

# Weight Loss in Combination With Physical Activity Improves Endothelial Dysfunction in Human Obesity

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**OBJECTIVE** — To test whether weight loss may improve endothelial dysfunction in human obesity, we recruited 28 healthy obese subjects, aged 30–46 years, with BMI 30–43 kg/m<sup>2</sup>.

**RESEARCH DESIGN AND METHODS** — Endothelium-dependent and -independent vasodilation were investigated by intra-arterial infusion of increasing doses of acetylcholine (ACh; 7.5, 15, and 30  $\mu\text{g} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ ) and sodium nitroprusside (0.8, 1.6, and 3.2  $\mu\text{g} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ ). Insulin resistance was estimated by homeostasis model assessment (HOMA). Weight loss was obtained by caloric restriction and physical activity.

**RESULTS** — We observed a significant reduction in BMI (from  $33.1 \pm 4.2$  to  $27.5 \pm 4.5$  kg/m<sup>2</sup>,  $-16.9\%$ ,  $P < 0.0001$ ) and in waist circumference (from  $108.2 \pm 12.1$  to  $96.8 \pm 12.9$  cm,  $-10.5\%$ ,  $P < 0.0001$ ). Weight loss was also associated with a significant increase in ACh-stimulated forearm blood flow (FBF), from  $7.4 \pm 2.8$  to  $12.9 \pm 3.4$  ml  $\cdot$  100 ml<sup>-1</sup> of tissue  $\cdot$  min<sup>-1</sup> kg/m<sup>2</sup> ( $P < 0.0001$ ). Multivariate regression analysis demonstrated that the only independent predictor of FBF was HOMA, accounting for 44.5% of the variation, whereas the addition of BMI explained another 2.3% of the variation.

**CONCLUSIONS** — Our data demonstrate that energy-restricted diet associated with physical activity induce a significant and clinically relevant improvement in ACh-stimulated vasodilation in obese healthy subjects.

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We recently reported that obesity and abdominal fat distribution are inversely related to endothelium-dependent vasodilation. We have also demonstrated that indexes of insulin sensitivity, which are linearly related to BMI and waist-to-hip ratio (WHR), predict the depressed acetylcholine (ACh)-stimulated forearm blood flow (FBF) in obese subjects (1). These findings are of

considerable clinical importance because endothelial dysfunction is considered the early manifestation of the atherosclerotic process (2–4). Impaired endothelium-dependent vasodilation, caused by insulin resistance (IR), may be the mechanism by which obesity confers increased risk for cardiovascular morbidity and mortality. In fact, IR represents a major underlying abnormality driving coronary and

extracoronary atherosclerosis and cardiovascular diseases, which are the principal worldwide causes of morbidity and mortality (5). Our previous observations (1) led us to hypothesize that weight loss might be useful in both improving endothelial dysfunction and reducing the risk of subsequent cardiovascular events. This hypothesis is also supported by recent evidence showing that both coronary (6,7) and forearm (8) endothelial dysfunction predict long-term atherosclerotic disease progression and cardiovascular event rates. Endothelial dysfunction associated with obesity is a very important medical problem in light of the evidence that the prevalence of obesity has significantly increased over the last few decades in developed and developing countries (9,10), becoming a major global public health problem (10). Many adverse clinical features associated with obesity are reversible with weight loss, but the effect of weight loss on ACh-stimulated vasodilation in human obesity is still unsettled.

In this study, we investigated the effect of weight loss on impaired endothelium-dependent vasodilation in obese subjects. In addition, we evaluated whether the increase in ACh-stimulated vasodilation is related to weight loss and reduction of IR.

## RESEARCH DESIGN AND METHODS

Of a total of 76 Caucasian healthy obese subjects previously reported (1), 39 accepted to participate in this study. Inclusion and exclusion criteria were previously described. In addition, subjects were considered eligible if they lost >10% of their initial body weight. 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. All subjects were clinically evalu-

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**Abbreviations:** ACh, acetylcholine; BP, blood pressure; FBF, forearm blood flow; HOMA, homeostasis model assessment; IR, insulin resistance; SNP, sodium nitroprusside; SNS, sympathetic nervous system; VR, vascular resistance; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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ated and were required to be able to understand and comply with diet and physical activity guidelines. Eligible participants were aged 30–46 years ( $42.6 \pm 7.5$  [mean  $\pm$  SD]) with a BMI between 30 and  $43 \text{ kg/m}^2$  ( $33.1 \pm 4.2$ ). To avoid underestimation of FBF measurements, the forearm circumference was required to be  $<28$  cm in all subjects. The institutional ethical committee approved the study, and all participants gave written informed consent.

### **Anthropometric measurements**

The same trained examiner (F.S.) collected anthropometric measurements at baseline and after weight loss. We used waist circumference as the best anthropometric correlate of the distribution of visceral adipose tissue because it provides a crude index of the absolute amount of abdominal fat and avoids misleading information provided by changes in only WHR (11). The waist was measured at its smallest point with the abdomen relaxed.

### **Dietary intervention**

The weight loss was obtained by an individual dietary program to reduce energy intake by 600–800 kcal, based on a macronutrient content  $<30\%$  fat and 15% protein as recommended by the World Health Organization (12). The aim of this dietary intervention was to achieve at least a 10% weight loss in 10–16 weeks. At the initial interview with a dietitian, obese subjects were given verbal and written instructions on how to keep diet records, with food weighed and measured. Dietary intake was monitored by the same dietitian (F.S.). Obese subjects were instructed to substitute low-fat alternatives for typical high-fat foods, to increase the consumption of vegetables and fresh fruits, and to substitute complex carbohydrates, such as whole-grain bread and cereals, according to the Mediterranean diet. The prescribed energy intake ranged between 1,200 and 1,700 kcal/day. Finally, an extra 30 min walking per day, for at least 3 days weekly, was recommended, with advice on behavioral modification. Dietetic help was given every 2 weeks by the dietitian when anthropometric measurements were performed; in addition, each subject was seen by a physician monthly to perform a clinical evaluation, standard electrocardiogram, and measurement of blood pressure (BP) and heart rate.

### **IR**

IR was estimated using the previously validated homeostasis model assessment (HOMA) (13). HOMA was calculated from the fasting glucose and insulin concentrations according to the following equation:  $\text{HOMA} = [\text{insulin } (\mu\text{U/ml}) \times \text{glucose } (\text{mmol/l})] / 22.5$ .

### **Measurements of FBF**

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 ( $22\text{--}24^\circ\text{C}$ ). Patients underwent an evaluation of vascular function before and after weight loss during a weight-stable period to avoid a possible interference between the hypocaloric diet and insulin and glucose levels. We used the protocol previously described by Panza et al. (14) and subsequently used by ourselves (1).

Briefly, the FBF was measured as the slope of the change in the forearm volume. 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 BP by FBF.

### **Vascular function**

#### **Endothelium-dependent and -independent vasodilation**

All subjects rested at least 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 -independent vasodilation was assessed by the dose-response curve to intra-arterial infusions at increasing doses of ACh ( $7.5$ ,  $15$ , and  $30 \mu\text{g} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ , each for 5 min) and sodium nitroprusside (SNP;  $0.8$ ,  $1.6$ , and  $3.2 \mu\text{g} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ , each for 5 min), respectively. The drug infusion rate, adjusted for the forearm volume of each subject, was 1 ml/min.

#### **Oxidative stress and vascular function**

To evaluate the effect of oxygen free radicals on endothelium-dependent and -independent vasodilation, both ACh and SNP were infused with either saline solution or vitamin C ( $24 \text{ mg/min}$ ), administered intrabrachially 5 min before the agonists, and continued throughout the study. This vitamin C concentration has been shown to both protect human

plasma from free radical-mediated lipid peroxidation (15) and improve impaired ACh-stimulated vasodilation in patients with various cardiovascular risk factors (16–18).

We have also 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  $500 \mu\text{g/min}$  starting 10 min before ACh administration and continued throughout the infusion.

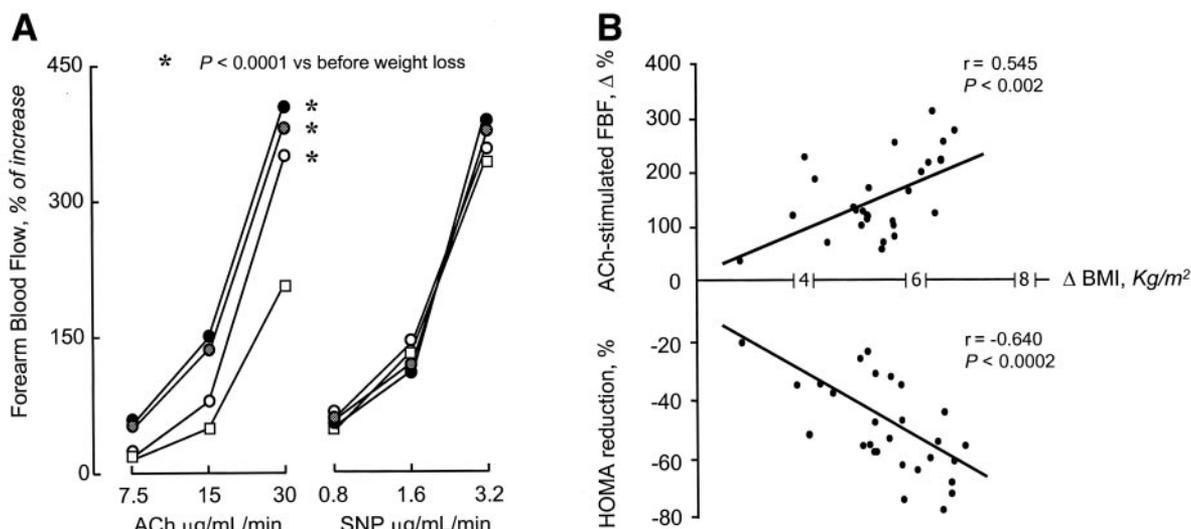
### **Statistical analysis**

ANOVA was performed for clinical and biological data, and the differences between means were compared using paired Student's *t* test. The responses to ACh and SNP were compared by ANOVA for repeated measurements and, when the analysis was significant, Tukey's test was applied. Simple linear regression analysis was performed to assess the relationship between the peak percent increase in FBF in response to ACh infusion and variables such as indexes of obesity (BMI and waist circumference), fasting insulin, HOMA, and other factors reported to impair endothelium-dependent vasodilation (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 vasoactive substances. In this analysis, we included only the HOMA values because fasting insulin levels may be affected by an impairment of the secretory function. Thus, we considered HOMA 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  $\pm$  SD.

## **RESULTS**

### **Study population**

Of the 39 obese subjects entering the dietary program (14–16 weeks), 28 were eligible for the subsequent evaluation because they lost  $>10\%$  of their initial body weight. The other 11 subjects were excluded because they stopped the dietary program. The energy-restricted diet resulted in a mean weight reduction of



**Figure 1**—A: Dose-response curves of ACh- and SNP-stimulated FBF before weight loss and during coinfusion of both vitamin C and indomethacin after weight loss. □, Before weight loss; ○, after weight loss; ⊕, weight loss + vitamin C; ●, weight loss + indomethacin. B: Top: the direct significant relationship between BMI reduction ( $\Delta$  BMI) and percent increase in FBF. Bottom: the inverse significant relationship between  $\Delta$  BMI and IR index (HOMA).

12.5  $\pm$  3.5 kg (from 89.3  $\pm$  16.5 to 76.8  $\pm$  15.4,  $P < 0.0001$ ). All subjects increased their physical activity, from 27  $\pm$  15 to 108  $\pm$  32 min weekly ( $P < 0.0001$ ). Clinical, anthropometric, and biochemical characteristics at baseline and after weight loss are summarized in Table 1.

**Anthropometric and metabolic changes**

The dietary program induced a significant reduction in BMI (from 33.1  $\pm$  4.2 to 27.5  $\pm$  4.5  $\text{kg/m}^2$ , -16.9%,  $P < 0.0001$ ) and waist circumference (from 108.2  $\pm$  12.1 to 96.8  $\pm$  12.9 cm, -10.5%,  $P < 0.001$ ). In contrast, forearm circumference was comparable before and after weight loss (27.6  $\pm$  1.1 vs. 27.1  $\pm$  1.2 cm,  $P = 0.11$ )

Compared with baseline values, a slight but significant decrease in systolic (135  $\pm$  5 vs. 130  $\pm$  7 mmHg,  $P < 0.005$ ) and diastolic (85  $\pm$  4 vs. 82  $\pm$  5 mmHg,  $P < 0.05$ ) BP was observed upon weight loss. Weight reduction did not induce significant changes in serum cholesterol (4.7  $\pm$  0.3 vs. 4.5  $\pm$  0.4 mmol/l,  $P = 0.06$ ), triglycerides (1.5  $\pm$  0.3 vs. 1.4  $\pm$  0.3 mmol/l,  $P = 0.32$ ), and fasting glucose (5.8  $\pm$  0.7 vs. 5.7  $\pm$  0.8 mmol/l,  $P = 0.62$ ). A significant decrease in fasting insulin (27.6  $\pm$  14.7 vs. 15.4  $\pm$  12.4  $\mu\text{U/ml}$ ,  $P < 0.001$ ) levels and HOMA (7.5  $\pm$  4.6 vs. 4.1  $\pm$  3.9,  $P < 0.005$ ) were observed after weight reduction.

**Endothelium-dependent and -independent vasodilation**

All evaluations were obtained after at least 2 weeks of stable BMI. Basal FBF did not differ at baseline and after weight loss: 3.5  $\pm$  0.6 and 3.6  $\pm$  0.6  $\text{ml} \cdot 100 \text{ ml}^{-1} \text{ tissue} \cdot \text{min}^{-1}$ , respectively. Weight loss significantly ( $P < 0.0001$ ) improved maximal vasodilator response to the highest dose of ACh, increasing from 7.4  $\pm$  2.8  $\text{ml} \cdot 100 \text{ ml}^{-1} \text{ tissue} \cdot \text{min}^{-1}$  (+211%) to 12.9  $\pm$  3.4  $\text{ml} \cdot 100 \text{ ml}^{-1} \text{ tissue} \cdot \text{min}^{-1}$  (+358%). In contrast, FBF during SNP infusions did not differ at baseline and upon weight loss: 11.9  $\pm$  4.3  $\text{ml} \cdot 100 \text{ ml}^{-1} \text{ tissue} \cdot \text{min}^{-1}$  (+340%) vs. 12.6  $\pm$  4.1  $\text{ml} \cdot 100 \text{ ml}^{-1} \text{ tissue} \cdot \text{min}^{-1}$  (+350%) (Fig. 1A).

Basal VR did not differ at baseline and after weight loss: 28.8  $\pm$  6.3 and 27.2  $\pm$  5.9 units, respectively. Similarly, after weight loss, VR significantly decreased in a dose-dependent manner in all subjects during ACh infusion (7.6  $\pm$  3.2 units,  $P < 0.0001$ ) as well as SNP administration (7.7  $\pm$  3.4 units,  $P < 0.0001$ ) (data not shown). ACh and SNP infusions did not affect BP or heart rate

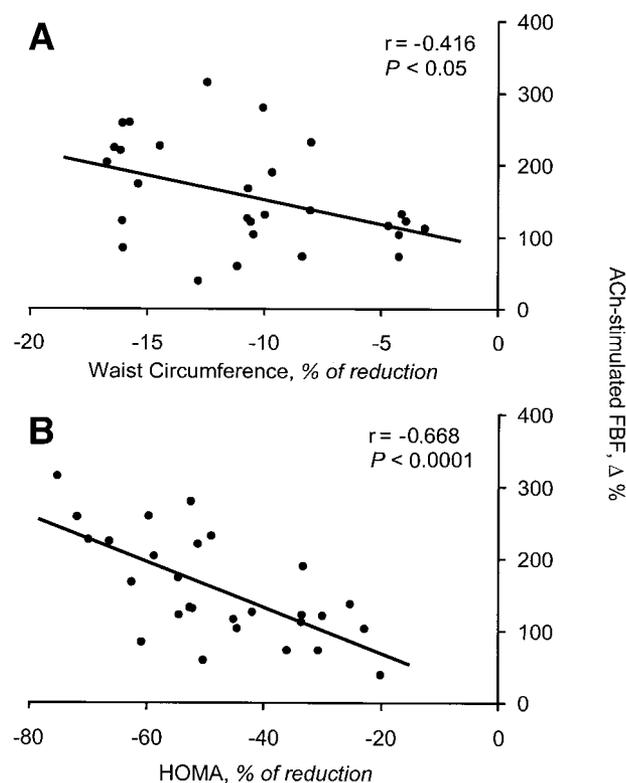
**Oxidative stress and FBF**

Before and after weight loss, the intrabradial coadministration of both vitamin C and indomethacin significantly increased the maximal ACh-stimulated vasodilation of obese subjects. Notably, percentage increments after weight loss were signifi-

**Table 1**—Subject characteristics before and after weight loss

	Before	After	P
Body weight (kg)	89.3 $\pm$ 16.5	76.8 $\pm$ 15.4	<0.0001
BMI ( $\text{kg/m}^2$ )	33.1 $\pm$ 4.2	27.5 $\pm$ 4.5	<0.0001
Waist circumference (cm)	108.2 $\pm$ 12.1	96.8 $\pm$ 12.9	<0.001
Systolic BP (mmHg)	135 $\pm$ 5	130 $\pm$ 7	<0.005
Diastolic BP (mmHg)	85 $\pm$ 4	82 $\pm$ 5	<0.05
Fasting glucose (mmol/l)	5.8 $\pm$ 0.7	5.7 $\pm$ 0.8	0.62
Cholesterol (mmol/l)	4.7 $\pm$ 0.3	4.5 $\pm$ 0.4	0.06
Triglyceride (mmol/l)	1.5 $\pm$ 0.3	1.4 $\pm$ 0.3	0.32
FBF ( $\text{ml} \cdot 100 \text{ ml}^{-1} \text{ tissue} \cdot \text{min}^{-1}$ )	3.5 $\pm$ 0.6	3.6 $\pm$ 0.6	0.54
VR (units)	28.8 $\pm$ 6.3	27.2 $\pm$ 5.9	0.33

Data are means  $\pm$  SD.



**Figure 2**—Relationship between percent waist circumference reduction and peak percent increase in FBF (A) and between percent IR reduction (HOMA) and peak percent increase in FBF (B).

cantly lower than those observed before weight loss. No significant differences were observed during coinfusion of SNP and vitamin C or indomethacin.

The Fig. 1A shows the dose-response curves of ACh- and SNP-stimulated FBF before weight loss and during coinfusion of vitamin C and indomethacin after weight loss.

### Correlational analyses

As shown in Fig. 1B, reduction in BMI was directly correlated with the peak percent increase in ACh-stimulated FBF ( $r = 0.545$ ,  $P < 0.002$ ) and inversely correlated with the percent reduction in HOMA ( $r = -0.640$ ,  $P < 0.0002$ ), accounting for 29.7 and 41.3% of the variation, respectively. Similarly, an inverse significant relationship was found between the reduction in BMI and the percent decrease in fasting insulin ( $r = -0.624$ ,  $P < 0.0003$ ), accounting for 39.1% of the variation.

We also tested the effect of fat distribution, as an index for central obesity, on endothelium-dependent vasodilation. Reduction in waist circumference was inversely related with the peak percent increase in FBF ( $r = -0.416$ ,  $P < 0.05$ ), accounting for 17.4% of the variation (Fig. 2A). When we investigated the effect

of insulin sensitivity on the peak increase in FBF after ACh infusion, we found an inverse significant relationship between the percent reduction in HOMA ( $r = -0.668$ ,  $P < 0.0001$ ), accounting for 44.6% of the variation (Fig. 2B). Taking into account that both BMI and fat distribution affect insulin sensitivity, these data suggest that IR is the underlying pathogenetic mechanism exerting the negative effects of BMI and central obesity on endothelium-dependent vasodilation. This was further supported by a stepwise multivariate regression analysis showing that the only significant independent predictor of the peak increase in FBF was HOMA, accounting for 44.5% of the variation, whereas the addition of BMI explained another 2.3% of the variation ( $P = 0.311$ ). Significant independent variables were identified by a stepwise approach; by this method, we constructed a statistical model with adequate statistical power (at least 14 patients for each variable in the final model).

**CONCLUSIONS**— Our data demonstrate that an energy-restricted diet in obese healthy subjects resulted in a significant and clinically relevant improvement in ACh-stimulated vasodilation. To our knowledge, this is the first study to pro-

spectively evaluate the effects of weight loss and physical activity on endothelium-dependent vasodilation of obese normotensive subjects. This dietary program and physical activity also induced a significant reduction of both fasting insulin and IR, expressed as the HOMA index. In addition, weight loss was associated with an important and significant reduction in oxidative stress, as evaluated by intra-arterial infusion of vitamin C, a potent antioxidant substance, and indomethacin, a well-established inhibitor of oxygen free radical production. These conclusions are based on indirect evidence reported in other human studies (15–18) because we did not directly measure free radicals. Interestingly, results obtained from multivariate regression analysis demonstrated that the only significant independent predictor of ACh-stimulated FBF resulted from the degree of IR, measured as the HOMA index, accounting for 44.6% of the variation. Thus, these data seem to suggest that weight loss may affect endothelium-dependent vasodilation not directly but by improving insulin sensitivity and by increasing the bioavailability of nitric oxide (NO). Increase of NO synthesis and/or its reduced inactivation by oxygen free radicals may account for the improvement of endothelium-dependent vasodilation after weight loss in obese subjects. In addition, we cannot exclude the possibility that physical activity also contributed to the improvement in ACh-stimulated FBF. In fact, the reduction of IR (19) and enhanced endothelial NOS gene expression (20) are the potential mechanisms by which regular aerobic exercise may increase endothelium-dependent vasodilation. The same mechanisms might be involved in the coronary and extracoronary endothelium-dependent vasodilation improvement reported in patients with chronic heart failure (21) and coronary artery disease (22).

Similar results were recently reported by Sasaki et al. (23) in a group of obese hypertensive patients. Even when the clinical characteristics of patients and the study protocol were different, weight loss induced a significant improvement in ACh-stimulated vasodilation. However, it is necessary to remark that in the study by Sasaki et al., the increase in endothelium-dependent vasodilation cannot only be attributed to weight loss because the low-calorie diet reduced BP (as was also found

in our study), cholesterol, and triglycerides, factors that all affect ACh-stimulated FBF.

Human obesity, and visceral adiposity in particular, is a clinical condition characterized by an increased risk for the development of coronary atherosclerosis and cardiovascular death (24,25). Notably, cardiovascular morbidity and mortality increase with body weight and visceral adiposity, irrespective of the presence of other risk factors associated, such as hypertension, type 2 diabetes, dyslipidemia, and smoking (9,10,12). This condition is clinically relevant and may be explained by the coexistence, in obese people, of IR/hyperinsulinemia, endothelial dysfunction, and activation of the sympathetic nervous system (SNS) (1,26,27), factors that all contribute to the development and progression of coronary atherosclerosis. In fact, substantial data show that depressed endothelium-dependent vasodilation may be considered an early event in atherogenesis that precedes the thickening of the vascular wall and the appearance of atherosclerotic plaques. A dysfunctioning endothelium increases vascular tone as well as platelet and monocyte adhesion, and it stimulates cell growth. Thus, it may be the earliest functional modification that promotes the vascular atherosclerotic process by stimulating the proliferation of vascular smooth muscle cells and fibroblasts. Moreover, the endothelium also has been shown to modulate several other important processes in the development of coronary atherosclerosis, including inflammation and thrombosis. In this regard, there is much data supporting the role of inflammation, as documented by an increase of proinflammatory cytokines such as interleukin-6, in the pathogenesis of cardiovascular diseases in general and in the atherogenetic process in particular (2,3). Consistent with this, recent data support the evidence that experimental inflammation impairs endothelium-dependent vasodilation in humans (28). In keeping with this, the recently demonstrated prognostic value of both coronary (6,7) and forearm (8) endothelial dysfunction in patients with coronary artery disease or essential hypertension appears clinically relevant. This point has a noteworthy clinical importance because Modena et al. (29) demonstrated that therapy-related endothelial improvement reduces

cardiovascular outcomes in hypertensive postmenopausal women.

Finally, the SNS overactivity associated with obesity contributes to obesity-related hypertension, IR/hyperinsulinemia, and lipid changes, a cluster of abnormalities that increases the cardiovascular risk in obese subjects (27).

### Conclusions and clinical implications

The relationship of obesity to IR and atherogenesis provided mechanistic insights into the understanding of this multifaceted syndrome that significantly increases the risk for cardiovascular morbidity and mortality. In this context, the vascular endothelium plays a key role because, first, it acts by producing several substances that are the biological markers of its activation, and, second, it represents the target on which inflammatory and growth factors interact, modifying the morphologic structure of the vascular wall. Therefore, findings provided by our study clearly demonstrate that weight loss attributed to both caloric restriction and physical activity may improve endothelium-dependent vasodilation by reducing oxidative stress and IR. These results are clinically relevant because they demonstrate that obesity-related endothelial dysfunction may be usefully reduced by lifestyle modifications.

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