Endothelial Function and Weight Loss: Comparison of Low-Carbohydrate and Low-Fat Diets

Emile R. Mohler III1, Alexandra A. Sibley1, Richard Stein2, Victor Davila-Roman2, Holly Wyatt3, Karen Badellino4, Daniel J. Rader1, Samuel Klein2 and Gary D. Foster3

Objective: The effect of weight loss on obesity-associated endothelial dysfunction is not clear because of conflicting data, demonstrating both improvement and no change in endothelial function after weight loss in obese subjects. A 2-year prospective study (n = 121) was conducted to examine: (1) the effect of obesity and weight loss (either a low-carbohydrate or and low-fat diet) on flow mediated vasodilatation (FMD), a measure of endothelial function.

Design and Methods: Participants reduced body weight by 7.1% ± 4.4%, 8.7% ± 6.8%, 7.1% ± 7.8%, and 4.1% ± 7.7% at 3, 6, 12, and 24 months, respectively with no significant differences between the low-fat and low-carbohydrate groups.

Results: Endothelial function was inversely correlated with waist circumference, triglyceride level, and directly correlated with leptin in obese persons prior to weight loss. These weight losses did not confer any improvements in FMD. There were no differences between the low-fat and low-carbohydrate diets in FMD at any time point. At 6 months (r = 0.26, P = 0.04) and 1 year (r =0.28, P = 0.03), there were positive correlations between change in FMD and change in leptin but not at 2 years.

Conclusion: There was no significant improvement in endothelial function after 7.1% ± 7.8% weight loss at 1 year and 4.1% ± 7.7% at 2 years, achieved by either a low carbohydrate or a low fat diet.

Introduction

Obesity is a serious, widespread problem in the United States and around the world. People who are overweight or obese are at risk for a variety of health complications, including coronary artery disease (CAD) and other cardiovascular conditions (1). One central factor whereby obesity per se is thought to initiate atherosclerosis is via endothelial dysfunction (2). Endothelial function is a marker of overall cardiovascular health and a predictor of future cardiovascular events (3). Methods have been developed to measure endothelial function both invasively—through the measurement of forearm blood flow (FFB) after the infusion of acetylcholine—and noninvasively. One commonly used non-invasive measure is flow-mediated dilatation (FMD), in which the amount of arterial dilatation caused by the hyperemic response to arterial occlusion is compared to arterial diameter at rest (4). Data from many studies examining endothelial dependent dilation in “healthy” obese patients (5-9), and patients with comorbid conditions (10-13), have found FMD is decreased compared with normal weight adults. FMD values in these studies ranged between ~4 and 10% (14), compared with typical values found in healthy nonobese adults 12-20% (15).

The effect of weight loss on obesity-associated endothelial dysfunction is not clear because of conflicting data from different studies, demonstrating both improvement (12,16-19) and no change (7,9,11,20-22) in endothelial function after weight loss in obese subjects. Additionally, several studies looking specifically at the effects of weight loss from high-carbohydrate vs. low-carbohydrate diets on endothelial function have produced differing results. Keogh et al. (9) found no improvement in FMD after a 52 week weight loss on either high-carbohydrate or low-carbohydrate diets, while Phillips et al. (23) reported decreased FMD after 6 weeks in subjects following a low-carbohydrate diet and increased FMD in subjects on a low-fat diet. Overall, studies evaluating the effect of weight loss on
vascular function were small (11 to 67 subjects) and short duration (2 weeks to 6 months) (2).

Considering the great variability in the literature on obesity, weight loss, and endothelial function, we conducted a large \( n = 121 \) 2-year study to examine: (1) the relationship between obesity and FMD in obese patients seeking weight loss treatment and (2) the effects of weight loss via either a low-carbohydrate or and low-fat diet on endothelial function as measured by FMD.

Methods

Study population

The study population was enrolled in a National Institute of Health sponsored 2-year, multicenter randomized trial of low-carbohydrate and low-fat diets on body weight. The study was approved by the Institutional Review Board of each respective institution. The subjects were randomized to either a low-carbohydrate diet or a low-fat diet as described in detail elsewhere (24). Approximately half of the participants were assigned to a low-carbohydrate diet, which limited carbohydrate intake but allowed unrestricted consumption of fat and protein. The other participants were assigned to consume a low-fat diet, which consisted of limiting energy intake to 1,200 to 1,500 kcal day\(^{-1}\) for women and 1,500 to 1,800 kcal day\(^{-1}\) for men, with \( \pm 55\% \) of calories from carbohydrate, 30% from fat, and 15% from protein. Both groups included group behavioral treatment and a prescribed physical activity plan of 200 min per week (24). The prescribed activity regimen was the same in both groups starting with 40 min per week (four bouts of 10 min) at week 3 and progressing to 200 min per week (four bouts of 50 min) by week 18. The primary form of physical activity was moderate to vigorous walking. Subjects with diabetes and heart disease were excluded from the study. This was a substudy of the larger study and involved two of the three sites (University of Pennsylvania and the Washington University). All subjects at these two institutions \( n = 201 \) were approached to see if they would undergo evaluation of endothelial function at baseline and at 3, 6, 12, and 24 months. A total of 133 subjects were assessed at baseline. Subjects on vasoactive medications \( n = 12 \) were excluded from all analyses. Of the remaining 121 subjects, follow-up assessments were conducted on 98 (81%) subjects at 3 months, 93 (77%) at 6 months, 80 (66%) at 1 year, and 59 (49%) at 2 years. There was a total of nine subjects with unreadable images (2 at 3 months, 4 at 6 months, and 3 at 1 year). These data were not analyzed.

Brachial artery reactivity

The evaluation of endothelial function was assessed via the brachial artery reactivity test using a previously published method (24). Sonographers were certified on this technique prior to patient enrollment. The brachial artery diameter was measured offline on B-mode ultrasound images.

Image analysis

Analyses of the images were completed using automated, edge-detection software (Brachial Analyzer for Research v 5.0.5, MIA Vascular Research Tools 5, Coralville, IA). Images were analyzed by an investigator proficient with the Brachial Analyzer software and blinded to patient information and study status.

Laboratory assays

The lipid profile was measured according to previously published method (24). Subjects were assessed at the respective clinical research centers after a 12-h overnight fast. Whole blood was drawn into EDTA-containing tubes. After the blood was centrifuged, the plasma was removed and stored at \(-80^\circ\)C until use. Plasma total cholesterol, HDL cholesterol, and triglyceride concentrations were measured enzymatically on a Cobas Fara II (Roche Diagnostic Systems, NJ) using Sigma reagents (Sigma Chemical, MO) in a CDC-standardized lipid laboratory. LDL cholesterol was calculated using the Friedewald formula.

Leptin was measured in a subset of individuals \( n = 66 \) with a complete set of samples at each time point. Plasma concentrations of leptin were determined using the human leptin RIA kit (HL-81K, Linco Research, St. Charles, MO) according to manufacturer’s instruction. Briefly, assay buffer, 100 μl standard, quality control or human sample (in duplicate), 100 μl \(^{125}\)I-labeled human leptin, and 100 μl human leptin antibody were combined in borosilicate tubes, vortexed and incubated 24 h at 4°C. Cold precipitating buffer was then added to each tube, incubated 20 min, then centrifuged at 2,500g for 20 min at 4°C. The supernatant was decanted and the pellets counted in a gamma counter for one minute. The concentration of leptin in each sample was determined by interpolation of a reference curve constructed from the known standards. The duplicate samples had CVs \(<10\%\) and the quality controls were within the expected range.

Statistical analysis

Differences in participant characteristics at baseline between the low carbohydrate and low fat groups were tested using Wilcoxon rank sum tests for continuous variables and chi square tests for categorical variables. A linear mixed-effects model with a treatment-by-time interaction term was used to evaluate between-group differences in endothelial function at a specific time point, as well as within-group differences between specific time points. Subject-specific random effects were used to account for the correlation due to repeated measurements. The model included research site to adjust for differences across sites. Because mixed-effects models does not depend on observed outcomes and treatment, no effort was made to impute the missing data (26). The two treatment groups were collapsed and bivariate correlations were used to assess the relationship between the endothelial function and variables measured at baseline and over time after controlling for treatment assignment. All statistical analyses were conducted using SAS version 9.1.3 (SAS Institute, Cary, NC).
TABLE 1 Participant characteristics at baseline

| Variable                  | Total participants | Low fat | Low carb | P value |
|---------------------------|--------------------|---------|----------|---------|
| N                         | 121                | 62      | 59       |         |
| Age (years)               | 45.7 ± 9.7         | 46.0 ± 10.6 | 45.4 ± 8.7 | 0.54    |
| Female gender, %          | 65                 | 63      | 66       | 0.71    |
| Race/ethnicity, %         |                    |         |          | 0.63    |
| African American          | 26.5               | 22.6    | 30.5     |         |
| Hispanic                  | 3.3                | 4.8     | 1.7      |         |
| Other                     | 1.7                | 1.6     | 1.7      |         |
| White                     | 68.6               | 71.0    | 66.1     |         |
| Systolic blood pressure (mmHg) | 126.5 ± 14.4       | 127.0 ± 14.8 | 126.4 ± 14.1 | 0.95 |
| Diastolic blood pressure (mmHg) | 75.0 ± 9.2        | 76.4 ± 0.2 | 73.5 ± 9.0  | 0.09 |
| BMI (kg m⁻²)              | 35.8 ± 3.8         | 35.7 ± 3.5 | 35.9 ± 4.1  | 0.76    |
| Weight (kg)               | 104.1 ± 14.8       | 103.6 ± 14.5 | 104.7 ± 15.3 | 0.82 |
| Waist circumference (cm)  | 111.2 ± 11.1       | 111.1 ± 11.2 | 111.3 ± 11.1 | 0.95 |
| Sagittal diameter (cm)    | 20.4 ± 5.4         | 20.5 ± 5.5 | 20.4 ± 5.4  | 0.98    |
| FMD                       | 9.6 ± 6.1          | 8.9 ± 5.7 | 10.4 ± 6.4  | 0.23    |
| Triglycerides (mg dl⁻¹)   | 108.1 ± 50.6       | 110.5 ± 49.7 | 105.6 ± 51.8 | 0.52 |
| Cholesterol (mg dl⁻¹)     |                    |         |          |         |
| Total cholesterol         | 193.0 ± 30.9       | 198.3 ± 30.8 | 187.5 ± 30.4 | 0.09 |
| Low-density lipoprotein   | 125.2 ± 28.6       | 130.1 ± 29.1 | 120.1 ± 27.3 | 0.07 |
| High-density lipoprotein  | 47.9 ± 12.7        | 57.6 ± 12.9 | 48.1 ± 12.5  | 0.70    |
| Leptin (ng ml⁻¹)          | 34.8 ± 18.0        | 33.7 ± 16.0 | 36.5 ± 20.8  | 0.65    |

Note: Summaries presented as mean ± standard deviation unless otherwise indicated as %. Differences between participants were tested using Wilcoxon rank sum tests for continuous variables and chi square tests for categorical variables.

Weight loss and blood pressure

Similar to the larger study (24), participants reduced body weight by 7.1% ± 4.4%, 8.7% ± 6.8%, 7.1% ± 7.8%, and 4.1% ± 7.7% at 3, 6, 12, and 24 months, respectively. There were no significant differences in weight loss between the two groups at any time during the study. There was a decrease in systolic and diastolic blood pressure over time; −5.15 mmHg systolic and −1.71 mmHg diastolic for high carb and −3.55 mmHg systolic and −1.52 diastolic for low carb diets at 2 years. The decreases in blood pressure did not differ between diet groups (P = 0.91 for systolic and P = 0.44 for diastolic) at any time point.

Diet and endothelial function

There were no differences between the low-fat and low-carbohydrate diets in FMD at any time point (week 0, P = 0.17; 3 months, P = 0.66; 6 months, P = 0.80; 1 year, P = 0.86; 2 years, P = 0.29) (Figure 1). There were no significant changes in FMD from week 0 to 2 years in either the low-carbohydrate group (P = 0.16) or the low-fat (P = 0.10) group. As in the larger parent study, attrition increased over time but did not differ between the two groups.

Results

Baseline demographics and endothelial function

The baseline study characteristics of study participants are listed in Table 1. There were no statistically significant differences between the low-fat and low-carbohydrate groups on any baseline variable, including FMD. As shown in Table 2, after collapsing the two treatment groups, there were statistically significant correlations between FMD and waist circumference (r = −0.27, P < 0.01), triglycerides (r = −0.27, P < 0.01), and leptin (r = 0.42, P < 0.001) at baseline and a marginally significant relationship with weight (r = −0.18, P = 0.05).

| Variable                  | n   | r   | P value |
|---------------------------|-----|-----|---------|
| Weight                    | 120 | −0.17 | 0.05    |
| BMI                       | 120 | 0.03 | 0.76    |
| Waist circumference       | 92  | −0.27 | <0.01   |
| Sagittal diameter         | 111 | −0.09 | 0.34    |
| Triglycerides             | 121 | −0.27 | <0.01   |
| Total cholesterol         | 121 | −0.12 | 0.18    |
| Low-density lipoprotein   | 121 | −0.14 | 0.13    |
| High-density lipoprotein  | 121 | 0.14  | 0.12    |
| Leptin                    | 66  | 0.42 | <0.001  |

Note: Partial correlations after controlling for treatment assignment; P values evaluate whether the partial correlation is equal to 0.
Obesity

1 year (was an inverse correlation between changes in triglycerides and FMD at between change in FMD and change in leptin but not at 2 years. There correlation at 2 years (0.04) and 1 year (¼ 0.03), there were positive correlations between change in FMD and change in leptin but not at 2 years. There was an inverse correlation between changes in triglycerides and FMD at 1 year (r = −0.23, P < 0.05) and a marginally significant inverse correlation at 2 years (r = −0.24, P = 0.08) for all subjects.

Discussion

To our knowledge, this is the largest and longest study of weight loss on vascular function reported to date. There are several findings from this study. First, waist circumference and triglyceride level were inversely associated with endothelial dysfunction and leptin was positively associated with FMD prior to weight loss. Second, at 1 year, there were positive correlations between change in FMD and change in leptin and inverse correlation between triglycerides and FMD. Third, diet-induced weight loss of 7% at 1 year and 4% at 2 years with either a low-carbohydrate or low-fat diet did not improve (or worsen) endothelial dependent dilatation.

The findings from the present study are consistent with several published studies showing an association with WHR and FMD in obese subjects (6,17,27,28). One study showed that in normal-weight healthy subjects, modest fat gain results in impaired endothelial function (29). Smaller published studies showed mixed results regarding endothelial function and weight loss. Most, however, do not show a significant improvement in endothelial function after weight loss (6,7,9,11-13,16-18,20,21). Our larger study also indicated there was no clinical improvement in endothelial function with weight loss at any point over 2 years.

The results of several clinical studies evaluating weight loss combined with exercise show improvement in endothelial function (8,12,13,30,31). There are data to suggest that improvement in glucose and/or insulin sensitivity (17,32) and the amount of LDL reduction (18) correlate with improvement in endothelial function for those enrolled in weight loss studies. Our data show no correlation with LDL. Thus, it seems that weight loss alone may not be enough to significantly improve endothelial function.

The type of diet subjects followed in obesity studies could potentially affect endothelial function discordantly. For example, it was reported that in hypercholesterolemic men, diets low in fat

| Variable | n | r  | P value |
|----------|---|----|---------|
| Weight   |   |    |         |
| 3 months | 95| −0.06| 0.56   |
| 6 months | 84| 0.08 | 0.47   |
| 1 year   | 68| −0.08| 0.53   |
| 2 years  | 57| −0.002| 0.99  |
| Waist circumference |   |    |         |
| 6 months | 54| 0.24 | 0.09   |
| 1 year   | 49| 0.11 | 0.45   |
| 2 years  | 38| −0.09| 0.60   |
| Sagittal diameter |   |    |         |
| 6 months | 66| −0.05| 0.68   |
| 1 year   | 50| −0.02| 0.91   |
| 2 years  | 25| −0.13| 0.55   |
| Triglycerides |   |    |         |
| 3 months | 95| −0.03| 0.75   |
| 6 months | 81| −0.16| 0.15   |
| 1 year   | 74| −0.23| 0.046  |
| 2 years  | 57| −0.24| 0.08   |
| Total cholesterol |   |    |         |
| 3 months | 95| 0.01 | 0.92   |
| 6 months | 81| 0.02 | 0.89   |
| 1 year   | 74| −0.05| 0.69   |
| 2 years  | 57| −0.13| 0.36   |
| LDL      |   |    |         |
| 3 months | 95| 0.05 | 0.62   |
| 6 months | 81| 0.02 | 0.88   |
| 1 year   | 74| −0.03| 0.79   |
| 2 years  | 57| 0.05 | 0.71   |
| HDL      |   |    |         |
| 3 months | 95| 0.04 | 0.69   |
| 6 months | 81| 0.14 | 0.23   |
| 1 year   | 74| 0.18 | 0.12   |
| 2 years  | 57| −0.17| 0.22   |
| Leptina* |   |    |         |
| 6 months | 59| 0.26 | 0.04   |
| 1 year   | 58| 0.28 | 0.03   |
| 2 years  | 50| 0.08 | 0.60   |

Note: Partial correlations after controlling for treatment assignment; P values evaluate whether the partial correlation is equal to 0.

*Variables were not collected at 3 months.
(especially saturated fat) and diets rich in monounsaturated fats improve endothelial function (33). In another study, endothelial function improved in patients with metabolic syndrome after eating a Mediterranean-style diet (34). However, diets that result in enhanced postprandial lipemia may adversely affect endothelial function (35). One small study showed endothelial function is markedly impaired by a high-fat meal that causes an acute hypertriglyceridemia and was evident in dyslipidemic patients with baseline hypertriglyceridemia but not in normotriglyceridemic controls (36). The results from our study did not demonstrate any significant difference in diet, either low carbohydrate or low fat, on endothelial function after weight loss. We did observe a significant association between changes in triglyceride levels and changes in endothelial function over time suggesting that lipemia likely affects endothelial function.

Leptin, a hormone derived from adipocytes, rises exponentially with body fat and is a vasodilator (2). Leptin receptors are present throughout the vascular system indicating importance in normal vascular physiology. A rat model (37) and a human study (38) showed leptin directly causes vasodilatation via a nitric oxide-independent mechanism. It is a paradox that obese patients generally have elevated leptin levels and yet a reduced capacity for endothelium mediated vasodilatation. The reason for this paradox is thought secondary to leptin resistance (39). Broek et al. studied the effect of weight loss over 3 months on FMD in 43 obese but otherwise healthy subjects (6). Although there was no overall significant change in FMD with weight loss, the change in endothelial function after weight loss was predicted only by the change in plasma leptin concentration. Our findings in larger sample confirm that changes in FMD are correlated with changes in leptin at both 6 months and 1 year. The reason for the leptin-FMD paradox with weight loss is unclear.

Limitations and strengths

At year 2, significant attrition had occurred which increases the possibility of type II statistical error at this time point. A study by Hambur et al. showed that obesity was associated with lower arterial shearing indicating a maladaptive vascular response which was reversed with weight loss (40). Thus, there may be beneficial vascular effects to weight loss that were not evident from measuring FMD such as change in postprandial triglyceride level with weight loss. In addition, it is possible that our study population, that was free of known cardiovascular disease, may not show a significant improvement above baseline FMD level. The strengths of our study include the large sample size, the 2-year duration, and participants randomized to two different diets.

Conclusions

The results of this study indicate that endothelial function is inversely correlated with waist circumference, triglyceride level, and directly correlated with leptin in obese persons before weight loss. There was no significant improvement in endothelial function after ~7% weight loss at 1 year and 4% at 2 years, achieved by either a low carbohydrate or a low fat diet, despite decreases in waist circumference, serum triglyceride concentrations and other CVD risk factors. Changes in leptin were associated with changes in FMD.

References

1. Lavie CJ, Milani RV, Ventura HO. Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. J Am Coll Cardiol 2009;53:1925-1932.
2. Blumenthal JA, Obesity, weight loss, and vascular function. Endocrine 2006;29:21-25.
3. Gerhardt-Herman M, Gardin JM, Jaff M, et al. Guidelines for noninvasive vascular laboratory testing: a report from the American Society of Echocardiography and the Society for Vascular Medicine and Biology. Vasc Med 2006;11:183-200.
4. Gerhardt-Herman M, Gardin JM, Jaff M, et al. Guidelines for noninvasive vascular laboratory testing: a report from the American Society of Echocardiography and the Society for Vascular Medicine and Biology. J Am Soc Echocardiogr 2006;19:955-972.
5. Arco G, Zamboni M, Rossi L, et al. Body fat distribution predicts the degree of endothelial dysfunction in uncomplicated obesity. Int J Obes Relat Metabol Disord 1999;23:936-942.
6. Brook RD, Bard RL, Glazewski L, et al. Effect of short-term weight loss on the metabolic syndrome and conduit vascular endothelial function in overweight adults. Am J Cardiol 2004;93:1012-1016.
7. Skilton MR, Sieveking DP, Harmer JA, et al. The effects of obesity and non-pharmacological weight loss on vascular and ventricular function and structure. Diabetics Obes Metab 2008;10:874-884.
8. Woo KS, Chook P, Yu CW, et al. Effects of diet and exercise on obesity-related vascular dysfunction in children. Circulation 2004;109:1981-1986.
9. Krog JB, Brinkworth GD, Noakes M, et al. Effects of weight loss from a very-low-carbohydrate diet on endothelial function and markers of cardiovascular disease risk in subjects with abdominal obesity. Am J Clin Nutr 2008;87:567-576.
10. Arkin JM, Alsdorf R, Biesmina S, et al. Relation of cumulative weight burden to vascular endothelial dysfunction in obesity. Am J Cardiol 2008;101:98-101.
11. Shechter M, Beigel R, Freidmark D, et al. Short-term subtherapeutic treatment is associated with weight loss and improved endothelial function in obese patients with coronary artery disease. Am J Cardiol 2006;97:1650-1653.
12. Handil O, Lebudry S, Mullolo C, et al. Lifestyle modification improves endothelial function in obese subjects with the insulin resistance syndrome. Diabetes Care 2002;26:2119-2125.
13. Goekse N, Vita JA, McDonnell M, et al. Effect of medical and surgical weight loss on endothelial vasomotor function in obese patients. Am J Cardiol 2005;95:266-268.
14. Roman MJ, Naqvi TZ, Gardin JM, et al. Clinical application of noninvasive vascular ultrasound in cardiovascular risk stratification: a report from the American Society of Echocardiography and the Society of Vascular Medicine and Biology. J Am Soc Echocardiogr 2006;19:943-954.
15. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelium-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257-265.
16. Sasaki S, Higashi Y, Nakagawa K, et al. A low-calorie diet improves endothelium-dependent vasodilatation in obese patients with essential hypertension. Am J Hypertens 2002;15:4, Part 1;302-309.
17. Raitakari M, Ilvonen T, Ahotupa M, et al. Reduction of weight burden with very low-calorie diet and endothelial function in overweight adults: role of plasma glucose. Arterioscler Thromb Vasc Biol 2004;24:124-128.
18. Bergelova R, Tiikkanen M, Vehkaviski S, et al. Lowering of LDL cholesterol rather than moderate weight loss improves endothelium-dependent vasodilatation in obese women with previous gestational diabetes. Diabetes Care 2003;26:1667-1672.
19. Williams IL, Chowcienycz PJ, Wheatcroft SB, et al. Endothelial function and weight loss in obese humans. Obes Surg 2005;15:1055-1060.
20. Clifton PM, Keogh JB, Foster PR, et al. Effect of weight loss on inflammatory and endothelial markers and FMD using two low-fat diets. Int J Obes (Lond) 2005;29:1445-1451.
21. Dengel DR, Kelly AS, Olson TP, et al. Effects of weight loss on insulin sensitivity and atherosclerotic function in overweight adults. Metabolism 2006;55:907-911.
22. Brook RD, Bard RL, Rubenfire M, et al. Usefulness of visceral obesity (waist/hip ratio) in predicting vascular endothelial function in healthy overweight adults. Am J Cardiol 2001;88:1264-1269.
23. Phillips SA, Jurva JW, Syed AZ, et al. Benefit of low-fat over low-carbohydrate diet on endothelial health in obesity. Hypertension 2008;51:376-382.
24. Foster GD, Wyatt HR, Hill JO, et al. Weight and metabolic outcomes after 2 years on a low-carbohydrate versus low-fat diet: a randomized trial. Ann Intern Med 2010;153:147-157.
25. Thijssen DH, Black MA, Pyke KE, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol 2011;300:H2-H12.
26. Laird NM. Missing data in longitudinal studies. Stat Med 1988;7:305-315.
27. Perticone F, Ceravolo R, Candigliota M, et al. Obesity and body fat distribution induce endothelial dysfunction by oxidative stress: precise effect of vitamin C. Diabetes 2001;50:159-165.
28. Williams IL, Chowcienycz PJ, Wheatcroft SB, et al. Effect of fat distribution on endothelium-dependent and endothelium-independent vasodilatation in healthy humans. Diabetes Obes Metab 2006;8:290-301.

© 2012 The Obesity Society
29. Romero-CorrA A, Sert-Kuniyoshi FH, Sierra-Johnson J, et al. Modest visceral fat gain causes endothelial dysfunction in healthy humans. *J Am Coll Cardiol* 2010;56:662-666.

30. Ziccardi P, Nappo F, Giugliano G, et al. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation* 2002;105:804-809.

31. Ribeiro MM, Silva AG, Santos NS, et al. Diet and exercise training restore blood pressure and vasodilatory responses during physiological maneuvers in obese children. *Circulation* 2005;111:1915-1923.

32. Sciacqua A, Candigliota M, Ceravolo R, et al. Weight loss in combination with physical activity improves endothelial dysfunction in human obesity. *Diabetes Care* 2005;28:1673-1678.

33. Fuentes F, Lopez-Miranda J, Sanchez E, et al. Mediterranean and low-fat diets improve endothelial function in hypercholesterolemic men. *Ann Intern Med* 2001;134:1115-1119.

34. Esposito K, Marfella R, Ciottola M, et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004;292:1440-1446.

35. Miller M, Beach V, Sorkin JD, et al. Comparative effects of three popular diets on lipids, endothelial function, and C-reactive protein during weight maintenance. *J Am Diet Assoc* 2009;109:713-717.

36. Giannattasio C, Zoppo A, Gentile G, et al. Acute effect of high-fat meal on endothelial function in moderately dyslipidemic subjects. *Arterioscler Thromb Vasc Biol* 2005;25:406-410.

37. Lembo G, Vecchione C, Fratta L, et al. Leptin induces direct vasodilation through distinct endothelial mechanisms. *Diabetes* 2000;49:293-297.

38. Nakagawa K, Higashi Y, Sasaki S, et al. Leptin causes vasodilation in humans. *Hypertens Res* 2002;25:161-165.

39. Martin SS, Qasim A, Reilly MP. Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. *J Am Coll Cardiol* 2008;52:1201-1210.

40. Hamburg NM, Mott MM, Bigornia SJ, et al. Maladaptive enlargement of the brachial artery in severe obesity is reversed with weight loss. *Vasc Med* 2010;15:215-222.