Obesity and Body Fat Distribution Induce Endothelial Dysfunction by Oxidative Stress

Protective Effect of Vitamin C

Francesco Perticone, Roberto Ceravolo, Mafalda Candigliota, Giorgio Ventura, Saverio Iacopino, Flora Sinopoli, and Pier L. Mattioli

Endothelial dysfunction has been reported in obese subjects, but its mechanism has not been elucidated. We have therefore investigated *1*) the possible relationship among BMI, waist-to-hip ratio (WHR), and endothelium-dependent vasodilation and *2*) whether oxidative stress participates in endothelial dysfunction. We recruited 76 healthy subjects (50 men and 26 women aged 21–45 years) and measured their BMI (kg/m^2), WHR, and insulin resistance (IR) estimated by the homeostasis model assessment (HOMA). Endothelium-dependent and -independent vasodilation were assessed by increasing doses of acetylcholine (ACh) (7.5, 15, and 30 µg · ml^{-1} · min^{-1}) and sodium nitroprusside (SNP) (0.8, 1.6, and 3.2 µg · ml^{-1} · min^{-1}) during saline and vitamin C coinfusion (24 mg/min). The effects of cyclooxygenase activity were evaluated by a dose-response curve to intrabrachial coinfusion of ACh and indomethacin (500 µg/min). Three different groups have been identified according to their BMI: group A (BMI <25), consisting of 10 men and 5 women; group B (BMI between 25 and 29), consisting of 16 men and 8 women; and group C (BMI ≥30), consisting of 24 men and 13 women. Obese subjects had significantly lower forearm blood flow (FBF) during ACh infusions (means ± SD): 19.8 ± 2.8, 10.8 ± 2.7, and 6.5 ± 1.8 ml · 100 ml^{-1} tissue · min^{-1} (P < 0.0001) for groups A, B, and C, respectively. SNP caused comparable increments in FBF in all groups. Regression analysis revealed a significant negative correlation between BMI (r = –0.676, P < 0.0001), WHR (r = –0.631, P < 0.0001), fasting insulin (r = –0.695, P < 0.0001), HOMA-IR (r = –0.633, P < 0.0001), and percent peak increase in FBF during ACh infusion. In obese subjects, both vitamin C and indomethacin increased the impaired vasodilating response to ACh, whereas the SNP effect was unchanged. In conclusion, in obese subjects, ACh-stimulated vasodilation is blunted, and the increase in FBF is inversely related to BMI, WHR, fasting insulin, and HOMA-IR. The effects of both vitamin C and indomethacin on impaired ACh-stimulated vasodilation support the hypothesis that oxidative stress contributes to endothelial dysfunction in human obesity. *Diabetes* 50:159–165, 2001

Obese subjects are at high risk for developing diabetes, dyslipidemia, hypertension, and cardiovascular diseases, which lead to an increased risk of mortality (1–3). Moreover, it has been demonstrated that obesity is associated with hyperinsulinemia, an independent predictor for coronary artery disease (4). In fact, hyperinsulinemia is linked to insulin resistance (IR) and potentially to atherogenic abnormalities.

The normal endothelium plays a key role in the regulation of vascular tone and in preventing the progression of atherosclerosis through the production and release of both contracting and relaxing factors (5). Nitric oxide (NO) represents the major endogenous relaxing factor (6–9), and its production is stimulated by physical stimuli (e.g., shear stress) (9) and by several agonists (e.g., acetylcholine [ACh], bradykinin, substance P, and serotonin) (8). The activation of guanylate cyclase and the subsequent accumulation of cGMP are the main mechanisms of NO-induced vasodilation. In contrast, sodium nitroprusside (SNP) is an endothelium-independent vasodilator capable of inducing vasodilation by providing an inorganic source of NO (10). Major risk factors for atherosclerotic vascular diseases (e.g., hypertension, smoking, diabetes, and hypercholesterolemia) have been associated with endothelial dysfunction due to increased oxidative stress (11–16). Recent reports have also indicated that insulin contributes to the maintenance of vascular tone through a selective physiological action in vasodilating skeletal muscle vasculature. Specifically, the insulin-mediated vasodilation has been attributed to endothelial NO release (17,18). Nevertheless, endothelial dysfunction has also been reported in obese insulin-resistant subjects, but the underlying mechanisms have not been clarified (19).

Given this information, we evaluated the relationship between body weight and endothelium-dependent vasodilation, and we investigated whether body fat distribution affects ACh-mediated vasodilation. Moreover, we evaluated whether oxygen free radicals could be responsible for the impairment in the L-arginine–NO pathway of obese subjects by studying the effects of a vitamin C infusion, a potent antioxidant compound. Finally, using indomethacin as an experimental tool, we also evaluated the possibility that oxygen free radicals could originate from cyclooxygenase activity.

From the Cardiovascular Disease Unit, Department of Experimental and Clinical Medicine G. Salvatore, University of Catanzaro Magna Graecia, Catanzaro, Italy.

Address correspondence and reprint requests to Francesco Perticone, MD, Dipartimento di Medicina Sperimentale e Clinica G. Salvatore, Policlinico Mater Domini–Via T. Campanella, 88100 Catanzaro, Italy. E-mail: perticone@unicz.it.

Received for publication 15 February 2000 and accepted in revised form 18 September 2000.

P.L.M. is deceased.

ACh, acetylcholine; ANOVA, analysis of variance, BP, blood pressure; FBF, forearm blood flow; HOMA, homeostasis model assessment; IR, insulin resistance; NO, nitric oxide; SNP, sodium nitroprusside; VR, vascular resistance; WHR, waist-to-hip ratio.

TABLE 1
Characteristics of the three BMI groups for healthy male and female subjects

	Men				Women			
	Group A	Group B	Group C	*P* (ANOVA)	Group A	Group B	Group C	*P* (ANOVA)
n	10	16	24		5	8	13	
Age (years)	40 ± 3	41 ± 4	41 ± 6	0.850	39 ± 7	40 ± 8	39 ± 5	0.935
BMI (kg/m^2)	24.6 ± 2	28.8 ± 1	35.4 ± 5	0.0001	23.8 ± 2	26.9 ± 5	37.8 ± 7	0.0001
Waist (cm)	88.4 ± 3.6	99.4 ± 4.0	111.5 ± 16.9	0.0001	86.4 ± 6.5	97.5 ± 6.2	118.5 ± 9.5	0.0001
WHR	0.90 ± 0.03	0.95 ± 0.05	1.01 ± 0.06	0.0001	0.76 ± 0.04	0.89 ± 0.06	0.93 ± 0.04	0.0001
Systolic BP (mmHg)	131 ± 6	133 ± 7	136 ± 6	0.092	129 ± 5	133 ± 9	135 ± 6	0.275
DiastolicBP (mmHg)	83 ± 7	85 ± 4	85 ± 5	0.554	82 ± 9	83 ± 8	82 ± 5	0.942
Fasting glucose (mmol/l)	5.6 ± 0.2	5.6 ± 0.7	6.1 ± 0.6	0.015	5.2 ± 0.3	5.7 ± 0.6	6.1 ± 0.7	0.032
Fasting insulin (μU/ml)	8.3 ± 2.6	15.3 ± 3.3	33.2 ± 15.6	0.0001	6.7 ± 2.6	17.3 ± 4.3	33.9 ± 11.2	0.0001
HOMA-IR	2.1 ± 0.7	3.8 ± 1.2	8.5 ± 4.7	0.0001	1.6 ± 0.7	4.6 ± 1.7	9.1 ± 3.7	0.0001
Cholesterol (mg/dl)	181 ± 16	183 ± 12	185 ± 11	0.679	167 ± 29	176 ± 35	184 ± 11	0.402
Triglycerides (mg/dl)	126 ± 27	128 ± 21	136 ± 25	0.438	111 ± 28	125 ± 30	124 ± 23	0.595

Data are means ± SD unless otherwise indicated.

RESEARCH DESIGN AND METHODS

Study population. For this study, we recruited 76 Caucasian volunteers (50 men and 26 women) aged 21–45 years (mean ± SD 40 ± 5). None of the subjects had a history of hypertension, diabetes, hyperlipidemia, peripheral vascular disease, or coagulopathy. Valvular, primary myocardial, and coronary artery diseases were excluded by history, physical examination, and standard diagnostic procedures. Other exclusion criteria were the presence of hematological, renal, or hepatic disease. None of the subjects was taking any pharmacological treatment, and none had ever smoked. The institutional ethical committee had approved the study, and all participants gave written informed consent for all procedures.

Anthropometric measurements. A trained examiner (F.S.) collected measurements of height, weight, and circumference according to a standardized protocol. BMI was calculated as kilograms per square meter, and the waist was measured at its smallest point with the abdomen relaxed. The hip circumference was measured at its largest point: the tape was held at the top of the patient's hipbone and then wrapped carefully around the torso.

Central fat distribution was defined on the basis of the sex-specific 85th percentile of the waist-to-hip ratio (WHR) values as indicated by the Italian Consensus Conference on Obesity (20). According to these guidelines, the cutoff value of central obesity was considered ≥0.81 for women and ≥0.92 for men.

IR evaluation. An oral glucose tolerance test was performed to exclude diabetes in undiagnosed patients. Subjects were seated for the test between 8:00 and 9:00 A.M. after fasting overnight for at least 12 h. Fasting glucose and insulin values were averaged from the values obtained 15 and 5 min before administration of a 75-g glucose solution.

IR was estimated using the homeostasis model assessment (HOMA) from the fasting glucose and insulin concentrations (21). The HOMA-IR is commonly used in clinical studies, and it was recently used in a population-based study (22,23).

Measurements of forearm blood flow. All studies were performed at 9:00 A.M. after subjects had fasted overnight, with the subjects lying supine in a quiet air-conditioned room (22–24°C). We used the study protocol previously described by Panza et al. (24) and subsequently used by our group (25,26).

The forearm blood flow (FBF) was measured as the slope of the change in the forearm volume (27). The mean of at least three measurements was calculated at each time point. Forearm vascular resistance (VR), expressed in arbitrary units, was calculated by dividing mean blood pressure (BP) by FBF. To avoid underestimation of FBF measurements, the forearm circumference in all subjects was required to be <28 cm.

Vascular function

Endothelium-dependent and endothelium-independent vasodilation. All participants rested ≥30 min after artery cannulation to obtain a stable baseline before data collection; FBF and VR were repeated every 5 min until stable. Endothelium-dependent and endothelium-independent vasodilation were assessed by the dose-response curve to intra-arterial infusions at increasing doses of ACh (7.5, 15, and 30 μg · ml^{-1} · min^{-1}, each for 5 min) and SNP (0.8, 1.6, and 3.2 μg · ml^{-1} · min^{-1}, each for 5 min), respectively. The drug infusion rate, adjusted for the forearm volume of each subject, was 1 ml/min.

Effects of vitamin C on endothelium-dependent and endothelium-independent vasodilation. To evaluate whether oxygen free radicals can selectively impair endothelium-dependent or endothelium-independent vasodilation in obese subjects, both ACh and SNP were infused under controlled conditions (saline infusion) and in the presence of intrabrachial vitamin C (24 mg/min), which was administered 5 min before the agonists and continued throughout. This vitamin C concentration has been shown to both protect human plasma from free radical–mediated lipid peroxidation (11) and improve impaired ACh-induced vasodilation in smokers (12) and hypercholesterolemic (11,13), hypertensive (14), and diabetic patients (15).

Effects of cyclooxygenase inhibition on ACh-stimulated vasodilation. We have evaluated the effects of cyclooxygenase activity (a source of oxygen free radicals) on endothelium-dependent vasodilation. A dose-response curve to intrabrachial ACh administration was performed during the coinfusion of indomethacin (a cyclooxygenase inhibitor) at a constant dose of 500 μg/min starting 10 min before ACh administration and continuing throughout.

Drugs. ACh (Sigma, Milan, Italy), vitamin C (Bracco, Milan, Italy), and indomethacin (Liometacen, Chiesi Farmaceutici SpA, Parma, Italy) were obtained from commercially available sources and diluted freshly to the desired concentration by the addition of saline. SNP (Malesci, Florence, Italy) was diluted in a 5% glucose solution immediately before each infusion and protected from light with aluminum foil.

Statistical analysis. Analysis of variance (ANOVA) was performed for clinical and biological data, and the differences between means were compared using unpaired Student's *t* tests. The responses to ACh and SNP were compared by ANOVA for repeated measurements, and when the analysis was significant, the Tukey's test was applied. Simple linear regression analysis was performed to assess the relationship between the peak increase in FBF in response to ACh infusion and variables such as indexes of obesity (i.e., BMI, waist circumference, and WHR), fasting insulin, HOMA-IR, and other factors reported to impair endothelium-dependent vasodilation (i.e., age, cholesterol, and systolic and diastolic BP). Subsequently, variables that achieved statistical significance were entered into a stepwise multiple regression model to assess the magnitude of their individual effect on the peak FBF response to intra-arterial infusions of ACh. In this analysis, we included only fasting insulin because HOMA-IR is a function of both fasting insulin and glucose. Thus, we considered fasting insulin the fittest variable to avoid a possible colinearity. Significant differences were assumed to be present at $P < 0.05$. All group data are reported as means ± SD.

RESULTS

Baseline demographic, hemodynamic, and humoral characteristics of the study population, stratified by sex and BMI, are summarized in Table 1.Three different groups have been identified according to their BMI: group A (BMI <25), consisting of 10 men and 5 women; group B (BMI between 25 and 29), consisting of 16 men and 8 women; and group C (BMI ≥30), consisting of 24 men and 13 women. Waist and hip circumferences as well as WHR in both sexes increased significantly ($P < 0.0001$ by ANOVA) with increasing BMI. In our study population, 76.5% of the subjects (76.0% of the men and 76.9% of the women) had central obesity according to the

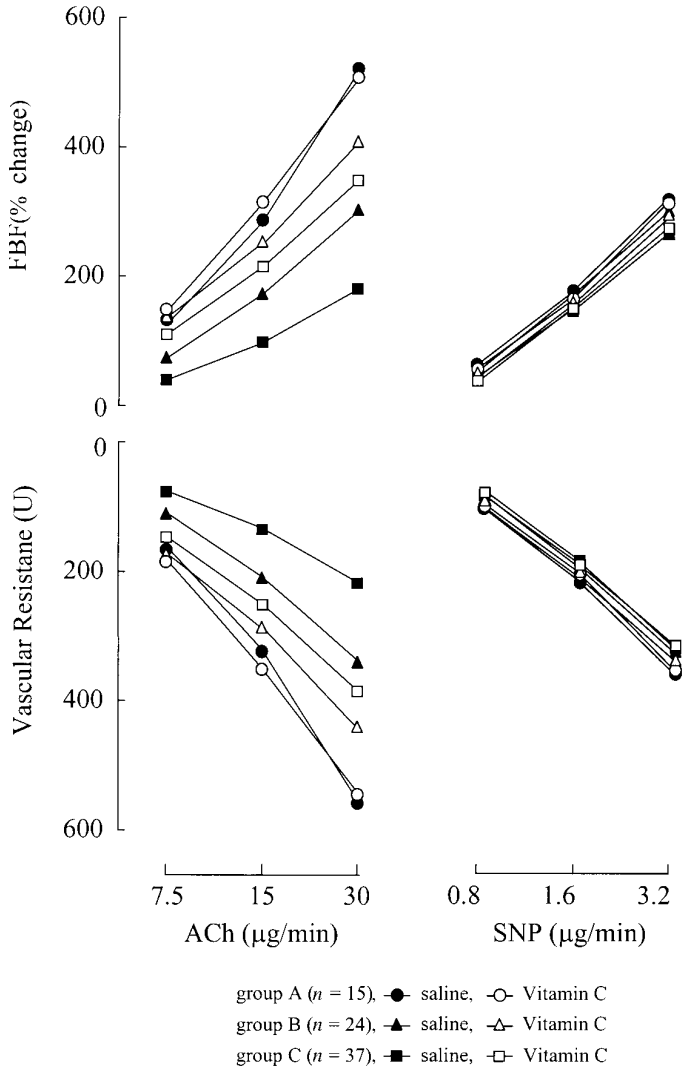

FIG. 1. Both FBF and VR during ACh and SNP infusions, before and during coinfusion of vitamin C.

previously defined criteria (20). Both fasting glucose values and insulin levels differed significantly among the groups. Compared with group A, insulin levels in men and women were 84 and 300% higher in group B and 158 and 308% higher in group C, respectively ($P < 0.0001$ by ANOVA). According to HOMA-IR (an index of insulin sensitivity), obese men and women showed higher levels of IR ($P < 0.0001$ by ANOVA). In the whole population, HOMA-IR linearly increased with the increase in BMI ($r = 0.663$, $P < 0.0001$) and WHR ($r = 0.524$, $P < 0.0001$) at an age-adjusted rate of both 0.452 per unit of BMI (CI 0.337–0.567, $P < 0.0001$) and 2.423 per 0.1 unit of WHR (CI 13.764–34.705, $P < 0.0001$). When the analysis was performed according to sex, HOMA-IR increased as a function of BMI more in women ($r = 0.773$, $P < 0.0001$) than in men ($r = 0.624$, $P < 0.0001$), but when the increase was evaluated as a function of WHR, IR was more consistent in men ($r = 0.695$, $P < 0.0001$) than in women ($r = 0.511$, $P < 0.0001$). Total cholesterol, triglyceride, and systolic and diastolic BP values were within the normal range and, though they were somewhat higher in group C than in the other groups, these differences did not reach statistical significance.

Endothelium-dependent and endothelium-independent vasodilation

ACh study. Basal FBF was 3.8 ± 0.6, 3.6 ± 0.7, and 3.6 ± 0.8 ml · 100 ml^{-1} of tissue · min^{-1} in groups A, B, and C, respectively ($P = 0.673$ by ANOVA). Similarly, no significant ($P = 0.268$ by ANOVA) differences in VR among the groups were observed: 26.5 ± 4.7 U for group A, 29.1 ± 5.6 U for group B, and 29.2 ± 6.0 U for group C.

In response to the intra-arterial infusion of ACh, FBF significantly increased ($P < 0.0001$) in a dose-dependent fashion in all groups. However, obese subjects (group C) had a significantly lower responsiveness to ACh (i.e., FBF from 3.6 ± 0.8 to a maximum of 6.5 ± 1.8 ml · 100 ml^{-1} tissue ·min^{-1}) than subjects included in both group A (3.8 ± 0.6 to a maximum of 19.8 ± 2.8 ml · 100 ml^{-1} tissue · min^{-1}) and group B (3.6 ± 0.7 to a maximum of 10.8 ± 2.7 ml · 100 ml^{-1} tissue · min^{-1}) (Fig. 1). VR significantly decreased ($P < 0.0001$) in a dose-dependent manner in all groups, and changes in VR were significantly more pronounced in group A than in groups B and C. Similarly, the changes induced by ACh in FBF and VR were significantly different between groups A and B (Fig. 1).

ACh and vitamin C coinfusion. The blunted vasodilation in response to ACh that was documented in obese subjects significantly increased during coinfusion of vitamin C (Fig. 1). However, the oxygen free radical scavenger did not significantly change the response to ACh in the subjects included in group A (FBF from 3.7 ± 0.5 to 19.1 ± 3.1 ml · 100 ml^{-1} tissue · min^{-1} with the highest dose), but in the subjects of both group B (3.6 ± 0.6 to 14.8 ± 2.5 ml · 100 ml^{-1} tissue · min^{-1} with the highest dose; $P < 0.0001$ vs. ACh during saline) and group C (3.5 ± 0.6 to 12.3 ± 2.4 ml · 100 ml^{-1} tissue · min^{-1} with the highest dose; $P < 0.001$ vs. ACh during saline), the intrabrachial administration of vitamin C significantly improved the vasodilating effect of the muscarinic agonist. In addition, the comparison among the groups was statistically significant ($P < 0.0001$ by ANOVA).

ACh and indomethacin coinfusion. ACh-stimulated vasodilation increased significantly during indomethacin coinfusion (Fig. 2). When the activity of the cyclooxygenase inhibitor was compared among the subjects of group A, ACh-dependent vasodilation was not significantly increased by indomethacin (FBF from 3.7 ± 0.6 to 19.3 ± 2.9 ml · 100 ml^{-1} tissue · min^{-1} with the highest dose; NS vs. ACh during saline). On the contrary, ACh-induced vasodilation was significantly increased by indomethacin in the subjects of group B (3.5 ± 0.5 to 15.2 ± 2.3 ml · 100 ml^{-1} tissue · min^{-1} with the highest dose; $P < 0.0001$ vs. ACh during saline) and group C (3.6 ± 0.5 to 12.5 ± 2.1 ml · 100 ml^{-1} tissue · min^{-1} with the highest dose; $P < 0.0001$ vs. ACh during saline). The enhancement of ACh-induced vasodilation exerted by indomethacin in obese subjects (groups B and C) was similar to the potentiation produced by coinfusion of vitamin C in the same subjects.

SNP study. Figure 1 shows the percent increase in FBF above baseline in response to intra-arterial infusions of SNP. The vasodilating responses to the endothelium-independent vasodilator SNP were similar in all groups.

SNP and vitamin C coinfusion. The vitamin C coinfusion did not significantly change either FBF or VR during SNP infusions (Fig. 1). The results also remained unchanged when data were analyzed according to WHR. Thus, all subjects displayed normal endothelium-independent vasodilation.

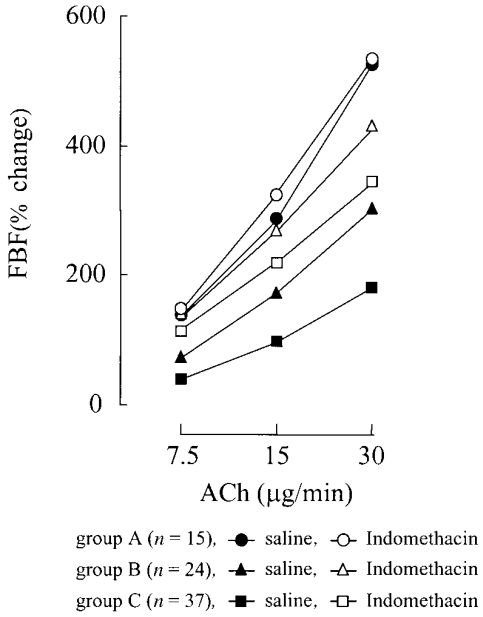

FIG. 2. ACh-induced FBF before and after indomethacin coinfusion.

Correlational analyses. Figure 3 shows the inverse relationship between the peak increase in FBF after ACh infusion and BMI ($r = –0.676$, $P < 0.0001$), which accounts for 45.7% of the variation in FBF response in the whole population. Subsequently, we tested the effect of fat distribution on endothelium-dependent vasodilation by using both waist circumference ($r = –0.581$, $P < 0.0001$) and WHR ($r = –0.631$, $P < 0.0001$) (Fig. 4) as criteria for central obesity. The last relationship accounts for 39.8% of the variation, and it is more evident in men ($r = –0.696$, $P < 0.0001$) than in women ($r = –0.538$, $P < 0.0001$). Therefore, our data suggest that central fat distribution may also affect endothelium-dependent vasodilation. When we investigated the effect of insulin sensitivity on the peak increase in FBF after ACh infusion, we found an inverse linear relationship between HOMA-IR (Table 2) and fasting insulin (graphically reported in Fig. 5). Taking into account that both BMI and WHR affect insulin sensitivity, these data suggest that IR indeed mediates the negative effects of BMI and WHR on endothelium-dependent vasodilation. Finally, we performed a simple linear regression analysis between the peak increase in FBF during ACh infusions and both waist circumference and IR for the subject population as a whole and subdivided by sex. This analysis (Table 2) demonstrated that increasing degrees of obesity, waist circumference, WHR, fasting insulin, and IR are associated with different degrees of endothelial dysfunction.

Furthermore, because impaired ACh-stimulated vasodilation has been associated with aging (27), hypercholesterolemia (22,24), and essential hypertension (25), we also investigated the relationship between the peak increase in FBF and age, cholesterolemia, and BP values. This analysis did not show any significant correlation with the maximal FBF response to ACh. In addition, in Table 3 we report the relationship between insulin sensitivity, indexes of obesity/body fat distribution, and Δ% changes in the FBF response to ACh caused by the coinfusion of vitamin C and indomethacin. The analysis demonstrates that greater improvement in FBF was observed in the subjects with the highest degree of obesity or IR whereas less improvement was seen in the leaner subjects, confirming that obesity and/or IR are the main causes of the endothelial dysfunction.

Finally, in a stepwise multivariate regression analysis (Table 4), the independent determinants of the peak increase in FBF were fasting insulin, WHR, and BMI in the total subject population and in the men; on the contrary, only BMI was significantly related to FBF in the women.

DISCUSSION

Our data confirm and extend previous findings (19) that human obesity and its correlates of abdominal fat distribution and indexes of IR are inversely related to FBF in response to ACh. In contrast, endothelium-independent vasodilation is unaffected, confirming the presence of an endothelial dysfunction in obese subjects. In this study, we also provide novel evidence that vitamin C coinfusion markedly improves the impaired endothelium-dependent vasodilation in obese subjects. This effect seems to be specific because it was observed neither in lean subjects (group A) nor during endothelium-independent vasodilation produced by SNP infusion. Moreover, our data demonstrate that intrabrachial infusion of indomethacin increases ACh-stimulated vasodilation in obese subjects (groups B and C) but not in lean subjects (group A), indicating that cyclooxygenase activity produces substances that can reduce NO bioavailability. These substances are probably oxygen free radicals because intrabrachial infusion of the antioxidant vitamin C significantly improves ACh-stimulated vasodilation. This hypothesis

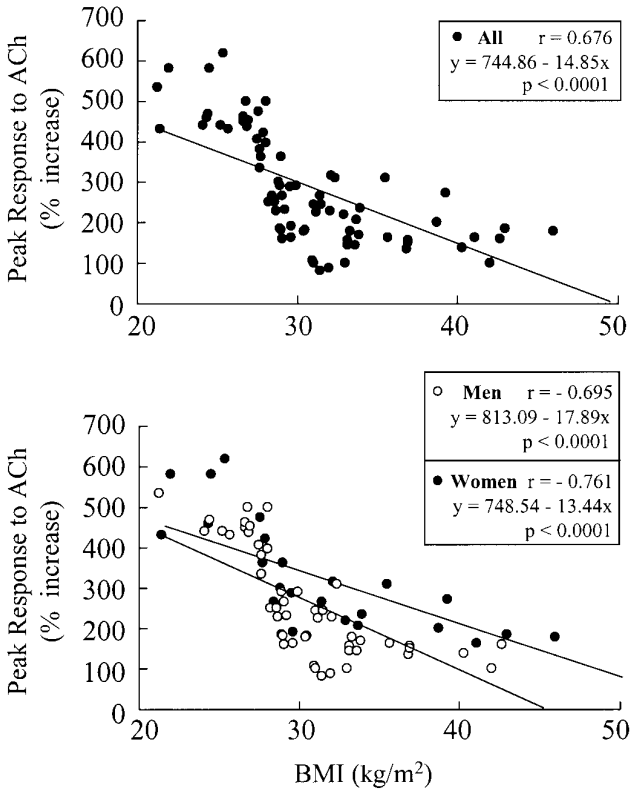

FIG. 3. Peak percent increases in FBF after ACh infusion versus BMI in all subjects and subdivided by sex.

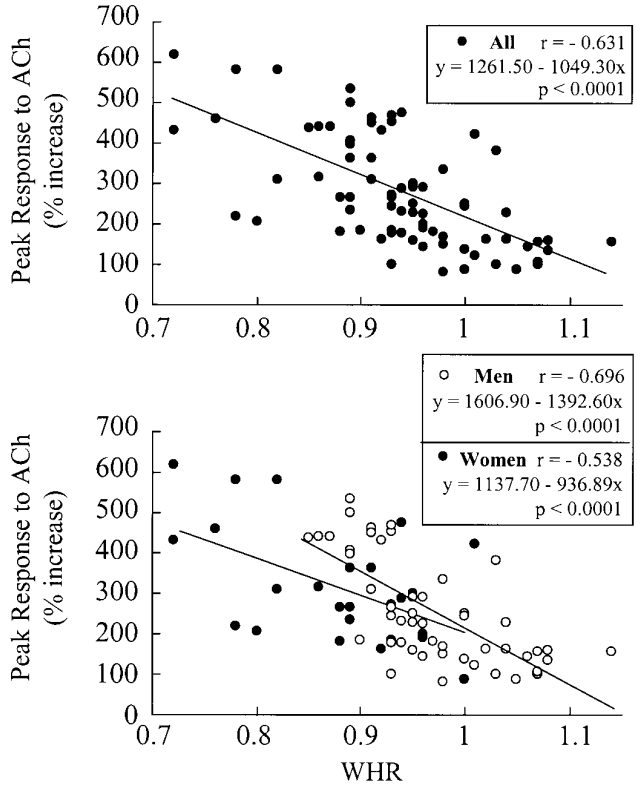

FIG. 4. Peak percent increases in FBF after ACh infusion versus WHR in all subjects and subdivided by sex.

may be strongly supported by data recently published by Jackson et al. (28), who demonstrated that arterial supraphysiologic ascorbic acid concentrations, like those used in our study, are effective in improving NO bioavailability by preventing its interaction with superoxide anions. Thus, the potentiating effect of vitamin C and indomethacin on ACh-stimulated FBF reinforces the hypothesis that oxidative stress may be considered the major, but not the only, mechanism responsible for endothelial dysfunction in human obesity. However, we must add that our conclusions are based on indirect evidence, similar to that reported in other human studies (11–15), because we did not directly measure the production of free radicals. Whether oxidative stress is caused by cyclooxygenase activity per se or by its derivatives remains to be investigated. Pryor and Squadrito (29) have shown that oxygen free radicals are constantly being combined with NO to form peroxynitrite, which has a greater oxidative capacity than any other compound.

Another finding of our study is that, like BMI, central fat distribution also affects the peak FBF response to intrabrachial infusion of ACh. This inverse relationship between FBF and both BMI and WHR is affected by sex, as shown in Figs. 3 and 4; in particular, BMI predicts 48.3 and 57.9% and WHR predicts 48.4 and 28.9% of the maximum response to ACh in men and women, respectively.

We also demonstrated that indexes of insulin sensitivity, which are linearly related to BMI and WHR, predict endothelial dysfunction in obese subjects. In fact, our data demonstrate that fasting insulin predicts 51.8 and 50.9% of the peak increase in FBF induced by ACh infusion in men and women, respectively. In addition, subsequent multiple regression analysis confirmed these data, as reported in Table 4. These findings are in agreement with the observation that the physiological vasodilating action of insulin is present in insulin-sensitive but not in insulin-resistant patients (30,31). In the same way, recent reports have shown that endothelial NO may mediate insulin-stimulated vasodilation in skeletal muscle (17,18), suggesting a direct physiological link between endothelial function and insulin sensitivity. Therefore, subjects who are relatively insulin-resistant appear to have a corresponding decrease in NO production. With respect to hypertension (14,24,26), hypercholesterolemia (11,13), diabetes (15), and aging (16)—well-known conditions associated with endothelial dysfunction—it is necessary to point out that all our subjects were normotensive, normoglycemic, and had cholesterol levels that did not exceed 200 mg/dl. Finally, all our subjects were relatively young, and we were unable to find a significant relationship between age and the peak increase in FBF ($r = 0.016$, $P = 0.892$). On the basis of this information, it is possible to affirm that indexes of insulin sensitivity, which are linearly related to indexes of obesity, largely explain the endothelial dysfunction in obese subjects.

However, our data do not completely clarify the association between endothelial dysfunction and both obesity and IR. In fact, the depressed ACh-stimulated vasodilation demonstrated in this study can be explained only in part by oxidative stress because the vitamin C coinfusion improved but did not completely normalize the endothelial response to ACh. Therefore, additional factors may be involved in the development

TABLE 2
Correlational analyses between the peak increase in FBF during ACh infusions and different parameters

	All			Men			Women		
	r	95% CI	*P*	*r*	95% CI	*P*	*r*	95% CI	*P*
BMI	–0.676	–18.60 to –11.61	0.0001	–0.695	–23.28 to –12.54	0.0001	–0.761	–18.26 to –8.62	0.0001
Waist	–0.581	–7.475 to –3.813	0.0001	–0.582	–7.662 to –3.244	0.0001	–0.670	–9.831 to –3.573	0.0001
WHR	–0.631	–1,347.6 to –749.05	0.0001	–0.696	–1,807.0 to –970.14	0.0001	–0.538	–1,554.5 to –319.20	0.004
Fasting insulin	–0.695	–8.761 to –5.382	0.0001	–0.720	–8.653 to –4.877	0.0001	–0.714	–11.417 to –4.744	0.0001
HOMA-IR	–0.633	–23.47 to –12.70	0.0001	–0.664	–27.46 to –12.93	0.0001	–0.624	–25.21 to –9.67	0.0001
Cholesterol	–0.072	–3.028 to 1.589	0.536	–0.180	–5.084 to 1.149	0.210	0.114	–2.606 to 4.557	0.579
Systolic BP	–0.153	–9.10 to 1.81	0.188	–0.226	–11.241 to 1.255	0.115	–0.073	–13.33 to 9.392	0.723
Diastolic BP	–0.142	–9.41 to 2.21	0.221	–0.200	–12.381 to 2.164	0.164	0.015	–9.92 to 10.672	0.941
Age	0.016	–5.96 to 6.83	0.892	0.0046	–7.716 to 7.969	0.974	0.78	–9.44 to 13.729	0.706

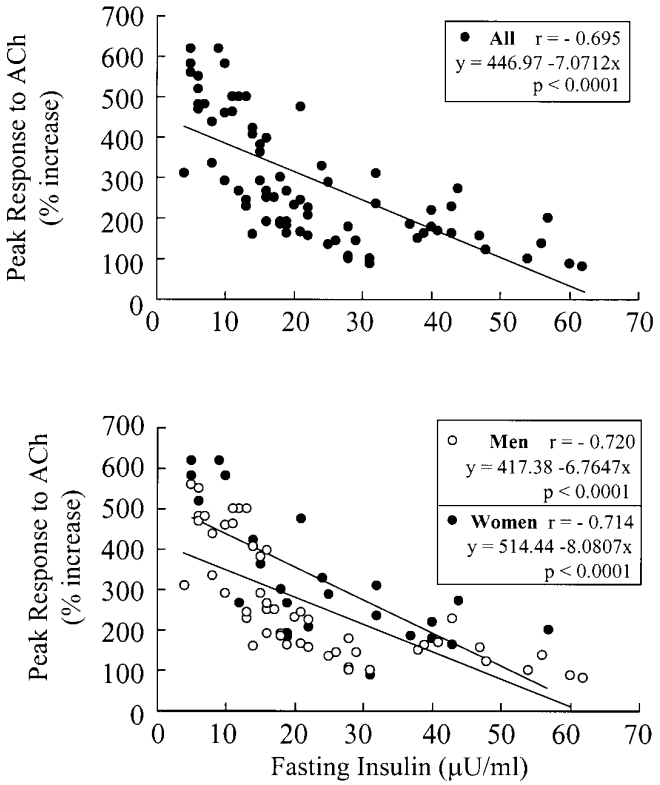

FIG. 5. Relationship between the maximum FBF responses to ACh infusion and fasting insulin, documented in all subjects and subdivided by sex.

of endothelial dysfunction in obese subjects. It is important to emphasize that, whereas insulin at low physiological levels increases endothelium-dependent vasodilation in normal subjects, much higher insulin levels, such as those detected in insulin-resistant obese and diabetic patients, fail to enhance endothelium-dependent vasodilation (19). This vasoactive action of insulin may be explained by its ability to act at the level of endothelial cells that modulate the production and release of NO. Nevertheless, this study fails to demonstrate the mechanisms by which the vasodilating effect of insulin is blunted in obese and diabetic patients, which suggests that the endothelium is probably resistant to insulin's modulating effect on NO production and release. On the other hand, there is evidence that sympathetic activity is increased in insulin-resistant states as a consequence of decreased activity of the inhibitory brain-stem center (32). The increase in vasoconstrictor substances could impair endothelium-dependent vasodilation and, thus, could reduce the FBF increase response to ACh. In the same way, Cardillo et al. (33) recently reported that in skeletal muscle, insulin stimulates both endothelin and NO activities. Therefore, on the basis of this evidence, it is possible to hypothesize that an imbalance between the release of ET-1 and NO in response to insulin may be involved in the endothelial dysfunction of obesity and other insulin-resistant states.

In conclusion, we have demonstrated that *1*) obesity/IR, independently of other risk factors, is associated with reduced ACh-stimulated vasodilation and *2*) the endothelial dysfunction is due, at least in part, to increased oxidative stress, which can be improved by the administration of intrabrachial vitamin C or indomethacin. Considering the key role of normal endothelium in antiproliferative and antiatherosclerotic processes, we suggest that endothelial dysfunction in human obesity may confer an increased risk of macrovascular diseases in obese insulin-resistant subjects. Finally, it is important to emphasize that this risk is higher in men than in women and that both fasting insulin and WHR, which were analyzed in multivariate stepwise logistic regression, appear as independent predictors of endothelial dysfunction. Thus, our analysis demonstrates that insulin sensitivity and central fat distribution are two interrelated predictors of endothelial dysfunction.

Clinical implications. The increased cardiovascular morbidity and mortality observed in human obesity may be caused by a quick progression of the atherosclerotic process in the presence of some risk factors for atherosclerosis. In this way, the endothelium has an important influence on vascular physiology and appears to be central in mediating damage to the vessel wall when patients are exposed to conventional risk factors for atherosclerosis. Thus, it is reasonable to hypothesize that caloric restrictions (by diet and/or pharmacological treatment) and metabolic and cardiovascular adaptations to weight loss might be useful in improving endothelial dysfunction and reducing cardiovascular risk. Nevertheless, further studies are required to determine the mechanistic explanation for the observed relationship between obesity/IR and depressed endothelium-dependent vasodilation.

Finally, we remark that we used intra-arterial vitamin C infusion to evaluate only the endothelial function because at

TABLE 3
Relationship between indexes of obesity/body fat distribution or insulin sensitivity and Δ changes on FBF response to ACh during coinfusion of vitamin C and indomethacin

	BMI			WHR			HOMA-IR			Fasting insulin		
	All	Men	Women	All	Men	Women	All	Men	Women	All	Men	Women
Vitamin C												
Pearson's r	0.568	0.561	0.699	0.596	0.673	0.549	0.467	0.458	0.534	0.656	0.697	0.590
R^2	0.323	0.315	0.489	0.355	0.453	0.301	0.218	0.210	0.285	0.430	0.485	0.348
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.003	0.0001	0.0008	0.004	0.0001	0.0001	0.001
Indomethacin												
Pearson's r	0.506	0.517	0.645	0.615	0.604	0.573	0.423	0.411	0.556	0.594	0.632	0.600
R^2	0.251	0.268	0.419	0.378	0.365	0.328	0.179	0.169	0.309	0.352	0.399	0.360
P	0.0001	0.0001	0.003	0.0001	0.0001	0.002	0.0001	0.003	0.003	0.0001	0.0001	0.001

TABLE 4
Independent predictors of peak increase in FBF during ACh infusions

Variable	Partial R^2	Total R^2	P
All			
Insulin	23.45	23.45	0.0001
WHR	16.59	40.04	0.0001
BMI	8.04	48.08	0.0003
Men			
Insulin	26.97	26.97	0.0001
WHR	13.28	40.25	0.0003
BMI	7.36	47.61	0.006
Women			
BMI	36.49	36.49	0.0001
WHR	6.29	42.78	0.082
Insulin	4.22	47.00	0.152

present there is no firm evidence that ascorbic acid supplementation decreases cardiovascular mortality.

ACKNOWLEDGMENTS

This work is dedicated to the memory of Prof. Mattioli, whose scientific and human support will always be remembered.

REFERENCES

1. Hubert HB, Feinleib M, McNamara PM, Castelli WP: Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation* 67:968–977, 1983
2. Manson JE, Colditz GA, Stampfer MJ, Willett WC, Rosner B, Monson RR, Speizer FE, Hennekens CH: A prospective study of obesity and risk of coronary heart disease in women. *N Engl J Med* 322:882–889, 1990
3. Van Itallie TB: Health implications of overweight and obesity in the United States. *Ann Intern Med* 103:983–988, 1985
4. Ducimetiere P, Eschwege E, Papoz L, Richard JL, Claude JR, Rosselin G: Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in a middle-aged population. *Diabetologia* 19:205–210, 1980
5. Vane JR, Anggard EE, Botting RM: Regulatory functions of the vascular endothelium (Review). *N Engl J Med* 323:27–36, 1990
6. Furchgott RF, Zawadzki JV: The obligatory role of the endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373–376, 1980
7. Palmer RMJ, Ferridge AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327:524–526, 1987
8. Lüsher TF, Vanhoutte PM: *The Endothelium*: *Modulator of Cardiovascular Function*. Boca Raton, FL, CRC Press, 1990
9. Vanhoutte PM, Rubanyi GM, Miller VM, Houston DS: Modulation of vascular smooth muscle contraction by the endothelium (Review). *Annu Rev Physiol* 48:307–320, 1986
10. Smith RP, Kruszyna H: Nitroprusside produces cyanide poisoning via reaction with hemoglobin. *J Pharmacol Exp Ther* 191:557–563, 1974
11. Ting HH, Timini FK, Haley EA, Roddy MA, Ganz P, Creager MA: Vitamin C improves endothelium-dependent vasodilation in forearm resistance vessels of humans with hypercholesterolemia. *Circulation* 95:2617–2622, 1997
12. Heitzer T, Just H, Munzel T: Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. *Circulation* 94:6–9, 1996
13. Anderson TJ, Meredith IT, Yeung AC: The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. *N Engl J Med* 332:488–493, 1995
14. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A: Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation* 97:2222–2229, 1998
15. Timimi FK, Ting HH, Haley EA, Roddy MA, Ganz P, Creager MA: Vitamin C improves endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 31:552–557, 1998
16. Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Pasolo CB, Sudano I, Salvetti A: Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation* 91:1981–1987, 1995
17. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD: Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric oxide release. *J Clin Invest* 94:1172–1179, 1994
18. Petrie JR, Ueda S, Webb DJ, Elliott HL, Connell JMC: Endothelial nitric oxide production and insulin sensitivity: a physiological link with implications for pathogenesis of cardiovascular disease. *Circulation* 93:1331–1333, 1996
19. 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* 97:2601–2610, 1996
20. Crepaldi G, Belfiore F, Bosello O, Caviezel F, Contaldo F, Enzi G, Melchionda N: Italian Consensus Conference: overweight, obesity, and health (Review). *Int J Obes* 15:781–790, 1991
21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
22. Ferrannini E, Mari A: How to measure insulin sensitivity (Review). *J Hypertens* 16:895–906, 1998
23. Haffner SM, Miettinen H, Stern MP: The homeostasis model in the San Antonio Heart Study. *Diabetes Care* 20:1087–1092, 1997
24. Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 323:22–27, 1990
25. Perticone F, Ceravolo R, Maio R, Ventura G, Zingone A, Perrotti N, Mattioli PL: Angiotensin-converting enzyme gene polymorphism is associated with endothelium-dependent vasodilation in never treated hypertensive patients. *Hypertension* 31:900–905, 1998
26. Perticone F, Maio R, Ceravolo R, Cosco C, Cloro C, Mattioli PL: Relationship between left ventricular mass and endothelium-dependent vasodilation in never-treated hypertensive patients. *Circulation* 99:1991–1996, 1999
27. Whitney RJ: Measurement of changes in human limb volume by means of a mercury-in-rubber strain gauge. *J Physiol* 109:5P–6P, 1948
28. Jackson TS, Xu A, Vita JA, Keaney JF Jr: Ascorbate prevents the interaction of superoxide and nitric oxide only at very high physiological concentrations. *Circ Res* 83:916–922, 1998
29. Pryor WA, Squadrito GL: The chemistry of peroxynitrite: a product from the action of nitric oxide with superoxide (Review). *Am J Physiol* 268:L699–L722, 1995
30. Laakso M, Edelman SV, Brechtel G, Baron AD: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese men. *J Clin Invest* 85:1844–1852, 1990
31. Baron AD, Brechtel-Hook G, Johnson A, Hardin D: Skeletal muscle blood flow: a possible link between insulin resistance and blood pressure. *Hypertension* 21:129–135, 1993
32. Reaven GM, Lithell H, Landsberg L: Hypertension and associated metabolic abnormalities: the role of insulin resistance and the sympathoadrenal system (Review). *N Engl J Med* 334:374–381, 1996
33. Cardillo C, Nambi SS, Kilcoyne CM, Choucair WK, Katz A, Quon MJ, Panza JA: Insulin stimulates both endothelin and nitric oxide activity in the human forearm. *Circulation* 100:820–825, 1999