Visceral obesity is characterized by impaired nitric oxide-independent vasodilation

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Background Endothelial dysfunction has been described in obesity. This study examines the impact of visceral obesity on nitric oxide-independent relaxation in the human forearm.

Methods and results In ten viscerally obese and ten matched controls forearm blood flow (FBF) was measured by venous occlusion plethysmography during intrabrachial infusion of: (1) sodium nitroprusside; (2) bradykinin, before and after inhibition of vasoactive prostaglandins and nitric oxide; (3) potassium; (4) ouabain (Na+/K+ ATPase inhibitor) alone or (5) in combination with BaCl₂ (KIR inhibitor). Baseline FBF and endothelium-independent vasodilatation were similar in the two groups. In obese patients, bradykinin-induced increase of FBF was significantly less than in controls (P<0.01). Irrespective of prostaglandins and nitric oxide inhibition, bradykinin response was lower in the viscerally obese. Intrabrachial potassium determined a significantly blunted response (P<0.05). Ouabain caused a similar, moderate decrease in basal FBF in the two groups; the coinfusion of BaCl₂ caused a more intense decline in FBF which was significantly relevant in obese (−24±5%, P<0.01).

Conclusions In obese patients there is a blunted nitric oxide-independent relaxation determined by a decreased response of inwardly rectifying potassium channels. © 2003 The European Society of Cardiology. Published by Elsevier Ltd. All rights reserved.

KEYWORDS Endothelium; Endothelium-derived hyperpolarizing factor; Obesity; Insulin resistance; Potassium channels

Introduction Central obesity, an important component of the plurimetabolic syndrome, is a powerful predictor of CHD in man.1 Several studies have shown that obesity is independently associated with endothelial dysfunction, in humans;2–4 moreover, the increase in forearm blood flow in response to acetylcholine (Ach) is inversely related to body mass index and waist to hip ratio (WHR).5,6

The important link between central obesity and endothelial function is further supported by the concept that insulin sensitivity is partly determined by the ability of endothelium to produce nitric oxide (NO).7 Thus, the haemodynamic resistance of endothelium to insulin in terms of NO production would further aggravate the metabolic insulin resistance and in general the metabolic/hemodynamic coupling.

However, studies have shown heterogeneous vascular relaxation in vessels of different sizes: small arteries contribute to vascular resistance and may exhibit different mechanisms of endothelium-dependent relaxation than large arteries:8,9 while NO-mediated relaxation is enhanced with increases in vessel size, endothelium-derived hyperpolarizing factor (EDHF) is a more prominent vasodilator in smaller vessels. Thus, in term of metabolic/hemodynamic coupling, the NO-independent vasorelaxation appear more relevant than the NO-dependent one.
Therefore, the present study was designed to examine the role of this metabolic condition on NO-independent relaxation in the vascular bed of the human forearm.

**Methods**

**Study population**

Ten non-smoking healthy male volunteers and ten obese male patients were recruited and participated in this study (Table 1). Exclusion criteria were considered: current tobacco use, established cardiovascular disease, medications for hypertension, and hyperlipidaemia. All subjects had blood pressures of ≤140/90 mmHg, fasting total cholesterol ≤200 mg/dl, fasting glucose ≤126 mg/dl, and no family history of premature cardiovascular disease. The presence of diabetes was excluded by an oral glucose tolerance test. The study was approved by the Ethical Committee of the University Hospital of Padova. After signing an informed consent form, subjects returned on two separate mornings in fasting state. On day 1, anthropometric measurements were determined. WHR was defined as the minimal abdominal circumference between the xiphoid process and the iliac crests (waist) divided by the circumference determined over the femoral heads (hip). A standardized questionnaire was completed regarding diet, exercise, and family history. Blood was drawn to measure triglycerides, insulin, glucose, total cholesterol, and high-density lipoprotein cholesterol.

All studies were performed in a temperature-controlled room.

**Study A and B**

These studies performed on day 1 assessed the endothelium-independent and the NO-independent vasodilations. The subjects were studied in the supine position. The day of the study, a plastic cannula (20-gauge) was inserted into the brachial artery of the non-dominant arm under local anaesthesia (xylocaine 2%) and used for the infusion of the test substances, the monitoring of arterial blood pressure and heart rate, and arterial blood sampling. Forearm blood flow (FBF) was measured in both forearms by strain-gauge plethysmography with a calibrated mercury-in-Silastic strain-gauge applied around both forearms and connected to a Microlab plethysmograph (Microlab, Padova). The data were monitored continuously with a dedicated software. Both arms were supported slightly above the heart level. During the measurement of FBF and blood sampling, a pediatric cuff was inflated around the wrist 100 mmHg above systolic blood pressure to exclude hand circulation from the measurements.

Sodium nitroprusside (SNP) was infused at the rate of 1, 3, and 9 µg of forearm volume (FAV)−1 min−1 to assess non-endothelium-mediated vasodilation. Each dose of the test substances was infused for 5 min, and FBF was measured during the last 2 min of infusion (Study A). A wash-out period of at least 30 min was allowed between Study A and B.

As previously shown by Honing et al. bradykinin (BK) causes a significant vasodilation in resistance vessels of the human forearm which seems to be largely independent of endothelium-derived NO and prostaglandins.10 Thus, dose-response curves to BK (50, 100, and 200 ng 100 ml FAV−1 min−1) were performed before and after inhibition of vasoactive prostaglandins and NO with indomethacin and N\(_2\)-mono-L-methyl-arginine (L-NMMA) (Study B). To accomplish this, 50 mg indomethacin was orally given 60 min before BK infusion; then we infused N\(_2\)-mono-L-methyl-arginine (L-NMMA; 200 µg 100 ml FAV−1 min−1; Clinalfa), a competitive inhibitor of NO synthase, throughout the experiment. After 20 min of L-NMMA infusion, the vasoconstriction by L-NMMA was subsequently counteracted by concurrent infusion of ascending doses of sodium nitroprusside (SNP; 30 to 180 ng 100 ml FAV−1 min−1) until blood flow had returned to baseline values (NO clamp). Thereafter, L-NMMA and SNP were coinjected at constant rates for the remainder of the experiment.
study. Blood flow measurements were performed in the contralateral (control) forearm as well, to ensure that systemic effects did not occur during the intra-arterial infusion of vasoactive agents. Therefore, the response of forearm blood flow was expressed as a percentage increase of the ratio infused-control arm. Blood pressure was recorded immediately before each measurement with a non-invasive technique (Finapress; Ohmeda, Englewood, CA).

Study C and D

In these studies, performed on day 2, we further characterized NO-independent vasodilation, and specifically tested the hypothesis that K⁺ act as EDHF. We used the approach proposed by Dawes and colleagues: FBF was measured as before at baseline and during the intrabrachial infusion of KCl (0.2 mmol/min), followed by a 15-min saline recovery period (Study C). Then the effects of either the infusion of ouabain alone, a Na⁺/K⁺ ATPase blocker, or in combination with BaCl₂, a rectifying potassium channel (Kir) blocker, on basal FBF was assessed. Ouabain was infused into the brachial artery at a rate of 2.7 nmol/min for 13 min; from min 9 BaCl₂ was co-infused at a rate of 4 µmol/min for next 4 min (Study D). FBF was measured from min 7 to min 9 and from min 11 to min 13. A wash-out period of at least 30 min was allowed between Study C and D.

Biochemical assays

Plasma glucose was determined with glucose oxidase method. Insulin was determined with conventional RIA. Insulin resistance was estimated using the homeostasis model assessment (HOMA) from the fasting glucose and insulin concentrations. Total cholesterol, HDL cholesterol and triglycerides in blood plasma were determined by standard enzymatic techniques.

Calculations and statistical analysis

Results are expressed as mean±SEM. The final ten blood flow recordings of each infusion step from both the measurement and the control arm were used to calculate the mean FBF. Statistical analysis was performed with two-way ANOVA for repeated measures. Unpaired t-test was used to compare the results between controls and patients. Statistical significance was taken at the 5% level (P<0.05).

Results

Metabolic parameters

Patients were selected because of the presence of visceral obesity: as expected in these patients, girth and WHR were significantly higher than in controls. Moreover, they had significantly higher plasma glucose, insulin and triglyceride concentrations. As verified by the HOMA, value they were also significantly more insulin resistant than controls (Table 1).

Endothelium-independent and effects of cyclooxygenase and NO synthase inhibition on vasodilation caused by bradykinin (Studies A and B)

Baseline FBF was similar in the two groups (1.7±0.1 in obese and 1.8±0.3 ml min⁻¹ 100 ml FAV⁻¹ min⁻¹ in controls). A slight increase in blood pressure, though not significant, was present in obese patients (128±3/79±3 mmHg vs 120±3/74±2 mmHg).

No differences were observed in response to SNP among each experimental condition. During the highest dose of SNP (10 µg min⁻¹) FBF increased by 560±58% in the obese and by 589±66% in controls (Fig. 1).

In the obese, cumulative doses of BK increased FBF by 329±34%, 452±31% and 614±40%, respectively for 50, 100, and 200 ng 100 ml FAV⁻¹ min⁻¹: at the highest BK infusion rate the FBF response was significantly less than that observed in controls (818±66%, P<0.01, Fig. 2, top). Inhibition of NO synthase with L-NMMA for 20 min caused a vasocconstriction in both groups that was counteracted with
incremental doses of SNP until baseline FBF was restored. After the inhibition of both cyclooxygenase and NO synthease activity, BK infusion elicited a similar blunted response in obese and in controls (Fig. 2, bottom) indicating that the altered FBF response in obese is apparently not dependent on NO and prostaglandin production.

Effects of potassium, ouabain and BaCl2 on basal forearm blood flow (Studies C and D)

The intrabrachial infusion of potassium had different effects on FBF of controls and obese: in the latter group this infusion elicited a significantly blunted response than in the former group (186±18% vs. 278±28%, P<0.05, Fig. 3, top).

Ouabain infusion (Fig. 3, bottom) caused a similar decrease in basal FBF of both groups (~19±3% in controls and −14±3% in obese). On the contrary, the coinfusion of ouabain with BaCl2 caused a much more intense decline in FBF in controls (~42±5%, P<0.01) than in obese (~24±5%, P<0.01).

Discussion

It is now well established that visceral obesity is an independent risk factor for the development of coronary artery atherosclerosis. Recent studies have produced persuasive evidence showing the presence of endothelial dysfunction in obese humans.13–15 From a pathophysiological point of view, recent studies have produced evidence showing that the presence of endothelial dysfunction in obese humans is due to a reduced NO bioavailability determined by an increased production of reactive oxygen species.16

The association between obesity/insulin resistance and endothelial dysfunction is strongly supported by the fact that endothelium-dependent vasodilation is impaired in proportion to insulin resistance and various indices of adiposity under baseline conditions.7 This concept has reinforced the hypothesis that a normal endothelial function is important to optimize the metabolism and haemodynamic coupling in muscle: this allows a normal substrate delivery to the site where they are normally oxidized. However, there is increasing evidence that vasodilation in smaller peripheral vessels is assured by the EDHF rather than by NO:17,18 this may be relevant in terms of substrates.
coupling and perfusion in human skeletal muscle since it is EDHF rather than NO which may act as a mediator coupling substrate metabolism to vasodilation. The exact identification of an EDHF remains to be an extremely contentious point. For this reason, a functional definition of EDHF has been proposed: 'agonist-induced, endothelium-dependent relaxations that are not blocked by inhibitors of NO synthase or cyclooxygenase but are inhibited by potassium channel blockers'. 19 To assess in patients with visceral obesity the NO-independent vasodilatation we administered BK before and during the so called NO clamp as proposed by Honing et al. 10 who suggest that the BK-dependent vasodilatation is mainly NO-independent. We show that in obese patients the FBF changes in response to BK administration were blunted irrespective of both nitric oxide synthase and cyclooxygenase inhibition. Although we did not quantify the effects of both nitric oxide synthase and cyclooxygenase inhibitors, we found that a substantial BK-mediated vasodilation of the forearm microcirculation persists, despite inhibition of cyclooxygenase and of NO: this reveals alternative vasodilator mechanism(s) such as EDHF release.

There is evidence that the activity of the so-called EDHF is mediated by the inwardly rectifying potassium channels (K_{IR}). 11,20 To clarify whether K_{IR} are involved in the blunted NO-independent dilation in these patients, we adopted the techniques proposed by Dawes et al. 11 Specifically, we evaluated the FBF response to the elevation of extracellular K\(^+\): at physiologic resting potential, an increase in extracellular levels of this cation leads to an increase in the resting outward K\(^+\) current through K_{IR} channels. 21 Our results show a substantial blunt of FBF increase in obese patients in response to K\(^+\): this implicates a reduced potassium-mediated dilation in these patients and the important role of K_{IR} in this altered response. This latter hypothesis was further substantiated by studies C and D in which we intrabrachially infused inhibitors of both K_{IR} and Na\(^+\)/K\(^+\) ATPase. This approach allowed us to demonstrate that: (a) Na\(^+\)/K\(^+\) ATPase is poorly implicated in this altered vasodilatation since ouabain infusion produced a slight decrease in FBF with no differences between the two groups; (b) the blunted vasoconstrictor response to Barium ion, a specific inhibitor of K_{IR}, in obese patients indicates not only that these channels contribute substantially to the physiological maintenance of the vascular vasorelaxation but also that their activity is reduced in these patients.

In our patients with visceral obesity, total cholesterol, triglyceride and blood pressure levels were somewhat higher than in the control group but were in the normal range and not statistically different from controls. These covariates could partly explain the decreased NO-independent vasodilation in obese: however, we cannot tell exactly how much their contribution is in the NO-independent vasodilatation. As far as we know, no studies explored the role of EDHF vasodilatation in hypercholesterolaemia/hypertriglyceridaemia: this aim would warrant further studies in the future. With respect to blood pressure, the obese exhibited somewhat higher levels of blood pressure than the controls: this may have contributed at least in part to the altered EDHF-mediated relaxation observed in these patients. Consistent with this hypothesis altered K_{IR} channels have been documented at least in animal models. 22 Alternatively, the higher blood pressure could be the expression of altered smooth muscle cell integrity: unfortunately we did not use an infusion of a calcium channel blocker such as Verapamil, in order to assess this parameter which is not dependent on endothelium-derived or exogenous NO. However Williams et al. found that, at least in Type 2 diabetic patients, the forearm blood flow response to graded Verapamil infusions was comparable to that observed in normal controls. 23 Clinically, our findings could have a potentially relevant implication in the metabolic syndrome: in these patients there is a general loss of normal vasodilatory capacity in the small forearm vessels where the coupling between blood flow and metabolism takes place. Therefore, the simultaneous loss of NO-mediated relaxation in the large conduit arteries but also the loss of NO-independent, potassium-mediated relaxation in the small arteries, not only may make these patients particularly prone to premature cardiovascular disease but also perpetrate insulin resistance by exerting a negative action on metabolism and haemodynamic coupling.

References


