20(6): 1430-42.

[121] Touyz RM, Briones AM. Reactive oxygen species and vascular biology: implications in human hypertension. Hypertens Res 2011; 34(1): 5-14.

[122] Marchesi C, Ebrahimian T, Angulo O, Paradis P, Schiffrin EL. Endothelial nitric oxide synthase uncoupling and perivascular adipose oxidative stress and inflammation contribute to vascular dysfunction in a rodent model of metabolic syndrome. Hypertension 2009; 54(6): 1384-92.

[123] Sánchez A, Contreras C, Martínez MP, *et al*. Role of neural NO synthase (nNOS) uncoupling in the dysfunctional nitrergic vasorelaxation of penile arteries from insulin-resistant obese Zucker rats. PLoS One 2012; 7(4): e36027.

[124] Erdös B, Snipes JA, Tulbert CD, Katakam P, Miller AW, Busija DW. Rosuvastatin improves cerebrovascular function in Zucker obese rats by inhibiting NAD(P)H oxidase-dependent superoxide production. Am J Physiol Heart Circ Physiol 2006; 290(3): H1264-70.

[125] Ketonen, J, Pilvi T, Mervaala; E. Caloric restriction reverses high-fat diet-induced endothelial dysfunction and vascular superoxide production in C57Bl/6 mice. Heart Vessels 2010; 25: 254-262.

[126] Ketonen J, Shi J, Martonen E, Mervaala E. Periadventitial adipose tissue promotes endothelial dysfunction via oxidative stress in diet-induced obese C57Bl/6 mice. Circ J 2010; 74(7): 1479-87.

[127] Frisbee JC, Maier KG, Stepp DW. Oxidant stress-induced increase in myogenic activation of skeletal muscle resistance arteries in obese Zucker rats. Am J Physiol Heart Circ Physiol 2002; 283(6): H2160-8.

[128] Romanko, OP, Stepp, DW. Reduced constrictor reactivity balances impaired vasodilation in the mesenteric circulation of the obese Zucker rat. Am J Physiol Heart Circ Physiol 2005; 289: H2097-H2102.

[129] Hodnett BL, Dearman JA, Carter CB, Hester RL. Attenuated PGI2 synthesis in Obese Zucker Rats. Am J Physiol Regul Integr Comp Physiol 2009; 296(3): R715-21.

[130] Du X, Edelstein D, Obici S, Higham N, Zou MH, Brownlee M. Insulin resistance reduces arterial prostacyclin synthase and eNOS activities by increasing endothelial fatty acid oxidation. J Clin Invest 2006; 116(4): 1071-080.

[131] Chinen I, Shimabukuro M, Yamakawa K, *et al*. Vascular lipotoxicity: endothelial dysfunction via fatty-acid-induced reactive oxygen species overproduction in obese Zucker diabetic fatty rats. Endocrinology 2007; 148(1): 160-5.

[132] Sonta T, Inoguchi T, Tsubouchi H, *et al*. Evidence for contribution of vascular NAD(P)H oxidase to increased oxidative stress in animal models of diabetes and obesity. Free Radic Biol Med 2004; 37(1): 115-23.

[133] Cardillo C, Kilcoyne CM, Cannon RO 3rd, Quyyumi AA, Panza JA. Xanthine oxidase inhibition with oxypurinol improves endothelial vasodilator function in hypercholesterolemic but not in hypertensive patients. Hypertension 1997; 30(1 Pt 1): 57-63.

[134] Pierce GL, Lesniewski LA, Lawson BR, Beske SD, Seals DR. Nuclear factor-{kappa}B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. Circulation 2009; 119(9): 1284-92

[135] Kralisch S, Sommer G, Stangl V, *et al*. Secretory products from human adipocytes impair endothelial function via nuclear factor kappaB. Atherosclerosis 2008; 196(2): 523-31.

[136] Zou MH, Shi C, Cohen RA. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J Clin Invest 2002; 109(6): 817-26.

[137] Zou MH, Hou XY, Shi CM, Nagata D, Walsh K, Cohen RA. Modulation by peroxynitrite of Akt- and AMP-activated kinase-dependent Ser1179 phosphorylation of endothelial nitric oxide synthase. J Biol Chem 2002; 277(36): 32552-7.

[138] Landmesser U, Dikalov S, Price SR, *et al*. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 2003; 111(8): 1201-9.

[139] Keaney JF Jr, Larson MG, Vasan RS, *et al*. Framingham Study. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol 2003; 23(3): 434-9.

[140] Furukawa S, Fujita T, Shimabukuro M, *et al*. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004; 114(12): 1752-61.

[141] Gollasch M, Dubrovska G. Paracrine role for periadventitial adipose tissue in the regulation of arterial tone. Trends Pharmacol Sci 2004; 25(12): 647-53.

[142] Santiago E, Contreras C, García-Sacristán A, *et al*. Signaling pathways involved in the H2O2-induced vasoconstriction of rat coronary arteries. Free Radic Biol Med 2013; 60C: 136-146.

[143] Tripathy D, Mohanty P, Dhindsa S, *et al*. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. Diabetes 2003; 52(12): 2882-7.

[144] Steinberg HO, Paradisi G, Hook G, *et al*. Free fatty acid elevation impairs insulin mediated vasodilation and nitric oxide production. Diabetes 2000; 49: 1231-8.

[145] Kim F, Tysseling KA, Rice J, *et al*. Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKKbeta. Arterioscler Thromb Vasc Biol 2005; 25(5): 989-94.

[146] Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006; 113(15): 1888-904.

[147] Inoguchi T, Li P, Umeda F, *et al*. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes 2000; 49(11): 1939-45.

[148] Esenabhalu VE, Schaeffer G, Graier WF. Free fatty acid overload attenuates Ca2+ signaling and NO production in endothelial cells. Antioxid Redox Signal 2003; 5: 147-153.

[149] Artwohl M, Roden M, Waldhäusl W, Freudenthaler A, Baumgartner-Parzer SM. Free fatty acids trigger apoptosis and inhibit cell cycle progression in human vascular endothelial cells. FASEB J 2004; 18(1): 146-1.

[150] Azekoshi Y, Yasu T, Watanabe S, *et al*. Free fatty acid causes leukocyte activation and resultant endothelial dysfunction through enhanced angiotensin II production in mononuclear and polymorphonuclear cells. Hypertension 2010; 56(1): 136-42.

[151] Bakker W, Sipkema P, Stehouwer CD, *et al*. Protein kinase C theta activation induces insulin-mediated constriction of muscle resistance arteries. Diabetes 2008; 57(3): 706-13.

[152] Symons JD, McMillin SL, Riehle C, *et al*. Contribution of insulin and Akt1 signaling to endothelial nitric oxide synthase in the regulation of endothelial function and blood pressure. Circ Res 2009; 104: 1085-94.

[153] Naruse K, Rask-Madsen C, Takahara N, *et al*. Activation of vascular protein kinase C-beta inhibits Akt-dependent endothelial nitric oxide synthase function in obesity-associated insulin resistance. Diabetes 2006; 55(3): 691-8.

[154] Zhang QJ, Holland WL, Wilson L, *et al*. Ceramide mediates vascular dysfunction in diet-induced obesity by PP2A-mediated dephosphorylation of the eNOS-Akt complex. Diabetes 2012; 61(7): 1848-59.

[155] Kougias P, Chai H, Lin PH, Lumsden AB, Yao Q, Chen C. Adipocyte-derived cytokine resistin causes endothelial dysfunction of porcine coronary arteries. J Vasc Surg 2005; 41: 691-698.

[156] Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995; 95: 2111-2119.

[157] Kim F, Gallis B, Corson MA. TNF-alpha inhibits flow and insulin signaling leading to NO production in aortic endothelial cells. Am J Physiol Cell Physiol 2001; 280(5): C1057-65.

[158] Mohamed F, Monge JC, Gordon A, Cernacek P, Blais D, Stewart DJ. Lack of role for nitric oxide (NO) in the selective destabilization of endothelial NO synthase mRNA by tumor necrosis factor-alpha. Arterioscler Thromb Vasc Biol 1995; 15(1): 52-7.

[159] Rask-Madsen C, Domínguez H, Ihlemann N, Hermann T, Køber L, Torp-Pedersen C. Tumor necrosis factor-alpha inhibits insulin's stimulating effect on glucose uptake and endothelium-dependent vasodilation in humans. Circulation 2003; 108(15): 1815-21.

[160] Hung MJ, Cherng WJ, Hung MY, Wu HT, Pang JH. Interleukin-6 inhibits endothelial nitric oxide synthase activation and increases endothelial nitric oxide synthase binding to stabilized caveolin-1 in human vascular endothelial cells. J Hypertens 2010; 28(5): 940-51.

[161] Chen K, Li F, Li J, *et al*. Induction of leptin resistance through direct interaction of C-reactive protein with leptin. Nat Med 2006; 12(4): 425-32.

[162] Procopio C, Andreozzi F, Laratta E, *et al*. Leptin-stimulated endothelial nitric-oxide synthasevia an adenosine 5′-monophosphate-activated protein kinase/Akt signaling pathway is attenuated by interaction with C-reactiveprotein. Endocrinology 2009; 150(8): 3584-93.

[163] Quehenberger P, Exner M, Sunder-Plassmann R, *et al*. Leptin induces endothelin-1 in endothelial cells *in vitro*. Circ Res 2002; 90(6): 711-8.

[164] Bełtowski J, Wójcicka G, Jamroz A. Stimulatory effect of leptin on nitric oxide production is impaired in dietary-induced obesity. Obes Res 2003; 11(12): 1571-80.

[165] Payne GA, Borbouse L, Kumar S, *et al*. Epicardial perivascular adipose-derived leptin exacerbates coronary endothelial dysfunction in metabolic syndrome via a protein kinase C-beta pathway. Arterioscler Thromb Vasc Biol 2010; 30(9): 1711-7.

[166] Korda M, Kubant R, Patton S, Malinski T.Leptin-induced endothelial dysfunction in obesity. Am J Physiol Heart Circ Physiol; 295(4): H1514-21.

[167] Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzma´n M, Brownlee M. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J Biol Chem 2001; 276: 25096 -25100.

[168] Kusminski CM, McTernan PG, Kumar S. Role of resistin in obesity, insulin resistance and Type II diabetes. Clin Sci (Lond) 2005; 109(3): 243-56.

[169] Mu H, Ohashi R, Yan S, *et al*. Adipokine resistin promotes *in vitro* angiogenesis of human endothelial cells. Cardiovasc Res 2006; 70: 146-157.

[170] Chemaly ER, Hadri L, Zhang S, *et al*. Long-term *in vivo* resistin overexpression induces myocardial dysfunction and remodeling in rats. J Mol Cell Cardiol 2011; 51(2): 144-55.

[171] Chen C, Jiang J, Lu JM, *et al*. Resistin decreases expression of endothelial nitric oxide synthase through oxidative stress in human coronary artery endothelial cells. Am J Physiol Heart Circ Physiol 2010; 299: H193-201.

[172] Jamaluddin MS, Weakley SM, Yao Q, Chen C. Resistin: functional roles and therapeutic considerations for cardiovascular disease. Br J Pharmacol 2012; 165: 622-32.

[173] Cheng KK, Lam KS, Wang Y, *et al*. Adiponectin-induced endothelial nitric oxide synthase activation and nitric oxide production are mediated by APPL1 in endothelial cells. Diabetes 2007; 56(5): 1387-94.

[174] Motoshima H, Wu X, Mahadev K, Goldstein BJ. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. Biochem Biophys Res Commun 2004; 315(2): 264-71.

[175] Zhang P, Wang Y, Fan Y, Tang Z, Wang N. Overexpression of adiponectin receptors potentiates the antiinflammatory action of subeffective dose of globular adiponectin in vascular endothelialcells. Arterioscler Thromb Vasc Biol 2009; 29(1): 67-74.

[176] Arita Y, Kihara S, Ouchi N, *et al*. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999; 257(1): 79-83.

[177] Hui X, Lam KS, Vanhoutte PM, Xu A. Adiponectin and cardiovascular health: an update. Br J Pharmacol 2012; 165(3): 574-90.

[178] Eringa EC, Bakker W, van Hinsbergh VW. Paracrine regulation of vascular tone, inflammation and insulin sensitivity by perivascular adipose tissue. Vascul Pharmacol 2012; 56(5-6): 204-209.

[179] Potenza MA, Addabbo F, Montagnani M. Vascular actions of insulin with implications for endothelial dysfunction. Am J Physiol Endocrinol Metab 2009; 297(3): E568-77.

[180] Clark MG, Wallis MG, Barrett EJ, *et al*. Blood flow and muscle metabolism: a focus on insulin action. Am J Physiol Endocrinol Metab 2003; 284(2): E241-58.

[181] Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S. Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. Am J Physiol Endocrinol Metab 2003; 285(1): E123-9.

[182] Montagnani M, Golovchenko I, Kim I, *et al*. Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. J Biol Chem 2002; 277(3): 1794-9.

[183] Houben AJ, Eringa EC, Jonk AM, Serne EH, Smulders YM, Stehouwer CD. Perivascular Fat and the Microcirculation: Relevance to Insulin Resistance, Diabetes, and Cardiovascular Disease. Curr Cardiovasc Risk Rep 2012; 6(1): 80-90.

[184] Muniyappa R, Sowers JR. Role of insulin resistance in endothelial dysfunction. Rev Endocr Metab Disord 2013; 14(1): 5-12.

[185] Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. J Clin Invest. 1990; 85(6): 1844-52.

[186] De Jongh RT, Serné EH, IJzerman RG, de Vries G, Stehouwer CD. Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance. Circulation 2004; 109(21): 2529-35.

[187] Wang H, Wang AX, Liu Z, Barrett EJ. Insulin signaling stimulates insulin transport by bovine aortic endothelial cells. Diabetes 2008; 57: 540-7.

[188] Kubota T, Kubota N, Kumagai H, *et al.* Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. Cell Metab 2011; 13(3): 294-307.

[189] Sjostrand M, Gudbjornsdottir S, Holmang A, Lonn L, Strindberg L, Lonnroth, P. Delayed transcapillary transport of insulin to muscle interstitial fluid in obese subjects. Diabetes 2002; 51: 2742-48.

[190] Ellmerer M, Hamilton-Wessler M, Kim SP, *et al.* Reduced access to insulin-sensitive tissues in dogs with obesity secondary to increased fat intake. Diabetes 2006; 55: 1769-1775.

[191] Jiang ZY, Lin YW, Clemont A, *et al*. Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats. J Clin Invest 1999; 104(4): 447-57.

[192] Cusi K, Maezono K, Osman A, *et al.* Insulin resistance differentially affects the PI3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest 2000; 105: 311-20.

[193] Lteif A, Vaishnava P, Baron AD, Mather KJ. Endothelin limits insulin action in obese/insulin-resistant humans. Diabetes 2007; 56(3): 728-34.

[194] Wheatcroft SB, Shah AM, Li JM, *et al.* Preserved glucoregulation but attenuation of the vascular actions of insulin in mice heterozygous for knockout of the insulin receptor. Diabetes 2004; 53(10): 2645-52.

[195] Okon EB, Chung AW, Rauniyar P, *et al.* Compromised arterial function in human type 2 diabetic patients. Diabetes 2005; 54: 2415-23.

[196] Kim F, Pham M, Luttrell I, *et al*. Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity. Circ Res 2007; 100(11): 1589-96.

[197] Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. Lancet 2010; 375: 2267-77.

[198] Dresner A, Laurent D, Marcucci M, *et al*. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest 1999; 103: 253-9.

[199] Meijer RI, Bakker W, Alta CL, *et al*. Perivascular adipose tissue control of insulin-induced vasoreactivity in muscle is impaired in db/db mice. Diabetes 2013; 62(2): 590-8.

[200] Maloney E, Sweet IR, Hockenbery DM, *et al.* Activation of NF-kappaB by palmitate in endothelial cells: a key role for NADPH oxidase-derived superoxide in response to TLR4 activation. Arterioscler Thromb Vasc Biol 2009; 29(9): 1370-5.

[201] Katakam PV, Snipes JA, Steed MM, Busija DW. Insulin-induced generation of reactive oxygen species and uncoupling of nitric oxide synthase underlie the cerebrovascular insulin resistance in obese rats. J Cereb Blood Flow Metab 2012; 32(5): 792-804.

[202] Ali MI, Ketsawatsomkron P, Belin de Chantemele EJ, *et al*. Deletion of protein tyrosine phosphatase 1b improves peripheral insulin resistance and vascular function in obese, leptin-resistant mice via reduced oxidant tone. Circ Res 2009; 105 (10): 1013-22.

[203] Bagi Z, Koller A, Kaley G. PPARgamma activation, by reducing oxidative stress, increases NO bioavailability in coronary arterioles of mice with Type 2 diabetes. Am J Physiol Heart Circ Physiol 2004; 286(2): H742-8.

[204] Oltman CL, Davidson EP, Coppey LJ, Kleinschmidt TL, Lund DD, Yorek MA. Attenuation of vascular/neural dysfunction in Zucker rats treated with enalapril or rosuvastatin. Obesity (Silver Spring) 2008; 16(1): 82-9.

[205] Nagashima H, Endo M. Pitavastatin prevents postprandial endothelial dysfunction via reduction of the serum triglyceride level in obese male subjects. Heart Vessels 2011; 26(4): 428-34

[206] Cao Y. Adipose tissue angiogenesis as a therapeutic target for obesity and metabolic diseases. Nat Rev Drug Discov 2010; 9(2): 107-15.

Received: April 30, 2013 **Revised: May 30, 2013** **Accepted: June 11, 2013**