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Obesity-Related Endothelial Dysfunction: moving from classical to emerging mechanisms

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ABSTRACT

Human obesity is associated with vascular endothelial dysfunction, caused by a reduced nitric oxide availability secondary to an enhanced oxidative stress production. Pro-inflammatory cytokine generation, secreted by perivascular adipose tissue, is a major mechanism whereby obesity is associated with a reduced vascular NO availability. Vasculature also represents a source of low-grade inflammation and oxidative stress which contribute to endothelial dysfunction in obese patients. Recently, a direct influence of ghrelin and arginase on endothelial function by interfering with nitric oxide availability was demonstrated in small vessels from patients with obesity.

Introduction

Vascular endothelium plays a pivotal role in modulating vascular homeostasis, since it represents an integrative signal transducer for circulating influences able to modify the vessel wall phenotype. This function is guaranteed by the generation of several factors that modulate vascular tone, cellular adhesion, smooth muscle cell proliferation, and vessel wall inflammation in response to a large variety of physical and chemical signals.

The integrity of vascular endothelial tissue guarantees a constant homeostasis of the vasculature, by acting as the central player in modulating the complex balance between substances with vasoconstricting/prothrombotic properties and vasodilating/anti-thrombogenic activities (Flammer et al. 2012). Nitric oxide (NO) represents the major compound produced by endothelial cells, deriving from the transformation of L-arginine into citrulline by the activity of the constitutive endothelial enzyme NO synthase (eNOS) and released under the influence of endothelial agonists (e.g. acetylcholine, bradykinin), and by shear stress (Virdis et al. 2016a). NO exerts multiple protective vascular effects by counterbalancing and/or preventing several mechanisms involved in the development of the atherosclerotic process, including leukocyte adhesion, adhesion molecules expression, platelet adhesion and aggregation, migration and proliferation of muscle cells (Virdis et al. 2008; Flammer et al. 2012).

Obesity is a chronic disease, whose growing worldwide incidence affects not only adults but also adolescents and children, thus reaching epidemic proportions in several countries (Flegal et al. 2010; MacPhee 2008). Obesity condition may favour/accelerate the atherosclerotic process. Endothelial dysfunction represents the earliest vascular alteration observed in obesity, a condition in which endothelial cells switch to a pro-atherosclerotic phenotype. This is characterised by a reduced NO availability because of its accelerated breakdown secondary to generating excess of reactive oxygen species (ROS) and its altered production by eNOS.

In obesity, endothelial dysfunction is also promoted by autocrine, paracrine and endocrine signals including low-grade vascular inflammation, which is strongly involved in favouring endothelial dysfunction and atherosclerosis. In details, in small vessels taken from patients with severe obesity, TNF-α has been demonstrated to be a major proinflammatory cytokine involved in reducing NO availability by inducing ROS generation (Virdis et al. 2011a).

The aim of this review was to give a brief overview of the known mechanisms involved in the pathogenesis of endothelial dysfunction at the level of microcirculation in obesity condition. In particular, the recognized classical, together with the so-called “emerging” mechanisms according to the most recent discoveries in peripheral circulation will be discussed.

Classical mechanisms of endothelial dysfunction: ROS and inflammation

Obesity is characterized by endothelial dysfunction, independently of concomitant CV risk factors usually associated with this clinical condition (Virdis et al. 2013). The first demonstration of a reduced endothelium-dependent vasodilation was observed in the leg microcirculation of obese patients (Steinberg et al. 1996), subsequently confirmed in the forearm microcirculation (Perticone et al. 2001).

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In patients with metabolic risk factors and suspected coronary artery disease, by utilizing the laser Doppler flowmetry the authors observed that body mass index was greatly and independently associated with a blunted endothelium-dependent vasodilatation (van der Heijden et al. 2017). These findings confirm the deleterious and independent impact of obesity on the vascular homeostasis at the level of peripheral circulation.

The *in vitro ex-vivo* myographic technique is a useful and reliable technique which allows the possibility to study the endothelial function of isolated small vells, also guaranteeing the possibility to explore several mechanisms accounting for this alteration (Virdis et al. 2011b; Virdis et al. 2014). By this technique, it has been possible to demonstrate the negative impact of obesity on endothelial function in isolated small vessels of abdominal fat of obese patients, also identifying the potential mechanisms involved. A large body of reports homogeneously documented a reduction of endothelial function in this vascular district (Grassi et al. 2010, Virdis et al. 2013, De Ciuceis et al. 2011). In details, endothelial dysfunction was resistant to the eNOS inhibitor Nω-nitro-L-arginine methylester (L-NAME) and potentiated by an antioxidant compound which in turn restored the inhibitory effect of L-NAME. These data indirectly highlight a central role played by ROS in reducing NO availability (Virdis et al. 2011a).

During the last decade, the concept that obesity is associated with a systemic low-grade vascular inflammation was consolidated (Virdis et al. 2016b). In details, the adipose tissue emerged as a central source of pro-inflammatory cytokine production. Several studies documented that human obesity may be considered an inflammatory disease and that specific adipokines produced by visceral fat have a major role in mediating the inflammatory exposure. 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 (Kershaw and Flier 2004), all of which can affect, by different mechanisms, vascular homeostasis. Leptin, the main protein produced by adipocytes, may stimulate the secretion of TNF-α and interleukin-6 (IL-6) which, in turn, favour endothelial dysfunction (Bullo et al. 2003) through their direct activity or inducing an increment of ROS production in endothelial cells (Bouloumie et al. 1999). Resistin, produced by adipogenesis, is involved in the development of insulin resistance and obesity (Steppan et al. 2001). When incubated with human recombinant resistin, endothelial cells enhance their production of Endothelin-1 and the expression of adhesion molecules, suggesting its direct impact on vascular endothelium (Verma et al. 2003). On the contrary, adiponectin, which is generated from mature adipocytes, is able to exert opposite effects than other adipokines. In fact, adiponectin is demonstrated to be able to reduce the proliferation of smooth muscle cells, the endothelial expression of adhesion molecules as well as the transformation of macrophages into foam cells (Matsuzawa et al. 2004). Reduced production and activity of adiponectin was demonstrated in obesity (Goldstein et al. 2004), and the reduced protective effects of this adipokine represent an important mechanism contributing to the development of endothelial dysfunction and the atherosclerotic process in this condition.

Of note, several animal and human studies revealed the mechanisms whereby PVAT induces vascular changes (Yudkin 2003). In particular, TNF-α may stimulate ROS production via activation of NAD(P)H oxidase (Dodd-o et al. 2004), one of the major ROS source (Griendling et al. 2003), or by the activation of nuclear transcription factor-kappa B (NF-kB) (Mercurio and Manning, 1999) which in turn mediates the expression of growth factors and inflammatory cytokines, leading to a favouring mobilization and activation of macrophages, migration and proliferation of smooth muscle cells, and induction of adhesion molecule expression by the endothelial and smooth muscle cells (Virdis et al. 2003). IL-6 is able to increase the ROS production by activating xanthine oxidase and NAD(P)H oxidase. IL-6 can also stimulate liver synthesis of C-reactive protein (CRP), which reduces NO production by decreasing the expression of eNOS (Ikeda et al. 2003).

Recent human studies investigated a possible link between TNF-α and obesity-related vascular dysfunction, a fundamental issue if considering that vasculature represents an important target for this cytokine (Zhang et al. 2003). In a study conducted in patients with obesity-related metabolic syndrome, Tesauro *et al* (Tesauro et al. 2008) assessed the effects of TNF-α neutralization by infliximab on vascular reactivity during hyperinsulinemia. At baseline, patients showed a blunted relaxing response to both acetylcholine and sodium nitroprusside during hyperinsulinemia, compared with control subjects. Infliximab significantly potentiated responsiveness to both agonists. Of note, the antioxidant vitamin C improved the vasodilator response to acetylcholine in obese patients, with no further potentiating effect by concomitant infliximab administration. These findings allowed the authors to conclude that TNF-α inhibition was able to ameliorate vascular reactivity in obese patients during hyperinsulinemia, an effect consequent to a decreased oxidative stress. (Tesauro et al. 2008).

A specific role played by PVAT-derived inflammation in the pathogenesis of vascular dysfunction is highlighted by a more recent study conducted in isolated resistance vessels, assessed in the presence or in the absence of PVAT. While in vessels of lean controls PVAT generated factors that potentiated NO availability, in obese patients the presence of PVAT did not improve vasodilation (Greenstein et al. 2009). 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 prevented these alterations (Greenstein et al. 2009). These results demonstrate that in physiological conditions adipocytes contribute to the regulation of local vascular tone by favouring NO availability. This important regulation is lost in obese patients, whose PVAT is characterised by adipocyte hypertrophy, ROS generation and increased production of TNF-α. Such data allow to definitively conclude that inflammation plays a central role in promoting vascular dysfunction, are represent an important mechanism whereby PVAT in obesity clinical condition exerts a deleterious effect on the surrounding vasculature.

Small vessels in obesity: target and also source of TNF-α

As mentioned, it has been demonstrated a direct role exerted by TNF-α from PVAT on the vascular tone of adjacent vasculature in obese patients. Nevertheless, the possibility that low-grade inflammation might be also generated within the vasculature in obesity condition is not yet determined. This issue was investigated in our laboratory. Small arteries were isolated from visceral fat immediately after biopsy sample, performed during laparoscopic surgery in obese patients and lean controls. Among obese group, small vessels revealed a blunted endothelium-dependent relaxation to acetylcholine, only slightly sensitive to the inhibitory effect of L-NAME on acetylcholine-induced responses. Such alterations were reversed by the superoxide scavenger tempol and, to a similar extent, by the anti-TNF-α monoclonal antibody infliximab (Virdis et al. 2011a). Moreover, it was observed an increased intravascular ROS generation, which was blunted by tempol or infliximab. These findings demonstrate that small arteries from patients with severe obesity show a reduced NO availability secondary to excess ROS generation. In such scenario TNF-α plays a primary role in promoting endothelial dysfunction, by triggering intravascular ROS generation. Such concept is strengthened by immunohistochemistry, which revealed a dramatic upregulation of TNF-α mainly in the media layer of these vessels (Virdis et al. 2011a). Possible pathway(s) involved in TNF-α-induced vascular ROS generation were also investigated. Indeed, NAD(P)H oxidase, inducible nitric oxide synthase (iNOS) and xanthine oxidase as hypothetical sources of ROS were examined by specific inhibitors apocynin, S-methylisothiourea (SMT) and allopurinol, respectively. Apocynin and SMT, but not allopurinol, ameliorated in part the endothelial dysfunction in the obese group. Likewise, apocynin, and similarly SMT, were

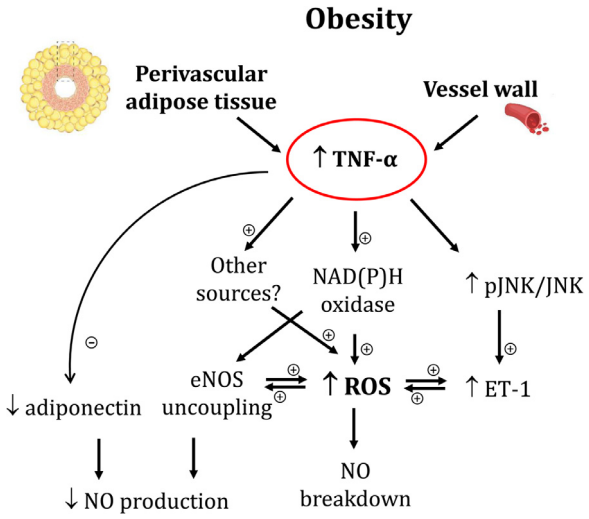

Fig. 1. Proposed mechanism whereby TNF-α induces ET-1/NO imbalance in small resistance arteries from obese patients. TNF-α -driven vascular NAD(P)H oxidase is a major source of ROS, leading to NO breakdown. Moreover, NAD(P)H oxidase acts as a trigger of endothelial NO synthase uncoupling, thus potentiating ROS generation. TNF-α also directly stimulates ET-1 generation via the c-Jun N-terminal kinase pathway, which in turn contributes to ROS generation. Reduced adiponectin expression in PVAT may participate in the TNF-α-mediated NO inhibition. TNF-α may promote ROS generation through still unknown mechanisms.

able to attenuate in part intravascular superoxide generation. In conjunction, these data indicate that both NAD(P)H oxidase and iNOS are major enzymatic pathways which, activated by TNF-α, mediate vascular ROS production in obesity (Virdis et al. 2011a). In obesity, it was recognized a vascular ET-1/NO imbalance, in favour of an abnormal activation of the endogenous ET-1 system. Thus, an increased ET-1-mediated vasoconstrictor tone, simultaneously with a reduced tonic NO release, is demonstrated in peripheral microcirculation of obese patients (Cardillo et al. 2004; Tesauro et al. 2009). Again, ROS induce ET-1 expression, and at the same time, ET-1 appears to stimulate ROS production (Pollock et al. 2005). Based on this knowledge, oxidant excess can be hypothesized as a mechanism whereby TNF-a interferes with the ET-1/NO system. Therefore, in our lab we recently assessed the possibility that TNF-α may contribute 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. Indeed, we documented that infliximab significantly potentiates the attenuated tonic NO release and, concomitantly, reduces the enhanced ETA-dependent contracting activity in small vessels from Obese (Virdis et al. 2015). These findings indicate that vascular and perivascular TNF-α excess, coupled with an increased vascular expression of ET-1 and ETA receptor, directly interferes with the vascular ET-1/NO system, thus greatly contributing to the imbalance of the vascular homeostasis in obesity condition. The up-regulated JNK pathway may represent a crucial molecular signalling involved in this process (Virdis et al. 2015). The complex cross-talk between TNF-α and ET-1/NO imbalance in obesity is tentatively schematized in Fig. 1.

In summary, small arteries from the PVAT of obese patients show a reduced NO availability, due to an increased vascular production and biological activity of TNF-α, which favours superoxide generation via both NAD(P)H oxidase and iNOS activation. Unfortunately, these findings do not allow to distinguish whether the small arteries and adipocytes, located within PVAT, are independent sites of TNF-α generation or, by contrast, there is a hierarchical relationship between these two districts.

These findings, while strengthening the concept that obesity is an inflammatory condition, identify the small vessels of PVAT as important sources of low-grade inflammation and ROS which, together with the PVAT, might directly contribute to the local development of insulin resistance, which characterizes obese patients.

Emerging mechanisms of endothelial dysfunction in obesity: ghrelin and arginase. Ghrelin is an identified growth hormone–releasing peptide, isolated from the stomach, initially described as an endogenous ligand for growth hormone secretagogue receptors. Although essentially a gastric hormone, additional growth hormone–independent cardiovascular actions have been attributed recently to this peptide. Among others, a significant impact on endothelial function has been identified, because ghrelin receptor expression has been documented in human endothelial **c**ells (Kleinz et al. 2006). Experimental reports indicated that exogenous ghrelin administration ameliorates endothelial dysfunction and reduces the vasoconstrictor effect of ET-1 and, at higher doses, also decreases arterial pressure. Obese patients with metabolic syndrome are characterized by reduced circulating ghrelin levels (Tesauro et al. 2005) These findings, together with evidence of compromised NO availability and enhanced ET-1–mediated vasoconstriction (Cardillo et al. 2004) make obesity a useful experimental model for investigating the impact of ghrelin on NO and ET-1. In fact, it was convincingly demonstrated that the unfavourable imbalance between NO and ET-1 was reversed by exogenous ghrelin. Thus, intra-arterial infusion of this peptide reduced enhanced ET-1–dependent vasoconstriction and reversed impaired NO-dependent vasodilation in patients. No effect was observed in control subjects (Tesauro et al 2009). These findings allow to conclude that, beyond its classic effect in regulating energy balance and food intake, ghrelin also greatly contributes to maintaining vascular homeostasis by restoration of a balance between endothelium-derived contracting and vasodilator forces (Taddei et al. 2009).

More recently, another interesting mechanism involving arginase, emerged in obesity-related vascular dysfunction. Arginase is a manganese metalloenzyme that hydrolyses L-arginine to urea and L-ornithine. Two distinct isoforms, named arginase I and II, have been identified. These isoforms have similar mechanisms, requirement of manganese as a co-factor and identical metabolites. Arginase I is cytoplasmic and mainly expressed in the liver. Arginase II is mainly located within mitochondria and highly expressed in kidney. Arginases can be expressed in many different cell types and can be induced by a wide variety of agents and conditions, depending on tissue and species. Both isoforms are found in the endothelium of the vasculature. An increased concentration or activity of arginase may cause eNOS uncoupling, resulting in reduced production of NO and endothelial dysfunction (Pernow and Jung 2013; Berkowitz et al. 2003). In different experimental models of endothelial dysfunction, including ageing and obesity, an increased vascular activity and expression of arginase has been documented (Michell, Andrews, and Chin-Dusting 2011; Ryoo et al. 2006; Chung et al. 2014; Johnson et al. 2015; Bhatta et al. 2017; Santhanam et al. 2008). In turn, arginase inhibition leads to an amelioration of endothelial function and increased NO availability in old rats (Kim et al. 2009). In a recent study, the impact of arginase on endothelial dysfunction in small vessels from obese patients and its relationship with ageing was investigated.

Results indicated that inhibition of arginase by preincubation with norNOHA induced a significant improvement of the vasodilatory response to Ach in obese subjects ($P<0.001$) but not in controls. This improvement was more evident in the young than the old obese group. The addition of L-NAME abrogated the significant improvement of vasodilation obtained with norNOHA observed in the young obese group, suggesting that the addition of norNOHA restored eNOS activity. Vascular levels of arginase I progressively increased by age in both the obese and control groups, and obese had higher levels of arginase compared with control groups. Similar differences were observed for vascular levels of arginase II between obese and controls, and a similar increase with aging was observed in both obese and control groups.

In addition, it was observed by immunofluorescent staining with DHE that older subjects in both the obese and control groups showed a higher amount of vascular superoxide anions compared with their

younger peers. Preincubation with gp91ds-tat (a selective inhibitor of NADPH oxidase) significantly reduced the amount of vascular superoxide anions in old obese subjects. Addition of norNOHA to the gp91ds-tat did not cause further reduction of the DHE signals, suggesting that only a minimal part of the superoxide anion production observed in this group was because of the uncoupling of the eNOS resulting from higher arginase activity/expression (Masi et al. 2018). In conclusion, arginase is involved in the regulation of NO availability in small vessels from obese, an effect modulated by aging. In young obese, inhibition of arginase was able to significantly improve microvascular endothelial function, whereas this response was attenuated in the old obese group, despite the increased levels of vascular arginase I and II expression. This is likely related to the progressive increase of vascular oxidative stress driven by NAD(P)H oxidase activity observed with aging.

Conclusions

In several vascular districts, obesity is characterised by a marked endothelial dysfunction evidenced by a reduced NO availability. In such a scenario, PVAT plays a direct major influence on the vascular homeostasis, since it generates pro-inflammatory cytokines, including TNF-α, which exert direct detrimental effect toward vasculature. This is because TNF-α promotes superoxide generation within the vascular wall via several pathways, mostly the NAD(P)H oxidase. Of importance, vasculature surrounding PVAT does not only represent the target of PVAT-derived pro-inflammatory cytokines but is also an important source of low-grade inflammation and oxidative stress which contribute to endothelial dysfunction in obesity condition. More recent reports put in evidence new lights on mechanisms involved in endothelial dysfunction in obese patients, identified as “emerging contributors.” In detail, ghrelin, beyond its classic effect in regulating energy balance and food intake, contributes to maintaining vascular homeostasis by restoration of a balance between endothelium-derived contracting and vasodilator factors. An increased activity of arginase may reduce the production of NO and inducing endothelial dysfunction is small vessels. Its impact is reduced by aging because of higher levels of vascular oxidative stress. If any, interaction between ghrelin and arginase needs further investigations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.endmts.2020.100063.

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