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REVIEW

Obesity, atherosclerosis and the vascular endothelium: mechanisms of reduced nitric oxide bioavailability in obese humans

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It is now well established that obesity is an independent risk factor for the development of coronary artery atherosclerosis. The maintenance of vascular homeostasis is critically dependent on the continued integrity of vascular endothelial cell function. A key early event in the development of atherosclerosis is thought to be endothelial cell dysfunction. A primary feature of endothelial cell dysfunction is the reduced bioavailability of the signalling molecule nitric oxide (NO), which has important anti atherogenic properties. Recent studies have produced persuasive evidence showing the presence of endothelial dysfunction in obese humans NO bioavailability is dependent on the balance between its production by a family of enzymes, the nitric oxide synthases, and its reaction with reactive oxygen species. The endothelial isoform (eNOS) is responsible for a significant amount of the NO produced in the vascular wall. NO production can be modulated in both physiological and pathophysiological settings, by regulation of the activity of eNOS at a transcriptional and post-transcriptional level, by substrate and co-factor provision and through calcium dependent and independent signalling pathways. The present review discusses general mechanisms of reduced NO bioavailability including factors determining production of both NO and reactive oxygen species. We then focus on the potential factors responsible for endothelial dysfunction in obesity and possible therapeutic interventions targetted at thses abnormalities.
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Introduction

Obesity is an independent risk factor for the development of cardiovascular atherosclerosis.[1–3] An individual with a body mass index (BMI) of greater 30 kg/m^2 is four times more likely to suffer from cardiovascular disease than an individual with a BMI of 25 kg/m^2 or less.[4,5] Currently, around 20% of the adult population in Europe is obese; as a consequence as the twenty-first century unfolds, obesity will become an increasingly important factor in the pathogenesis of cardiovascular atherosclerosis.[6]

The development of atherosclerosis involves a complex interaction between the vascular endothelium, serum lipids, inflammatory cells, platelets and vascular smooth muscle cells.[7] A critical early event in the pathogenesis of atherosclerosis is endothelial cell dysfunction, a key feature of which is reduced bioavailability of the signalling molecule, nitric oxide (NO).[7] It is now well established that obesity is associated with endothelial dysfunction. The mechanisms underlying endothelial dysfunction in obese humans remain unclear and incompletely explored. In this review we discuss the potential role of endothelial dysfunction in the excess cardiovascular risk of obese humans, the putative mechanisms underlying this endothelial dysfunction and potential targets for therapeutic intervention.

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Nitric oxide biosynthesis

NO is generated from L-arginine by a family of nitric oxide synthases (NOSs) (see Figure 1).[8] The endothelial isoform, eNOS, is a calcium/calmodulin-dependent enzyme[9] requiring tetrahydrobiopterin (BH_4),[10] NAD(P)H, flavin adenine dinucleotide and flavin mononucleotide[11] as co-factors. Many of the vascular actions of NO are mediated via the

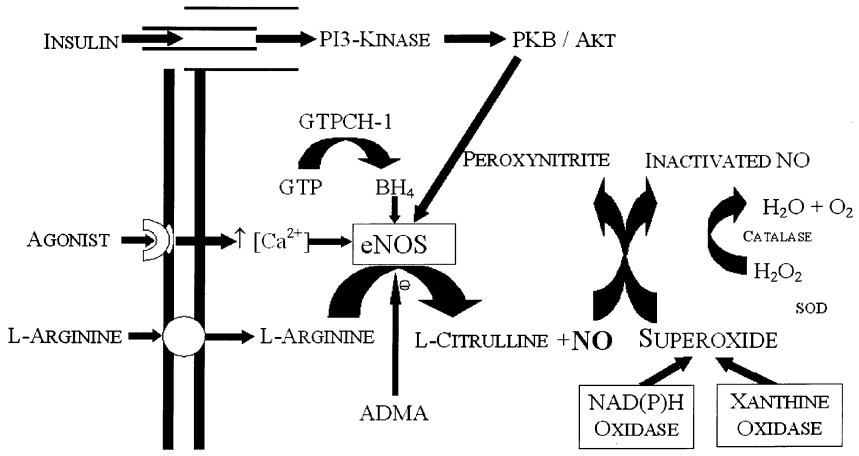

Figure 1 Determinants of nitric oxide bioavailability. Nitric oxide is synthesised by eNOS from L-arginine. Production is dependent on L-arginine provision, via specific cell membrane amino-acid transporters, and the availability of co-factors, notably BH_4, which is synthesised from GTP by the action of GTP cyclohydrolase-1 (GTPCH-1). Asymmetric dimethyl arginine (ADMA) competitively inhibits L-arginine binding to eNOS. eNOS activity may be stimulated via a Ca^{2+}-dependent pathway, or, as in the case of insulin and shear stress, a Ca^{2+}-independent pathway, mediated via PI3-kinase and Akt. Reactive oxide species may combine with NO, to inactivate it, and potentially produce the peroxynitrite radical. Superoxide production is determined by a number of factors that may increase production or which may generate other reactive species via the actions of superoxide dismutase and catalase, eg H_2O_2.

activation of soluble guanylate cyclase, which in turn leads to a rise in cyclic guanosine monophosphate (cGMP).

Physiological regulation of eNOS activity

Transcriptional and post-transcriptional regulation of eNOS

A number of factors can regulate eNOS expression.[12] *In vitro* studies have shown for example that shear stress,[13,14] lysophosphatidylcholine,[15] low concentrations of oxidised LDL,[16] physiological levels of insulin[17] and exercise[18] can lead to increased eNOS expression. The expression of eNOS can also be regulated by factors influencing its mRNA half-life. Of note, the cytokine tumour necrosis factor-α (TNF-α) has been shown to decrease the mRNA half-life of eNOS.[19]

Post-translational regulation of eNOS

eNOS is unique amongst the NOS isoforms in being dually acylated by myristate and palmitate, and like myristolation of other proteins this modification of eNOS is irreversible. In contrast with the myristolation of eNOS, the enzyme's palmitoylation is readily reversible. Palmitoylation is thought to be important in modulating subcellular localisation and function of eNOS.[20]

In the inactive state eNOS is closely associated with the inhibitory protein-caveolin-1, contained within caveolae, although the significance of caveolin-1 as an inhibitory regulator of eNOS remains controversial. Caveolae are invaginations of the plasma membrane, which bind and organise a number of signalling molecules.[21] This protein–protein interaction may play an important role in regulating eNOS activity. Binding of eNOS to caveolin-1 may both facilitate targeting of the enzyme to caveolae and dynamically regulate eNOS activity,[22] It has been proposed that a number of stimuli, including vascular endothelial growth factor (VEGF), may induce the disruption of the caveolin–eNOS complex, allowing the subsequent association of eNOS with other structural proteins that promote its activation.[23]

One such factor thought to be important in eNOS regulation is the heat shock protein, Hsp90. Hsp90, a molecular chaperone, acts as scaffolding to facilitate the interaction of higher order proteins by inducing structural changes and reducing protein sub-unit turnover.[24,25] A receptor-mediated rise in Ca^{2+}, VEGF stimulation or shear stress leads to increased association of eNOS with Hsp90 and, as a result, augmented NO production.[26] In particular, it has been suggested that this permits the recruitment of protein kinases, in turn leading to eNOS phosphorylation and subsequent activation.[23]

Calcium dependent/independent eNOS activation

Agonists activating eNOS include acetylcholine, substance P and 5-HT. These proteins, acting via cell membrane G-protein-coupled receptors, stimulate a rise in cytosolic [Ca^{2+}]. This calcium then binds to the regulatory protein, calmodulin. The resulting calcium–calmodulin complex binds to eNOS, in association with Hsp90, leading to its activation.[27] Ca^{2+} influx is another factor that may lead to dissociation of eNOS from the inhibitory influence of caveolin,[22,28] allowing its transfer to the cytosol.[29] A decline in Ca^{2+} is thought to lead to re-association of eNOS with caveolin.

Recently a calcium-independent regulatory pathway for eNOS has been described which may be of particular relevance to the obese state.[30,31] Both shear stress[32] and agonists acting on tyrosine kinase receptors, such as insulin and insulin-like growth factor-1 (IGF-1) promote eNOS activity via the activation of phosphatidylinositol 3-kinase (PI3-kinase)[33] and protein kinase-B (PKB)/Akt. Activated Akt, in turn, phosphorylates eNOS, leading to an increased sensitivity to the calcium-calmodulin complex and an increase in NO production.[30,31] Two sites, Ser-1179 and Thr-497, have been proposed as central to the regulation of eNOS through phosphorylation.[34] Phosphorylation at the Ser-1179 site by a number of protein kinases, including Akt, cAMP-dependent protein kinase (PKA) and the AMP-activated protein kinase (AMPK), leads to activation of eNOS, whereas phosphorylation at the Thr-497 site, by, for example, PKC, leads to inhibition of its activity.[35] The Thr-497 site is important in binding to the Ca–calmodulin complex and is phosphorylated in the basal state, thus inhibiting their association. Phosphorylation at one site appears to be associated with a concomitant, but independently coordinated dephosphorylation, via the phosphatases PP1 or PP2A, at the other.[36] Whether the mechanism by which eNOS activation occurs in this pathway is truly calcium-independent, or whether the process of phosphorylation simply augments the enzyme's sensitivity to low levels of calcium remains controversial.[37,38] Of note is the fact that the PI3-kinase/Akt signalling pathway is the same pathway as that responsible for GLUT-4-mediated glucose uptake and has been shown to be blunted in the vasculature of obese mammals, whereas the pathway responsible for the mitogenic action of insulin remains intact.[39–41]

Insulin as an NO dependent vasoregulatory peptide

When investigating the significance of changes in skeletal muscle blood flow in glucose homeostasis, Baron's group first demonstrated that insulin induced an increase in leg blood flow.[42–44] Further, this effect was shown to be blunted in insulin-resistant states[45] and abolished by pre-infusion of the specific NOS inhibitor, L-NMMA, suggesting an NO-mediated effect.[46] Insulin at physiological concentrations has been shown in some studies to lead to vasorelaxation in different vascular territories[47–49] as well as to enhance the vasodilatory response to endothelium dependent vasodilators.[50] Direct evidence of vasodilatation is, however, limited, with some studies showing no response to physiological levels of insulin[49,51] or differential responses in different vascular beds and different animal models. It has been suggested that this may be due to the concomitant release of endothelin-1, a factor that may contribute to hypertension in insulin resistant states.[52] Intriguingly, the same group demonstrated that the vasodilatory response was seen only in the presence of systemic and not local hyperinsulinaemia, suggesting that the process may involve additional mechanisms.[53] Further, using an *in vitro* porcine coronary artery model, insulin and IGF-1 were shown to regulate vasomotor tone via a non-endothelium-dependent mechanism, possibly through an influence on smooth muscle cell-derived inducible NOS (iNOS), a mechanism that may explain the response to L-NMMA, or modulation of potassium channels.[54]

Despite this, eNOS activation in response to insulin has, more recently, been shown to lead to a rapid rise in NO concentration in bovine and human endothelial cells.[37,55] Prolonged exposure to physiological levels of insulin for 4–6 h leads to increased eNOS expression and a sustained increase in NO production.[17] This is thought to be mediated by PI3-kinase, probably via an increase in eNOS mRNA transcription, as well as enhanced eNOS activity through Ser-1179 phosphorylation, and appears to be regulated by the inhibitory action of protein kinase C (PKC).[55] The PKCβ isoform, which has greatest inhibitory effect in this setting, has been associated with a number of markers of endothelial dysfunction, and is activated in insulin resistant states.[55] In obesity, increased PKCβ may be a contributory factor to insulin resistance.[56] Thus with a blunting of the PI3-kinase pathway and increased PKC activity, both eNOS activity and expression may be reduced in insulin resistance.

The anti-atherogenic effects of nitric oxide

NO is a potent vasodilator and there is unequivocal evidence demonstrating that there is basal NO-dependent vasodilatation in humans, which, at least in part, counters the effects of the renin–angiotensin, sympathetic nervous and other vasoconstrictor systems.[57] This vasodilator tone plays an important role in regulation of blood flow in healthy humans.[58]

Inflammation of the vessel wall is an important event in early atherosclerosis (for review see Cannon[59]). NO has been shown to blunt monocyte adhesion to the endothelial surface.[60,61] Furthermore, by reducing oxidative stress NO may inhibit the transcription of MCP-1 and VCAM-1,[62–64] proteins which are of central importance in initiating inflammation of the vascular wall.

Migration and proliferation of VSMC play an important role in the pathogenesis of the atherosclerotic plaque, effects that may also be inhibited by NO.[65] Supportive evidence for this has been shown in *in vitro* studies of human VSMC.[66] In these studies both NO and the transfection of cells with eNOS induced G_1-phase arrest in the cell cycle of VSMC.

General mechanisms of endothelial dysfunction

NO bioavailability is determined by the balance between NO production and its reaction with reactive oxygen species (ROS).[67] A number of factors adversely affect endothelial function by reducing NO biosynthesis.[68]

eNOS expression

Raised concentrations of oxidised LDL,[69] TNF-α[19] and hypoxia[70] all lead to down-regulation of eNOS expression,

via a reduction in its mRNA half-life. High LDL levels are also associated with increased caveolin expression, leading to competitive inhibition of calmodulin-mediated eNOS activation. Disruption of the PI3-kinase/Akt pathway in insulin resistant states may impair insulin's stimulus to increased eNOS expression.

Agonist-mediated activation of eNOS

G-protein-coupled receptor-mediated eNOS activation can be influenced by atherosclerotic risk factors. Hypertension, hypercholesterolaemia,[71] and the presence of oxidised LDL[72] have all been shown to inhibit G-protein sub-unit expression and may inhibit association of the sub-units via an influence on cell membrane fluidity.[73]

Provision of substrate/co-factors

NO production is dependent on the provision of L-arginine, which is regulated by a membrane bound cationic amino acid transporter that actively transports L-arginine into the endothelial cell against a concentration gradient. This process can be blunted by cytokines such as TNFα.[68]

Asymmetric dimethyl arginine (ADMA) is an endogenous competitive inhibitor of the binding of L-arginine to eNOS, and therefore may play a role in dysregulation of the L-arginine/NO pathway.[74,75] Levels of ADMA have been shown to be positively correlated with a number of endothelial risk factors including insulin resistance and type 2 diabetes. One mechanism by which this is thought to occur is the inhibition of ADMA breakdown by the action of TNFα.[76] It is possible that, by overcoming this competitive inhibition, L-arginine dietary supplementation, despite adequate L-arginine reserves, restores endothelial function[77] in conditions of excessive ADMA/endothelial dysfunction.

The provision of the co-factor BH_4 is a key regulatory mechanism of eNOS activity. In the relative absence of BH_4, needed for effective binding of L-arginine, eNOS may instead generate superoxide radicals.[78] Non-insulin-dependent diabetes mellitus (NIDDM), insulin-resistant states (such as fructose-fed rats), increasing age and hypercholesterolaemia have all been associated with a reduced bioavailability of BH_4. Importantly, in situations of severe oxidative stress, BH_4 may be oxidised, leading to its depletion.[79]

Superoxide production

There are persuasive data supporting a pivotal role for ROS in the pathophysiology of atherosclerosis.[80,81] ROS are produced by a number of enzymes within the vascular wall, notably NAD(P)H and xanthine oxidases, cyclo-oxygenase, lipoxygenase and, as discussed above, eNOS itself.[82] There is a well-established relationship between ROS and NO.[83] NO has a direct effect on oxidative stress by scavenging ROS, and NO inactivation is enhanced in the presence of excess ROS. Relatively small changes in ROS production may have a substantial effect on NO bioactivity and hence endothelial function. NO may also modulate the production of endogenous anti-oxidants[81] and the process of lipid oxidation within the vascular wall.[84] Risk factors for atherosclerotic disease, such as hypertension, hypercholesterolaemia and diabetes mellitus are known to be associated with an increased production of ROS.[80,85,86]

Endogenous vascular superoxide dismutase (SOD) acts to prevent the accumulation of ROS, by conversion to hydrogen peroxide, which is subsequently converted to molecular oxygen and water. However, its relatively slow rate of reaction in comparison with that between NO and O_2^- is such that highly reactive radicals may continually be produced, and NO function inhibited.[82] In healthy humans endogenous antioxidant defence mechanisms are able to protect against this accumulation. This delicate balance is sensitive to disruption by pathological states in which ROS production is increased.[87]

There is up-regulation of expression of the $p22^{phox}$ sub-unit of NAD(P)H oxidase in atheromatous arteries[88,89] and in response to angiotensin II (AII),[90] platelet-derived growth factor and TNF-α.[91] The endothelial and vascular smooth muscle cell isoforms produce a continuous low level output that, at this concentration, have a regulatory role on eNOS and NO activity.[68]

NO has a direct effect on oxidative stress by scavenging ROS. Perhaps the more important corollary of this is that NO inactivation is enhanced in the presence of oxidative stress. Relatively small changes in superoxide production may have a substantial effect on NO activity and hence endothelial function. It is not necessarily the absolute levels of NO or O_2^-, but the balance between the two that is of importance, in particular by determining whether NO, in combining with ROS, acts as an anti-oxidant, or forms the highly reactive peroxynitrite radical.[84]

ROS within the vascular wall contribute to LDL-oxidation, leading to the process of LDL accumulation, increased uptake into macrophages, the evolution of an inflammatory process and the progression of atherosclerosis.[82] Oxidised LDL is capable of impairing endothelial function in isolated porcine coronary arteries, whereas native LDL is not. NO may directly disrupt the process of LDL oxidation by reacting with lipid peroxyl radicals. Thus there is a complex interdependence within the vascular wall between NO production, ROS and lipid oxidation.

The influence of the renin–angiotensin system on NO bioavailability

There are emerging data supporting an important role for the renin–angiotensin system (RAS) in the pathophysiology of atherosclerosis,[92] and furthermore for an intimate relationship between the RAS and NO bioavailability. Inhibition of ACE leads to an increase in NO bioactivity, and increased eNOS expression, whereas ROS production possibly by

activation of NAD(P)H oxidase is augmented by AII, at levels not leading to an increase in systemic blood pressure.

Interestingly there appears to be an inverse relationship between NO and ACE expression or activity. NO has been shown to blunt ACE expression in the vasculature,[93,94] while conversely chronic eNOS inhibition leads to up-regulation of ACE activity.[95] In the rat carotid artery, NO release is associated with a reduction in the conversion of angiotensin I to angiotensin II.[94]

The role of insulin resistance in endothelial dysfunction

Obesity is the insulin resistant state *par excellence*. There are now convincing data showing that insulin resistance is associated with endothelial dysfunction and is an independent risk factor for the development of coronary artery disease (CAD).[96] The magnitude of risk associated with insulin resistance is of the same order as that of hypercholesterolaemia.[97] The IRAS study group showed that insulin sensitivity is inversely proportional to the development and extent of atherosclerosis, as assessed by carotid artery ultrasound.[98] In patients with newly diagnosed type 2 diabetes, hyperinsulinaemia, as a marker of insulin resistance, may be a better predictor of vascular disease than hyperglycaemia.[99]

Insulin resistance has been shown to be associated with endothelial dysfunction in obese and type II diabetic humans.[50,100] In addition relatively insulin resistant first-degree relatives of patients with type 2 diabetes have also been shown to have endothelial dysfunction.[101] Interestingly, in healthy human subjects, Petrie *et al*[102] demonstrated a close positive correlation between insulin sensitivity and basal endothelial NO production.

Chronic feeding of rats with a high fructose diet induces insulin resistance, which is then followed by impairment of endothelium-dependent vascular relaxation *in vitro*.[103] Consistent with these data, mice with gene-targeted deficiency of insulin receptor substrate-1 (IRS-1), which results in insulin resistance with marked hyperinsulinaemia and hypertriglyceridaemia, develop significant endothelial dysfunction as assessed by the response to acetylcholine in aortic vascular rings.[104]

Evidence supporting a detrimental effect of obesity on endothelial function

The association between obesity and CAD is well established. The evidence that obese humans have endothelial dysfunction is similarly compelling. Agonist-stimulated, calcium-dependent NO production is blunted in obesity. Steinberg *et al* demonstrated that the increase in blood flow into the leg in response to methacholine, a muscarinic agent, is blunted in obese humans, the degree of dilatation being inversely proportional to the degree of obesity.[50] Consonant with this Laine *et al* showed that the ED_{50} for bradykinin to increase leg blood flow measured using PET is double in obese subjects compared to lean.[100] In both of these studies the increment in blood flow in response to the exogenous NO donor sodium nitroprusside was no different between obese and lean subjects.

Calcium-independent NO production is also probably abnormal in obese humans. Arcaro *et al* showed that the blood flow response to shear stress is blunted in obese subjects.[105] In support of these data Tack *et al* demonstrated that the vasodilatory response in the forearm in response to insulin is also blunted[106] and Westerbacka *et al* confirmed a similar response in large vessels showing an association between endothelial dysfunction and the level of obesity.[107] While these studies provide persuasive evidence for the presence of endothelial dysfunction in obesity they do not indicate the underlying mechanisms.

Potential factors leading to endothelial dysfunction in obese humans

Elevated non-esterified fatty acids

Central obesity and type 2 diabetes are associated with increased plasma levels and turnover of NEFA.[108] Recent data have shown that exposure to pathophysiological concentrations of NEFAs may impair endothelial function as measured by agonist stimulated and flow-mediated vasodilatation.

Steinberg *et al* demonstrated that the administration of exogenous fatty acids, in the form of a 2 h infusion of Intralipid, was associated with a reduction in methacholine-induced vasodilatation[109] in the leg. This effect was similar to that seen in obese, insulin-resistant individuals in whom comparable NEFA levels are seen clinically. Lundman *et al*, using flow-mediated vasodilatation as their marker for endothelial function, showed a similar impairment in response to Intralipid infusion, although they also demonstrated impaired endothelium-independent vasodilatation.[110]

This effect of NEFAs has been shown to be independent of chain length or prostaglandin synthesis, in a study using acetylcholine as a stimulus to endothelium-dependent vasodilatation.[111] However, this study induced NEFA levels far in excess of those seen in insulin-sensitive or insulin-resistant individuals.

Concordant with these data, a recent study by de Man *et al* demonstrated blunting of vasodilatation in hypertriglyceridaemic patients in response to 5-HT, an NO-dependent vasodilator. This study, however, raised the interesting possibility that the effect of NEFAs on endothelial function may be receptor-dependent as the authors demonstrated an increased vasodilator response to acetylcholine.[112]

A link between specific NEFAs and endothelial dysfunction has been proposed by Davda *et al*, who showed impaired eNOS activity in the presence of oleic and linoleic acids, but not stearic or elaidic acids.[113] Lu *et al* have recently proposed another pathway by which NEFAs may induce endothelial dysfunction.[114] Using fluorescence techniques they demonstrated that oleic acid induced a time-dependent increase in

ROS production from VSMC, via a mechanism that was blocked by catalase, suggesting that H_2O_2 was the principal ROS generated. The effect of more chronic perturbation of NEFA levels on vascular endothelial function remains unexplored.

Adipose tissue and cytokines

Recent studies have demonstrated that adipose tissue, acting in a paracrine fashion, may be a significant source of IL-6, TNF-α, complement factor C3, angiotensin II and plasminogen activator inhibitor-1 (PAI-1). There is a clear association between adiposity and CRP, IL-6 and TNFα.[115] TNFα has a major metabolic role in adipose tissue and there is evidence of up to a three-fold increase in TNFα mRNA, protein[116] and circulating levels[117] in obese individuals. In turn, these cytokines have been shown to be, at least in part, responsible for some of the manifestations of insulin resistance. TNF-α, by affecting phosphorylation both at the level of the insulin receptor tyrosine kinase[118] and also through an inhibition of IRS-1 phosphorylation,[119] impairs the insulin-signalling pathway and through this may have a direct action on NO release and eNOS expression. Further, it may directly activate NAD(P)H oxidase and increase ROS production in vascular smooth muscle.[120] TNF-α may provide a link between obesity, insulin resistance and endothelial dysfunction.[117] This possible relationship warrants further investigation.

Leptin

Leptin, the product of the *ob* gene, is a plasma protein secreted by adipocytes and is involved in the control of body weight, mainly through its hypothalamic effects. Obese humans have been shown to have up to a 10-fold greater plasma leptin concentration than lean subjects.[121] Leptin receptors have been demonstrated on endothelial cells.[122] *In vitro* stimulation of cultured endothelial cells with leptin leads to an increase in the production of ROS, and it is suggested by Busse's group that this is through the production of hydrogen peroxide. However, it has also been suggested that leptin stimulates the production of superoxide from mitochondria.[123] In contrast to this, it has been demonstrated that leptin evokes a dose-dependent relaxation of both aortic and mesenteric vessels[124] and may restore endothelium-dependent relaxation in the vasculature of obese rats.[125] The role of leptin in obesity-related endothelial dysfunction and its mechanisms warrants detailed *in vivo* and *ex vivo* studies.

Abnormalities of LDL in obesity/insulin resistance

The metabolic changes associated with insulin resistance and obesity, notably hyperinsulinaemia, increased very low-density lipoprotein (VLDL), NEFAs and triglycerides are well established. LDL levels often remain unchanged in obesity. There are, however, emerging data supporting the intriguing possibility that the qualitative characteristics of LDL in insulin resistance/obesity are altered.

James *et al* have demonstrated modifications in the atherogenic nature of lipoprotein distribution and composition in obese subjects.[126] Further, Tack *et al* have shown that in obese, insulin resistant subjects troglitazone, a thiazolidinedione insulin-sensitising agent, improved this proatherogenic profile.[127] The influence of lipid modifications in obese subjects on endothelial function has not been explored; however LDL from type 2 diabetic men, with a mean BMI of 27, has been shown to have a more significant adverse effect on endothelial function.[128] In this study McNeill *et al* first demonstrated impaired endothelial function in the otherwise fit type 2 diabetic group as compared to normal controls using forearm blood flow studies. Native LDL was then isolated and shown to inhibit endothelial function in a rabbit aortic ring bioassay. The characteristics of LDL leading to this finding remain unclear, but may include smaller denser subfractions, oxidation, glycosylation and desialylation;[129] some of these changes have been described in obesity.[130,131]

Increased hepatic VLDL-associated apoB secretion is seen in visceral obesity and this has been shown to be associated both with a proatherogenic lipoprotein profile and endothelial dysfunction, as measured by strain-gauge plethysmography. Weight loss is associated with a concomitant fall in apoB secretion and a subsequent improvement in endothelial function.[132]

The renin–angiotensin system in obese humans

All the components of the renin–angiotensin system (RAS) necessary to generate AII are expressed in human adipose tissue.[133] Increased activity of the RAS has been demonstrated in obesity, both systemically and within adipose tissue, and this may relate directly to the mass of adipose tissue.[134,135] Further, weight loss is associated with falls in renin and aldosterone levels that parallel a fall in blood pressure.[134] This increased RAS activity may contribute to endothelial dysfunction via an influence on both NO and ROS production. In rats, angiotensin II has been shown to lead to an up-regulation of NAD(P)H oxidase expression, an effect associated with endothelial dysfunction and smooth muscle hypertrophy.[136] At a cellular level, AII has been shown to increase NEFA turnover, possibly contributing to the increased NEFA release seen in obesity.[137]

Therapeutic interventions and future areas of research

Obesity is a major contributor to the prevalence of cardiovascular disease in the developed world, and yet has only recently been afforded the same level of attention as other risk factors for CAD. It is now well established that endothelial dysfunction is an early event in cardiovascular atherosclerosis. Obesity like other risk factors for atherosclerosis is

characterised by endothelial dysfunction. As discussed, endothelial dysfunction results mainly from reduced NO production and/or an increase in its breakdown by ROS. This simple principle provides a logical approach to improve endothelial function in obese humans.

A variety of drugs, including statins, angiotensin converting enzyme (ACE) inhibitors and antioxidants have been shown to improve endothelial function in different experimental conditions.[86,138,139]

Angiotensin-converting enzyme inhibitors

There are persuasive data supporting an important role of the RAS in determining endothelial function.[92] Recent exciting data from the HOPE study suggest that ACE inhibitors may have particular benefit in insulin-resistant conditions. This study demonstrated not only a reduction in adverse cardiovascular events in patients at risk of atherosclerosis but also a decreased rate of progression to diabetes in at-risk individuals.[140] The effect of ACEi on endothelial function in obesity remains unexplored.

Insulin sensitising agents

In overtly hyperglycaemic patients, tight diabetic control as a general principle, to reduce vascular risk, has been supported by recent studies.[141] Certain insulin-sensitising drugs have been shown to improve endothelial function, raising the question of whether these should be used in obese patients with a view to reducing the risks of cardiovascular disease. Metformin, for example, corrects the abnormal endothelium-dependent vasodilator responses in fructose-fed insulin-resistant rats, probably through a direct effect of the drug rather than secondary metabolic changes.[142] The effect of metformin on endothelial function in obesity remains unexplored.

Thiazolidinediones (TZDs) are, in experimental models, very effective at improving insulin sensitivity and may have anti-atherogenic properties, although these appear to be mediated by effects on gene transcription and smooth muscle proliferation rather than vasomotor responses.[143] These actions are mediated through the stimulation of peroxisome proliferator-activated receptor gamma (PPARγ), an adipocyte nuclear receptor, to which TZDs bind with high affinity. The increased expression of specific genes, some of which are also controlled by insulin, has the effect of amplifying insulin stimulation. The improvement in insulin sensitivity is predominantly through both reduced hepatic glucose production and increased peripheral disposal. There is also evidence for the modulation of adipocyte-derived TNF-α by pioglitazone.[144] The effect of these agents on endothelial function in obese subjects was explored by Tack *et al*, who found no change in forearm vasomotor responses *in vivo*.[106] However, there are few studies exploring the effect of these agents on endothelial function *in vitro* to guide the best approach if any to their use. Therefore currently more work needs to be carried out before an answer to this important question is found.

HMGCoA reductase inhibitors

The value of the HMGCoA reductase inhibitors/statins to reduce cardiovascular risk in patients with atherosclerosis and those at risk of its development is now clear. Statins have been shown to improve endothelial function by both their cholesterol-lowering effect and by other actions on the endothelial cell. Recent evidence supports a direct action of statins to augment eNOS activity,[145] by a mechanism which may be a post-transcriptional prolongation of eNOS mRNA half-life.[146] Their use in insulin-resistant patients was accompanied by an impressive benefit in mortality and morbidity in large clinical trials; however, their effect *per se* in obesity/insulin resistance remains unclear.

Antioxidants

An area that remains controversial is the pharmacological modulation of oxidative stress. Despite a formidable body of evidence implicating ROS in the development of atherosclerosis,[147,148] and experimental data to support the role of antioxidant agents to improve endothelial function,[149,150] clinical trials using vitamins C and E have not demonstrated any benefit.[151] It is possible though that more specific, effective and carefully directed agents may succeed in demonstrating clinical benefit.

Conclusion

In conclusion, it is now clear that obesity is associated with endothelial cell dysfunction, an association that, at least in part, accounts for the increased risk of developing cardiovascular atherosclerosis. The challenge now for clinicians dealing with obese patients is to unravel the mechanisms underlying this association and design effective therapies to maintain endothelial cell homeostasis and its cardioprotective actions.

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