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Microvascular endothelial dysfunction in human obesity: role of TNF-α

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Context. Endothelium guarantees the vascular homeostasis by the opposite action of substances with vasodilating/anti-thrombogenic and vasoconstricting/prothrombotic activities. Obesity is characterized by endothelial dysfunction associated with a condition of vascular low-grade inflammation.
Evidence Acquisition. Analysis of available basic or clinical papers published in peer-reviewed international journals on microcirculation and obesity.
Evidence Synthesis. Vascular low-grade inflammation which characterizes obesity is secondary to abnormal production of proinflammatory cytokines, including tumour necrosis factor-alpha (TNF-α). TNF-α, generated either in small vessels or within the perivascular adipose tissue (PVAT) of obese patients, stimulates the reactive oxygen species generation, mainly through NAD(P)H oxidase activation, which in turn reduces nitric oxide (NO) availability. These aspects are lighted by the insulin resistance status and macronutrient intake which characterize the obesity condition. Oxidant excess has also been proposed as a mechanism whereby TNF-α interferes with the Endothelin-1/NO system at the level of small vessels from obese patients.
Conclusions. In obesity, microvasculature from visceral fat is an important source of low-grade inflammation and oxidative stress that, together with the PVAT, directly contribute to vascular changes, favouring the development and acceleration of the vascular atherothrombotic process in this clinical condition.

the aim of the present review is to report critically the state of the art of the mechanisms accounting for the functional alterations of the microvasculature in human obesity.

INTRODUCTION

The microcirculation, which includes small arteries, arterioles and capillaries, represents a key network to support adequate tissue perfusion and metabolism. The tone of resistance vessels has a crucial role in dissipating the pulsatile energy generated by the heart contraction, thus protecting small capillaries from pressure damage. This energy dissipation is achieved at the cost of an increase in peripheral vascular resistances, making the regulation of the vascular tone at this level one of the most important determinants of blood pressure values (1). Accordingly, vascular dysfunction which characterises several pathological conditions largely involves adaptive changes at this vascular bed.

The endothelium represents a fundamental tissue which guarantees the vascular homeostasis by the opposite action of substances with vasodilating/anti-thrombogenic properties and vasoconstricting/prothrombotic activities (2). In healthy conditions, the most important compound generated by endothelial cells and influencing vascular homeostasis is nitric oxide (NO). This is a gas produced by the activity of the constitutive endothelial enzyme NO synthase (eNOS) under the influence of chemical or mechanical forces. Among these, the most characterised are the activities of endothelial agonists (e.g. acetylcholine, bradykinin) acting on specific endothelial receptors, and the shear stress (3). NO exerts its cardiovascular (CV) protective role by relaxing media-smooth muscle cells, inhibiting platelet adhesion and aggregation, preventing leukocyte adhesion and migration into the arterial wall, muscle cell proliferation, and adhesion molecule expression (2, 3).

In the presence of major CV risk factors, the endothelium loses its protective role, switching to a pro-atherosclerotic phenotype. This condition, called “endothelial dysfunction”, is characterised by either a reduced production of NO or a predominant vascular generation of vasoconstricting and pro-atherosclerotic substances, generically termed endothelium-derived contracting factors (EDCFs) (4). These include reactive oxygen species (ROS), mainly superoxide, that are generated by different intracellular sources according to clinical conditions and vascular districts, including nicotinamide dinucleotide phosphate (NADPH) oxidase, cyclooxygenase, xanthine oxidase, and the mitochondria (5, 6). Superoxide rapidly reacts with NO to form the highly reactive intermediate peroxynitrite which, in turn, determines vascular changes limiting NO bioavailability. Superoxide may also stimulate the expression of endothelin-1 (ET-1), another EDCF that binds to specific receptors located on both endothelial and smooth muscle cells (ET_A and ET_B), mediating the vast majority of its biological effects (6, 7).

Recently, a key role played by low-grade vascular inflammation on endothelial dysfunction and atherosclerosis emerged (8, 9). In particular, TNF-α has been suggested as a key cytokine involved in reducing NO availability, an effect exerted by both inducing ROS generation and direct inhibition of eNOS activity (8).

Obesity is a complex chronic disease associated with increased CV morbidity and mortality (10). The earliest manifestation of CV disease in obesity is endothelial dysfunction, an alteration that has been documented in several different vascular beds (11-15). A condition of chronic low-grade inflammation secondary to the abnormal production of proinflammatory cytokines, including TNF-α (16, 17), is considered the main mechanisms leading to endothelial dysfunction in obesity. This review aims to give a brief overview of the known mechanisms involved in the pathogenesis of endothelial dysfunction in the microcirculation of patients with obesity, along with an attempt to address the complex links between visceral adiposity, TNF-α and microcirculation.

OBESITY AND ENDOTHELIAL DYSFUNCTION

To date, some experimental and human studies have homogeneously documented the presence of endothelial dysfunction in obesity (18, 19). A large body of literature indicates that obesity is responsible for direct detrimental effects on the vasculature, independently from the concomitant presence of traditional CV risk factors commonly detected in obesity, including hypertension, diabetes mellitus or dyslipidaemia. The first demonstration that obesity *per se* can cause endothelial dysfunction was reported by Steinberg *et al* (11), who documented reduced endothelium-dependent vasodilation to methacholine in the leg microcirculation of obese patients. In line with these results, Perticone et al identified a linear relationship between severity of obesity and reduction of endothelial function in the forearm microcirculation of overweight-obese patients (13). Importantly, in this study the intra-arterial infusion of ascorbic acid was able to ameliorate response to acetylcholine, thus

suggesting a role of oxidative stress in mediating endothelial dysfunction. Similarly, de Jongh *et al.* (20) demonstrated by iontophoresis technique that small cutaneous vessels of obese women have a reduced vasodilating response to acetylcholine, but not to sodium nitroprusside, as compared to healthy controls, and that this alteration was detectable in both basal conditions and during hyperinsulinemia. In this study, vascular response to acetylcholine was directly related to insulin sensitivity and inversely related to blood pressure values, suggesting that a reduced endothelial function might contribute to the development of microangiopathy associated to obesity and insulin resistance in this vascular bed. Growing literature also investigated the endothelial function in resistance vessels isolated from a biopsy of abdominal subcutaneous tissue or visceral fat of patients with severe obesity, by an *in vitro ex-vivo* technique. These studies concordantly documented a dramatic reduction of endothelium-dependent relaxation of small resistance arteries from obese patients compared to lean controls (14, 15, 21). In particular, it was observed that the blunted response to acetylcholine was resistant to the eNOS inhibitor N^{ω}-nitro-L-arginine methylester (L-NAME), and potentiated by the antioxidant tempol, which also restored the inhibitory effect of L-NAME on the endothelial agonist (15). These findings demonstrate that oxidative stress might have a greater influence on the NO availability in such vascular bed than the intrinsic activity of the eNOS.

More recently, the presence of an independent impact of obesity on microcirculation was further confirmed by van der Heijden et al (22). These authors showed that, in patients with treated metabolic risk factors and suspected coronary artery disease, body mass index was significantly associated with decreased endothelium-dependent vasodilatation measured with peripheral arterial tonometry and laser Doppler flowmetry. Notably, this relationship remained highly significant even after adjustment for confounding risk factors, including diabetes mellitus, hypertension, hypercholesterolemia, and smoking.

In conclusions, concordant findings emerging from several vascular beds and consequent to different endothelial stimuli demonstrate that microvascular circulation represents a major target for obesity-related endothelial dysfunction. In isolated resistance vessels, in which the mechanisms accounting for endothelial dysfunction have been explored, a blunted activity of NO secondary to oxidant excess was demonstrated.

Obesity and Inflammation: the role of perivascular adipose tissue (PVAT)

Over the past decade, a fundamental breakthrough in the obesity-related cardiovascular disease was the discovery that obesity is associated with a chronic, low grade, systemic inflammatory exposure. While multiple pathways could account for this elevated inflammatory burden detected in obesity, the adipose tissue is emerging as a central source of pro-inflammatory cytokine production. This affirmation was first proposed by Hotamisligil *et al* (23) showing that TNF-α was constitutively expressed by adipose tissue, over-expressed in obesity, and acting as a potential mediator of insulin resistance in several animal models. From this pioneering observation, many studies confirmed that human obesity is an inflammatory disease, also providing important insights into the mechanisms underlying the chronic activation of the immune-inflammatory response in obese patients. It has been documented that circulating mononuclear cells and inflammatory cells infiltrating the adipose tissue of obese patients adopt a proinflammatory phenotype (24), which is characterised by an elevated and dysregulated production of proinflammatory cytokines. Beyond the contribution of the immune-inflammatory cells, the visceral fat is increasingly recognised as a major source of pro-inflammatory chemokines, named adipokines. The generation of adipokines by fat cells can be regarded as an endocrine function and makes the adipose tissue the largest endocrine organ of the body, particularly in patients affected by severe obesity. Major adipokines generated by fat cells include leptin, resistin and adiponectin (25), all of which can influence, through several mechanisms, vascular homeostasis. Leptin is the main protein

produced by adipocytes, and its blood concentration is proportional to the amount of adipose tissue. I*n vitro* studies suggested that leptin could cause an increment of oxidative stress production in endothelial cells (26). Moreover, leptin can stimulate the secretion of TNF-α and interleukin-6 (IL-6) which, in turn, may induce endothelial dysfunction (27). Other reports, conversely, suggested that leptin might induce vasorelaxation, an effect which seemed to be mediated by mechanisms involving the production of the endothelium-derived hyperpolarizing factor (28, 29). The ability of leptin to stimulate vasodilation by NO - independent or -dependent mechanisms requires, however, further investigation, as available evidence is conflicting. For example, while Matsuda et al showed a NO-independent vasorelaxation of human coronary arteries after infusion of leptin (30), Tsuda et al documented an influence of NO-dependent pathways on the endothelial vasorelaxation obtained with leptin (31).

Resistin, produced by adipogenesis, is involved in the development of insulin resistance and obesity (32). Recently, it was observed that endothelial cells incubated with human recombinant resistin increase their production of ET-1 and the expression of adhesion molecules (33), a finding suggesting a direct effect of resistin on vascular endothelium. Most convincing evidence on the effects of adipokines on the vascular wall involves the role of adiponectin. This adipokine, produced in mature adipocytes, has opposite effects than leptin and resistin. Indeed, adiponectin stimulates insulin sensitivity (34), reduces the expression of adhesion molecules on the endothelium, inhibits the transformation of macrophages into foam cells and reduces the smooth muscle cells proliferation. A study including hypertensive patients documented that circulating levels of adiponectin are inversely related to the severity of the endothelial dysfunction measured at the level of forearm microcirculation (35). Obesity is characterised by reduced production and activity of adiponectin (36), potentially reducing the protective effects of this adipokine against the development of endothelial dysfunction and the atherosclerotic process.

A major mechanism whereby obesity can induce vascular changes involves the PVAT, which exerts deleterious effects on the vasculature by secreting TNF-α and IL-6 (37). Experimental reports indicated TNF-α might stimulate ROS production via activation of NAD(P)H oxidase (38), or by the activation of nuclear transcription factor-kappa B (NF-kB) (39). This leads to mobilisation and activation of macrophages, migration and proliferation of smooth muscle cells, and induction of adhesion molecule expression by the endothelial and smooth muscle cells of the vascular wall (40). IL-6 can increase the ROS production by activating xanthine oxidase and NAD(P)H oxidase. IL-6 also stimulates liver synthesis of C-reactive protein, which reduces NO production by decreasing the expression of eNOS (41). An increased expression of TNF-α and the acquisition of a pro-oxidant phenotype by PVAT might represent common mechanisms leading to vascular changes in condition of insulin resistance. Indeed, PVAT from a rodent model of metabolic syndrome shows an increased TNF-α generation, together with a decreased adiponectin expression, a greater NADPH oxidase activity and a reduced expression of antioxidant ·O2− dismutase (-1, -2, and -3). (42).

In humans, many studies contribute to our understanding a possible link between TNF-α and obesity-related vascular dysfunction. In patients with obesity-related metabolic syndrome, Tesauro *et al* (43) assessed the effects of TNF-α neutralisation by infliximab, a monoclonal antibody blocking the TNF-α signalling, on vascular reactivity during hyperinsulinemia. At baseline, patients showed a reduced relaxation to both acetylcholine and sodium nitroprusside during hyperinsulinemia, compared with control subjects. Infliximab potentiated the vascular responses to both agonists. Of note, the antioxidant ascorbic acid improved the vasodilator response to acetylcholine in obese patients, with no further potentiating effect when infliximab was concomitantly administrated. These results indicate

that TNF-α inhibition may ameliorate vascular reactivity in obese patients during hyperinsulinemia, an effect consequent to a decreased oxidative stress (43).

More recently, an interesting study conducted in isolated resistance vessels evidenced a specific role of PVAT-derived inflammation in the pathogenesis of vascular dysfunction. Small arteries with and without PVAT were obtained from a subcutaneous gluteal fat biopsy of obese and control subjects. While in vessels of lean controls PVAT was able to generate factors that potentiated NO availability, in obese subjects, which showed an increased area of adipocytes, the presence of PVAT did not improve vasodilation (44). Acute injection of TNF-α and IL-6 around healthy blood vessels reduced dilator activity of PVAT, resulting in no longer differences compared to the obese group. Pre-incubation of ROS scavengers or cytokine antagonist was able to prevent these alterations (44). Taken together, these results evidence that in physiological conditions adipocytes contribute to the regulation of local vascular tone by modulating NO availability. Adipocyte hypertrophy, oxidant excess and an increased accumulation of TNF-α in the PVAT of obese subjects result in a loss of this important regulations. These data highlight the pivotal role of inflammation as a promoter of vascular dysfunction, being an important mechanism whereby the pathological adipose tissue exerts a deleterious effect on the surrounding vasculature in a paracrine manner.

Does vasculature represent a mere target of PVAT-derived TNF-α?

Based on current evidence, therefore, PVAT is emerging as a direct regulator of vascular tone throughout the generation of pro-inflammatory cytokines with documented direct deleterious effects on the vasculature, including TNF-α. These data strengthen the concept of obesity as an inflammatory state and indicate vasculature as a potential target of adipose tissue-derived inflammatory cytokines. However, whether low-grade inflammation result also from the generation of pro-inflammatory products at the level of the vasculature, as well as their possible effects on vascular endothelial function in human obesity remain to be elucidated. A recent study conducted in our laboratory attempted to respond to these uncertainties. Small arteries were isolated from visceral fat immediately after collection of a biopsy sample during laparoscopic surgery, in patients with and without severe obesity. Vessels from the obese group showed a reduced endothelium-dependent relaxation, which was resistant to L-NAME. This blunted relaxation was reversed by the superoxide scavenger tempol and, to a similar extent, by infliximab (15). These data were corroborated by the direct measure of the vascular wall superoxide anion concentration performed at the confocal microscope after staining with the fluorescent probe dihydroethidium. Vessels from the obese group showed higher levels of superoxide anions compared to the non-obese group, which was dramatically reduced by incubation with tempol or infliximab. Immunohistochemistry indicated a marked up-regulation of TNF-α mainly in the media layer of these vessels, demonstrating for the first time that the vascular wall may also be a source of TNF-α involved in endothelial dysfunction. Taken together, these results demonstrate that small vessels from obese patients show a reduced NO availability secondary to excess ROS generation and that TNF-α promotes endothelial dysfunction by stimulating intravascular ROS generation. In the same study, we explored the potential pathway(s) involved in TNF-α-induced vascular ROS generation, focusing on the role of NAD(P)H oxidase, inducible nitric oxide synthase (iNOS) and xanthine oxidase. Using the specific inhibitors of these enzymes (apocynin, S-methylisothiourea and allopurinol), we found that only the inhibition of NAD(P)H oxidase and iNOS, but xanthine oxidase, could restore the impaired endothelial function in the obese group. Similarly, the inhibition of NAD(P)H oxidase and iNOS led to a significant reduction of the intravascular superoxide generation as assessed by dihydroethidium. Comprehensively, these results suggest that NAD(P)H oxidase and iNOS are the two major enzymatic pathways mediating the increase of vascular wall oxidative stress induced by TNF-α in obesity (15).

Role of insulin and insulin resistance status on vascular inflammation

In the past two decades, several evidence suggested that insulin might have an important role in regulating vascular homeostasis, over and above the control of local and systemic metabolism. It is now recognised that insulin has vasodilatory effects on arteries, veins and microcirculation (45-47). Several studies convincingly documented that such effects are mainly mediated by an increased stimulation and expression of eNOS within endothelial cells, which results in an increased NO availability (48, 49). Over and above its direct effect on eNOS, insulin might influence NO availability also by controlling systemic and local levels of inflammation and oxidative stress. The expression of the key pro-inflammatory transcription factor NF-KB is suppressed by insulin, particularly within the endothelial cell, and this leads to a reduced vascular inflammation (50-52). Insulin also suppresses ROS generation by inhibiting the NADPH oxidase activity (51). Based on these findings, it is not surprising that conditions of insulin resistance (such as in human obesity) lead to ROS generation, vascular inflammation and inhibition of eNOS expression (53). TNF-α has an important role in promoting vascular inflammation and oxidative stress due to an altered vascular activity of insulin, as it can reduce the insulin signal by inhibiting the expression of the insulin receptors (54). Thus, over and above its direct effects, the TNF-α might also influence the vascular homeostasis of obese patients by reducing the insulin signal and its anti-inflammatory and anti-oxidative effects on the vascular wall.

Additional support to the presence of a strong relationship between inflammation, insulin resistance and vascular changes in obesity is provided by the evidence that an excessive macronutrient intake, primary cause of an excessive body weight, results in an increase in ROS generation and inflammatory response (52, 55), as well as increment in plasma endotoxin concentrations and the expression of Toll-Like receptors (56). Long-term caloric restriction and diet-induced weight loss, by contrast, result in the fall of pro-inflammatory cytokines, including TNF-α, and this is accompanied by a reduced insulin release after glucose challenge (16, 17). Similarly, the reduced inflammation and insulin resistance which follows bariatric surgery (57) are associated to a fall in the circulating levels of molecules with a negative impact on the vascular homeostasis, including ET-1, elements of the renin-angiotensin system, and neprilysin (58).

Local inflammation in obesity: the role of ET-1

In healthy conditions, ET-1 participates to the vascular homeostasis by binding with two receptor subtypes, ET_A and ET_B. ET_A receptors are represented only on smooth muscle cells and mediate contractions and promote growth. In contrast, ET_B receptors are located on smooth muscle cells (where they evoke contractions) and on endothelial cells (where they induce relaxation stimulating NO production). Although in physiological condition the NO counterbalances the contracting effect of ET-1 (7, 59), in obesity a vascular ET-1/NO imbalance is commonly detected. Indeed, Cardillo C *et al* (60) observed that intra-arterial infusion of an ET_A receptor blocker (BQ-123) resulted in significant vasodilation at the level of the forearm microcirculation from overweight and obese but not in lean hypertensive subjects or normotensive control subjects irrespective of their body mass. The BQ-123-induced vasodilation was significantly related to body mass in hypertensive subjects but not in control subjects, thereby indicating a selective enhancement of ET_A-dependent vasoconstrictor tone in hypertensive patients with increased body mass (60). These findings suggest a potential role of the activated ET-1 system in the pathophysiology of complications of obesity-related hypertension. The upregulated production of ET-1 in obesity might result from the activation of various vascular pathways. Among these, experimental evidence indicated that oxidative stress is one of the most important factors stimulating the increase of ET-1 synthesis in human coronary artery smooth muscle cells. In turn, ET-1 signal is able to stimulate ROS production in the vascular wall, potentially creating a vicious cycle.

The evidence obtained by Amiri et al in transgenic mice with endothelium-targeted overexpression of human preproET-1 strengthened the hypothesis of a positive feedback loop between ET-1 signal and oxidative stress. In this model, preproET-1 overexpression causes remodelling and inflammation of resistance vessels in the absence of a blood pressure elevation, an effect mediated by multiple mechanisms, including the activation of vascular NAD(P)H oxidase (61, 62). The increased production of ET-1 under conditions of increased vascular oxidative stress also raises the hypothesis that local inflammation might interfere with the ET-1-mediated vascular tone in obesity. Oxidant excess can be hypothesized as a mechanism whereby TNF-α interferes with the ET-1/NO system. Such possibility was recently addressed in our laboratory, aiming to evaluate whether TNF-α contributes to the vasoconstriction induced by endogenous ET-1 in small arteries isolated from the visceral abdominal fat of patients with severe obesity, and whether this effect might be indirectly mediated by a modulation of tonic NO release. Endogenous ET-1 activity was assessed by the ETA blocker BQ-123, while the anti-TNF-α infliximab and L-NAME were utilised to test TNF-α and NO, respectively. Obese showed a blunted L-NAME-induced vasoconstriction, which was improved by pre-incubation with infliximab. Also, BQ-123 induced a greater relaxation in the obese group, which resulted to be unaffected by L-NAME but attenuated by infliximab. Of note, PVAT removal reversed these effects. Incubation of the vessel with the NAD(P)H oxidase inhibitor apocynin, L-NAME, or BQ-123 induced a significant reduction of the superoxide excess within the vascular wall of obese patients, and a similar effect was obtained when the TNF-α signalling was blocked with infliximab (63). We also detected an increased vascular expression of ET-1, ETA, and ETB receptors, and higher vascular/perivascular TNF-α and TNF-α receptor expression (63). These complex results provided the first evidence that in small arteries from obese patients, the ET-1/NO imbalance is mediated by vascular and perivascular TNF-α excess, coupled with an increased vascular expression of ET-1 and ETA receptor. Such alterations induce endothelial dysfunction, which is characterised by ROS excess and impair tonic NO release in the vascular wall. These findings also support the concept that in obesity PVAT loses its vascular protective properties switching towards a functionally active pro-contractile inflammation source.

Conclusions

Obesity is characterised by a marked endothelial dysfunction caused by a reduced NO availability. In this clinical condition, PVAT plays a crucial role in generating pro-inflammatory cytokines, including TNF-α, with a documented direct deleterious effect toward the vasculature. In particular, while TNF-α promotes superoxide generation in the vascular wall via different pathways, the most important contribution might be due to the hyperactivation of the NAD(P)H oxidase. In such a scenario, crucial roles are played by the insulin resistance status and the chronic macronutrient intake, which contribute to the obesity-associated chronic inflammatory status. TNF-α also participates in the ET-1/NO imbalance in small arteries from obese patients (Figure 1). At present, the available literature does not enable establishing whether the small arteries and adipocytes located within PVAT should be regarded as independent and coexisting sites of TNF-α generation or, by contrast, whether there is a hierarchical relationship or mutual interplay between these two districts. Any extrapolation of the potential effects of visceral fat on the small vessels to other vascular beds requires caution, as the vascular responses to the autocrine/paracrine functions of the endothelium can vary depending on the anatomical location of blood vessels. Nevertheless, there is no doubt that these findings recognise obesity as an inflammatory condition and identify the microvasculature from visceral fat an important source of low-grade inflammation and oxidative stress that, together with the PVAT, directly contribute to the local development of insulin resistance.

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The authors have nothing to disclose.

Bibliography

1. Mulvany MJ, Aalkjaer C**.** Structure and function of small arteries. *Physiol Rev*. 1990;70(4):921-961.
2. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, Luscher TF, Shechter M, Taddei S, Vita JA, Lerman A**.** The assessment of endothelial function: from research into clinical practice. *Circulation.* 2012;126(6):753-767.
3. Virdis A, Ghiadoni L, Versari D, Giannarelli C, Salvetti A, Taddei S**.** Endothelial function assessment in complicated hypertension. *Curr Pharm Des.* 2008;14(18):1761-1770.
4. Virdis A, Taddei S**.** Endothelial Dysfunction in Resistance Arteries of Hypertensive Humans: Old and New Conspirators. *J Cardiovasc Pharmacol*. 2016;67(6):451-457.
5. Leopold JA, Loscalzo J**.** Oxidative enzymopathies and vascular disease. *Arterioscler Thromb Vasc Biol*. 2005;25(7):1332-1340.
6. Munzel T, Gori T, Bruno RM, Taddei S**.** Is oxidative stress a therapeutic target in cardiovascular disease? *Eur Heart J*. 2010;31(22):2741-2748.
7. Dhaun N, Goddard J, Kohan DE, Pollock DM, Schiffrin EL, Webb DJ**.** Role of endothelin-1 in clinical hypertension: 20 years on. *Hypertension*. 2008;52(3):452-459.
8. Zhang H, Park Y, Wu J, Chen X, Lee S, Yang J, Dellsperger KC, Zhang C**.** Role of TNF-alpha in vascular dysfunction. *Clin Sci (Lond).* 2009;116(3):219-230.
9. Libby P, Ridker PM, Hansson GK**.** Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol.* 2009;54(23):2129-2138.
10. Klein S, Burke LE, Bray GA, Blair S, Allison DB, Pi-Sunyer X, Hong Y, Eckel RH**.** Clinical implications of obesity with specific focus on cardiovascular disease: a statement for professionals from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: endorsed by the American College of Cardiology Foundation. *Circulation.* 2004;110(18):2952-2967.
11. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD**.** Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J Clin Invest*. 1996;97(11):2601-2610.
12. 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(6):1523-1528.
13. Perticone F, Ceravolo R, Candigliota M, Ventura G, Iacopino S, Sinopoli F, Mattioli PL**.** Obesity and body fat distribution induce endothelial dysfunction by oxidative stress: protective effect of vitamin C. *Diabetes*. 2001;50(1):159-165.
14. Grassi G, Seravalle G, Scopelliti F, Dell'Oro R, Fattori L, Quarti-Trevano F, Brambilla G, Schiffrin EL, Mancia G**.** Structural and functional alterations of subcutaneous small resistance arteries in severe human obesity. *Obesity (Silver Spring).* 2010;18(1):92-98.
15. Virdis A, Santini F, Colucci R, Duranti E, Salvetti G, Rugani I, Segnani C, Anselmino M, Bernardini N, Blandizzi C, Salvetti A, Pinchera A, Taddei S**.** Vascular generation of tumor necrosis factor-alpha reduces nitric oxide availability in small arteries from visceral fat of obese patients. *J Am Coll Cardiol*. 2011;58(3):238-247.

16. Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T**.** Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab.* 1998;83(8):2907-2910.
17. Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R**.** Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation.* 2005;111(11):1448-1454.
18. Brook RD**.** Obesity, weight loss, and vascular function. *Endocrine*. 2006;29(1):21-25.
19. Avogaro A, de Kreutzenberg SV**.** Mechanisms of endothelial dysfunction in obesity. *Clin Chim Acta*. 2005;360(1-2):9-26.
20. de Jongh RT, Serne EH, RG IJ, de Vries G, Stehouwer CD**.** Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance. *Circulation*. 2004;109(21):2529-2535.
21. De Ciuceis C, Porteri E, Rizzoni D, Corbellini C, La Boria E, Boari GE, Pilu A, Mittempergher F, Di Betta E, Casella C, Nascimbeni R, Rosei CA, Ruggeri G, Caimi L, Rosei EA**.** Effects of weight loss on structural and functional alterations of subcutaneous small arteries in obese patients. *Hypertension.* 2011;58(1):29-36.
22. van der Heijden DJ, van Leeuwen MAH, Janssens GN, Lenzen MJ, van de Ven PM, Eringa EC, van Royen N**.** Body Mass Index Is Associated With Microvascular Endothelial Dysfunction in Patients With Treated Metabolic Risk Factors and Suspected Coronary Artery Disease. *J Am Heart Assoc*. 2017;6(9).
23. Hotamisligil GS, Shargill NS, Spiegelman BM**.** Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science.* 1993;259(5091):87-91.
24. Ghanim H, Aljada A, Hofmeyer D, Syed T, Mohanty P, Dandona P**.** Circulating mononuclear cells in the obese are in a proinflammatory state. *Circulation.* 2004;110(12):1564-1571.
25. Kershaw EE, Flier JS**.** Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*. 2004;89(6):2548-2556.
26. Bouloumie A, Marumo T, Lafontan M, Busse R**.** Leptin induces oxidative stress in human endothelial cells. *Faseb J*. 1999;13(10):1231-1238.
27. Bullo M, Garcia-Lorda P, Megias I, Salas-Salvado J**.** Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. *Obes Res*. 2003;11(4):525-531.
28. Lembo G, Vecchione C, Fratta L, Marino G, Trimarco V, d'Amati G, Trimarco B**.** Leptin induces direct vasodilation through distinct endothelial mechanisms. *Diabetes*. 2000;49(2):293-297.
29. Vecchione C, Maffei A, Colella S, Aretini A, Poulet R, Frati G, Gentile MT, Fratta L, Trimarco V, Trimarco B, Lembo G**.** Leptin effect on endothelial nitric oxide is mediated through Akt-endothelial nitric oxide synthase phosphorylation pathway. *Diabetes*. 2002;51(1):168-173.
30. Matsuda K, Teragawa H, Fukuda Y, Nakagawa K, Higashi Y, Chayama K**.** Leptin causes nitric-oxide independent coronary artery vasodilation in humans. *Hypertens Res*. 2003;26(2):147-152.
31. Tsuda K, Nishio I**.** Leptin and nitric oxide production in normotensive and hypertensive men. *Obes Res*. 2004;12(8):1223-1237.
32. Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, Enders GH, Silberg DG, Wen X, Wu GD, Lazar MA**.** A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci U S A*. 2001;98(2):502-506.
33. Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, Mickle DA**.** Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. *Circulation.* 2003;108(6):736-740.

34. Matsuzawa Y, Funahashi T, Kihara S, Shimomura I**.** Adiponectin and metabolic syndrome. *Arterioscler Thromb Vasc Biol*. 2004;24(1):29-33.
35. Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, Kumada M, Ohashi K, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Nagasawa A, Kobayashi H, Hiraoka H, Komai N, Kaibe M, Rakugi H, Ogihara T, Matsuzawa Y**.** Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension*. 2003;42(3):231-234.
36. Goldstein BJ, Scalia R**.** Adiponectin: A novel adipokine linking adipocytes and vascular function. *J Clin Endocrinol Metab*. 2004;89(6):2563-2568.
37. Yudkin JS**.** Adipose tissue, insulin action and vascular disease: inflammatory signals. *Int J Obes Relat Metab Disord*. 2003;27 Suppl 3:S25-28.
38. Dodd-o JM, Welsh LE, Salazar JD, Walinsky PL, Peck EA, Shake JG, Caparrelli DJ, Ziegelstein RC, Zweier JL, Baumgartner WA, Pearse DB**.** Effect of NADPH oxidase inhibition on cardiopulmonary bypass-induced lung injury. *Am J Physiol Heart Circ Physiol*. 2004;287(2):H927-936.
39. Mercurio F, Manning AM**.** Multiple signals converging on NF-kappaB. *Curr Opin Cell Biol*. 1999;11(2):226-232.
40. Virdis A, Schiffrin EL**.** Vascular inflammation: a role in vascular disease in hypertension? *Curr Opin Nephrol Hypertens*. 2003;12(2):181-187.
41. Ikeda U, Takahashi M, Shimada K**.** C-reactive protein directly inhibits nitric oxide production by cytokine-stimulated vascular smooth muscle cells. *J Cardiovasc Pharmacol*. 2003;42(5):607-611.
42. 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):1384-1392.
43. Tesauro M, Schinzari F, Rovella V, Melina D, Mores N, Barini A, Mettimano M, Lauro D, Iantorno M, Quon MJ, Cardillo C**.** Tumor necrosis factor-alpha antagonism improves vasodilation during hyperinsulinemia in metabolic syndrome. *Diabetes Care.* 2008;31(7):1439-1441.
44. Greenstein AS, Khavandi K, Withers SB, Sonoyama K, Clancy O, Jeziorska M, Laing I, Yates AP, Pemberton PW, Malik RA, Heagerty AM**.** Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. *Circulation.* 2009;119(12):1661-1670. Epub 2009 Mar 1616.
45. Baron AD**.** Hemodynamic actions of insulin. *Am J Physiol*. 1994;267(2 Pt 1):E187-202.
46. Grover A, Padginton C, Wilson MF, Sung BH, Izzo JL, Jr., Dandona P**.** Insulin attenuates norepinephrine-induced venoconstriction. An ultrasonographic study. *Hypertension*. 1995;25(4 Pt 2):779-784.
47. Clerk LH, Vincent MA, Lindner JR, Clark MG, Rattigan S, Barrett EJ**.** The vasodilatory actions of insulin on resistance and terminal arterioles and their impact on muscle glucose uptake. *Diabetes Metab Res Rev*. 2004;20(1):3-12.
48. Zeng G, Quon MJ**.** Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J Clin Invest*. 1996;98(4):894-898.
49. Aljada A, Dandona P**.** Effect of insulin on human aortic endothelial nitric oxide synthase. *Metabolism*. 2000;49(2):147-150.
50. Aljada A, Ghanim H, Saadeh R, Dandona P**.** Insulin inhibits NFkappaB and MCP-1 expression in human aortic endothelial cells. *J Clin Endocrinol Metab*. 2001;86(1):450-453.
51. Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, Ahmad S**.** Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear

cells in obese subjects: evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab.* 2001;86(7):3257-3265.
52. Dandona P, Chaudhuri A, Mohanty P, Ghanim H**.** Anti-inflammatory effects of insulin. *Curr Opin Clin Nutr Metab Care*. 2007;10(4):511-517.
53. Aljada A, Ghanim H, Assian E, Dandona P**.** Tumor necrosis factor-alpha inhibits insulin-induced increase in endothelial nitric oxide synthase and reduces insulin receptor content and phosphorylation in human aortic endothelial cells. *Metabolism.* 2002;51(4):487-491.
54. Dandona P, Aljada A, Bandyopadhyay A**.** Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol*. 2004;25(1):4-7.
55. Aljada A, Mohanty P, Ghanim H, Abdo T, Tripathy D, Chaudhuri A, Dandona P**.** Increase in intranuclear nuclear factor kappaB and decrease in inhibitor kappaB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect. *Am J Clin Nutr*. 2004;79(4):682-690.
56. Ghanim H, Abuaysheh S, Sia CL, Korzeniewski K, Chaudhuri A, Fernandez-Real JM, Dandona P**.** Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. *Diabetes Care*. 2009;32(12):2281-2287.
57. Monte SV, Caruana JA, Ghanim H, Sia CL, Korzeniewski K, Schentag JJ, Dandona P**.** Reduction in endotoxemia, oxidative and inflammatory stress, and insulin resistance after Roux-en-Y gastric bypass surgery in patients with morbid obesity and type 2 diabetes mellitus. *Surgery*. 2012;151(4):587-593.
58. Ghanim H, Monte S, Caruana J, Green K, Abuaysheh S, Dandona P**.** Decreases in neprilysin and vasoconstrictors and increases in vasodilators following bariatric surgery. *Diabetes Obes Metab*. 2018.
59. Cardillo C, Kilcoyne CM, Cannon RO, Panza JA**.** Interactions between nitric oxide and endothelin in the regulation of vascular tone of human resistance vessels in vivo. *Hypertension.* 2000;35(6):1237-1241.
60. 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.
61. Amiri F, Virdis A, Neves MF, Iglarz M, Seidah NG, Touyz RM, Reudelhuber TL, Schiffrin EL**.** Endothelium-restricted overexpression of human endothelin-1 causes vascular remodeling and endothelial dysfunction. *Circulation.* 2004;110(15):2233-2240.
62. Amiri F, Paradis P, Reudelhuber TL, Schiffrin EL**.** Vascular inflammation in absence of blood pressure elevation in transgenic murine model overexpressing endothelin-1 in endothelial cells. *J Hypertens*. 2008;26(6):1102-1109.
63. Virdis A, Duranti E, Rossi C, Dell'Agnello U, Santini E, Anselmino M, Chiarugi M, Taddei S, Solini A**.** Tumour necrosis factor-alpha participates on the endothelin-1/nitric oxide imbalance in small arteries from obese patients: role of perivascular adipose tissue. *Eur Heart J*. 2015;36(13):784-794.

Figure 1. Schematic diagram showing the hypothetic mechanisms whereby TNF-α induces vascular changes in small vessels from obese patients. In condition of visceral obesity, chronic low-grade vascular inflammation generated by PVAT and/or within vasculature stimulates the reactive oxygen species (ROS) generation, through NAD(P)H oxidase activation and other sources, which in turn reduces the NO availability. TNF-α also directly stimulates ET-1 generation, that exerts a mutual stimulation with ROS.