**Weight Reduction With Very-Low-Caloric Diet and Endothelial Function in Overweight Adults: Role of Plasma Glucose**

Maria Raitakari, Thomas Ilvonen, Markku Ahotupa, Terho Lehtimäki, Aimo Harmoinen, Pauli Suominen, Juhani Elo, Jaakko Hartiala, Olli T. Raitakari

**Objective**—Obesity is associated with endothelial dysfunction that may contribute to the development of atherosclerosis. We studied whether weight reduction improves endothelial function in overweight individuals.

**Methods and Results**—Flow-mediated endothelium-dependent vasodilation of the brachial artery was measured in 67 adults (age: 46±7 years, body mass index: 35.2±5.4 kg/m²) before and after a 6-week weight reduction program induced by very-low-calorie diet (daily energy: 580 kcal/2.3 MJ). Caloric restriction reduced body weight from 101±18 to 90±17 kg. Flow-mediated vasodilation increased from 5.5%±3.7 to 8.8%±3.7% (P<0.0001). Nitrate-mediated vasodilation was not significantly affected. The improvement in flow-mediated dilation was associated with the reduction in plasma glucose concentration (P=0.0003). This relationship was independent of changes in weight, serum lipids, oxidized LDL, C-reactive protein, adiponectin, blood pressure, and insulin.

**Conclusions**—Weight reduction with very-low-calorie diet improves flow-mediated vasodilation in obese individuals. This improvement is related to the reduction in plasma glucose concentration. These observations suggest that changes in glucose metabolism may determine endothelial vasodilatory function in obesity. (Arterioscler Thromb Vasc Biol. 2004; 24:124-128.)

**Key Words:** endothelium ■ obesity ■ risk factors ■ glucose ■ weight reduction

The vascular endothelium plays an important role in the regulation of arterial tone, thrombosis, and inflammation. Endothelial dysfunction may predispose arteries to the development of atherosclerotic lesions and is pathophysiologically linked to acute cardiovascular syndromes. A common condition associated with endothelial dysfunction is obesity. Endothelial-dependent vascular responses to both agonist-stimulated and flow-mediated vasodilation have been shown to be blunted in obese individuals. The mechanisms of obesity-induced endothelial dysfunction may be multifactorial, because excess adipose tissue induces several metabolic changes that may interfere with normal endothelial function. These may include dyslipidemia, elevated blood pressure, increased inflammation, oxidative stress, and changes in glucose metabolism. Although weight reduction is known to reduce several of these risk factors for endothelial dysfunction, it is inadequately known whether endothelial function can be improved by reducing weight. We hypothesized that weight loss induced by very-low-calorie diet would enhance flow-mediated endothelium-dependent vasodilation in overweight adults. To gain insight for possible mechanisms of weight-loss-mediated changes in flow-mediated dilation, we also measured changes in several potential biochemical determinants of endothelial function.

**Methods**

**Subjects**

We recruited overweight (body mass index ≥27kg/m²) men and women from an occupational health service clinic. The exclusion criteria included diabetes, pregnancy, gout, gall stone disease, alcohol/drug abuse, liver/kidney disorder, psychiatric disorder, and use of cholesterol lowering medication.

Of the 74 subjects enrolled, 47 women and 20 men completed the 6-week program (9.5% dropout rate). Sixteen women were postmenopausal (2 using hormone replacement therapy), 19 subjects had treatment for hypertension, and there were 7 smokers and 13 ex-smokers. Most participants (65%) were sedentary, engaging in physical activities less than once per week, and were instructed to maintain their exercise levels during the diet. The study was conducted according to the guidelines of the Helsinki declaration, and the study protocol was approved by the Joint Ethics Committee.

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of Turku University and Turku University Central Hospital. All subjects gave their informed consent.

Study Design
After recruitment, the participants underwent a physical examination with measurements of height, weight, waist/hip circumferences, and blood pressure with a standard mercury sphygmomanometer. After a 1-week run-in period with instructions to eat less to adjust for smaller food portions, the participants were prescribed a low-calorie diet. All daily meals were replaced by diet products for a period of 6 weeks (Nutristar; Leiras, Finland) (2.3 MJ, 4.5 g fat, 59 g protein, and 72 g carbohydrate per day). Alcohol consumption was not allowed. The physical examination was repeated within 3 days after the cessation of the diet.

Ultrasound Studies
We used Acuson Sequoia 512 mainframe (Mountain View, CA) with 8.0-MHz linear array transducer. Studies were performed after an overnight fast on the previous day before initiating the diet and on the last day of the diet. Subjects were instructed to discontinue any vasoactive medications 3 days before the study. Brachial artery diameter was measured as previously described. In brief, a resting scan above the elbow was performed. Thereafter, hyperemia was induced by inflation of a cuff placed around the forearm, followed by release. Subsequent scan was performed at 60 seconds after the cuff release. Flow-mediated dilatation (FMD), as a marker of endothelial function, was calculated as percent increase in arterial diameter during hyperemia compared with the resting value. The brachial arterial dilator response to hyperemia has been shown to be caused mainly by endothelial release of nitric oxide. Nitrate-mediated endothelium-independent vasodilatation was tested by scanning the artery 4 minutes after a sublingual dose of 1.25 mg isosorbide dinitrate. In our laboratory, the between-interobserver variation (coefficient of variation) of FMD measurements was 8.6%, and the between-visits variation was 9.0%.

Biochemical Analyses
Fasting blood samples were taken on the same day as the ultrasound study. Serum total cholesterol, HDL cholesterol, triglycerides, and plasma glucose concentrations were measured using standard enzymatic methods. LDL cholesterol was calculated. Oxidized LDL was measured by determining the level of LDL diene conjugation, as previously described. Serum high-sensitivity C-reactive protein was analyzed with sandwich immunoassay method using Innotrac Aio1 immunoanalyzer (Innotrac Diagnostic, Turku, Finland). Serum insulin was measured with time-resolved fluoroimmunoassay method using Wallac AutoDELFIA analyzer (Wallac, Turku, Finland). Serum adiponectin was measured using an immunoenzymometric assay based on a standard 96-well microtiterplate, and the generated absorbances were read at 450 nm using PlateReader (Wallac/Perkin-Elmer, Turku, Finland). Purified native adiponectin from human serum was used in both calibrators and controls (low and high).

Statistical Methods
The effects of intervention were tested by repeated-measures ANOVA. Correlation and multivariate regression analyses were used to examine the relationships between baseline and 6-week changes in measured parameters. All statistical analyses were performed using the statistical analysis system.

Results
Characteristics of study subjects are shown in Table 1. At baseline, FMD correlated with vessel size \((r = 0.32, P = 0.008)\), waist-to-hip ratio \((r = -0.29, P = 0.02)\), and pack-years of cigarettes \((r = -0.28, P = 0.02)\). Men had lower FMD \((3.7\% \pm 2.1\% \text{ versus } 6.3\% \pm 4.2\%, P = 0.01)\) compared with women. Subjects with hypertension had similar FMD \((5.9\% \pm 4.4\% \text{ versus } 5.4\% \pm 3.4\%, P = 0.57)\) compared with normotensives.

After diet, total weight reduction was 11.3 kg \((-12\%)\). Total cholesterol \((-22\%)\), LDL cholesterol \((-26\%)\), triglycerides \((-25\%)\), oxidized LDL \((-31\%)\), glucose \((-8\%)\), insulin \((-36\%)\), C-reactive protein \((-31\%)\), and systolic \((-7\%)\) and diastolic \((-9\%)\) blood pressure were significantly reduced from baseline. Adiponectin concentration was significantly increased \((26\%)\) (Table 1). Weight loss correlated with reductions in diastolic \((r = 0.48, P < 0.001)\) and systolic blood pressure \((r = 0.46, P < 0.001)\), insulin \((r = 0.3, P = 0.004)\), oxidized LDL \((r = 0.34, 0.007)\), triglycerides \((r = 0.3, P = 0.01)\), LDL cholesterol \((r = 0.3, P = 0.02)\), and glucose \((r = 0.24, P = 0.05)\).

After diet, FMD increased by 60% from 5.5% \(\pm 3.7\%\) to 8.8% \(\pm 3.7\%\) \((P < 0.0001)\). The increase was similar between women and men, between normotensive and hypertensive subjects, between smokers and non-smokers, and between pre-menopausal and post-menopausal women (all pairwise comparisons: \(P > 0.1\)). Nitrate-mediated dilatation and brachial artery diameter were not affected (Table 1). The improvement in FMD correlated with the reduction in plasma glucose concentration \((r = 0.44, P = 0.0003)\) but not with changes in weight \((r = 0.01, P = 0.92)\) or other risk variables. FMD change in each tertile of plasma glucose change is shown in the Figure.

In multivariate regression models, glucose change remained a significant correlate for FMD after adjustments for sex, age, hypertension, smoking, baseline FMD, initial weight, weight change, and changes in LDL cholesterol.

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of 67 Study Subjects Before and After 6-Week Weight Reduction Program</th>
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<tbody>
<tr>
<td><strong>Week 0</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Oxidized LDL (μmol/L)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
</tr>
<tr>
<td>Adiponectin (mg/L)</td>
</tr>
<tr>
<td>Vessel size (mm)</td>
</tr>
<tr>
<td>Flow-mediated dilatation (%)</td>
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<tr>
<td>Nitrate-mediated dilatation (%)</td>
</tr>
</tbody>
</table>

Repeated-measures ANOVA: *P < 0.001, †P < 0.05.
obesity is associated with endothelial dysfunction, which is thought to predispose obese individuals to the development of atherosclerosis and acute cardiovascular events. Whether weight reduction improves endothelial function, however, is inadequately known. We found that a 6-week weight reduction program with very-low-calorie diet significantly improves endothelial-dependent vasodilation in overweight adults. This improvement was similarly seen in women and men, in normotensive and hypertensive subjects, and in smokers and non-smokers.

Because co-morbidities, such as dyslipidemia, insulin resistance, hypertension, increased inflammatory state, and increased oxidative stress, often exist in obesity, the causes of endothelial dysfunction in obese individuals may be multifactorial. To gain insight for the potential mechanisms how weight-loss-induced by short-term hypocaloric diet might improve endothelial function, we measured changes in several biochemical variables potentially related to changes in endothelial function. As expected from previous observations, weight loss was accompanied by significant reductions in serum lipids, oxidized LDL, C-reactive protein, blood pressure, glucose and insulin/insulin resistance, and an increase in adiponectin concentration. Earlier studies indicate that all these metabolic changes could be potential determinants of endothelial vasodilatory function. In the present study, however, the strongest correlate for the improvement in FMD was the reduction in plasma glucose concentration. Thus, our study is the first to our knowledge to demonstrate that a decrease in plasma glucose concentration induced by weight loss is associated with improvement in endothelial function. This finding may be explained by earlier findings of the effects of glucose on endothelial cells. Chronic hyperglycemia has been implicated as the underlying mechanism that causes endothelial dysfunction in diabetes and other insulin-resistant conditions. In addition, studies in non-diabetic humans have consistently shown that acute hyperglycemia impairs endothelium-dependent vasodilation.

Potential mechanisms for glucose-mediated endothelial dysfunction may include reduced nitric oxide bioavailability caused by increased formation of reactive oxygen intermediates, glucose auto-oxidation, activation of protein kinase C, formation of advanced glycosylation end products, decreased nitric oxide synthase expression, and direct chemical inactivation of nitric oxide by glucose.

Acute oxidant stress possibly plays a role in glucose-mediated endothelial dysfunction, because hyperglycemia induced impairment in endothelial function can be reversed by pretreatment with antioxidant vitamins. The molecular effects of glucose in many of the experimental studies have usually been observed using glucose concentrations >20 mmol/L. Therefore, one must keep in mind that the relevance of these observations in explaining the vascular effects of glucose within the non-diabetic range are not clear.

Three previous studies have examined the effect of weight reduction on endothelial vasodilatory function. Sasaki et al measured forearm blood flow responses in 11 obese hypertensive patients before and after a 2-week low-calorie diet (average weight reduction: 4.3 kg). Caloric restriction was associated with enhanced forearm blood flow response to endothelium-dependent vasodilator acetylcholine. Another study by Sciacqua et al showed that weight reduction in combination with physical activity was effective in improving acetylcholine-stimulated vasodilation in obese but healthy individuals. This study is confirmatory to our findings but is limited because the effects of weight reduction and physical activity were not studied separately. A recent study by Bergholm et al found that a weight loss (average: 7.3 kg) achieved by combining a hypocaloric diet with orlistat, compared with identical weight loss achieved by a hypocaloric diet and placebo, improved acetylcholine-stimulated endothelium-dependent vasodilation in previously gestationally diabetic obese women. Despite identical weight loss, serum LDL cholesterol concentration decreased significantly more in the orlistat group than in the placebo group. Also, the change in the blood flow response to acetylcholine-stimulated blood flow after weight loss correlated with the change in LDL cholesterol but not with changes in body composition or other metabolic parameters. A study by Ziccardi et al also

### TABLE 2. Association Between Changes in Flow-Mediated Dilation and Plasma Glucose Concentration (Δglucose) in Multivariate Analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>β±SE*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Δglucose</td>
<td>-1.99±0.53</td>
<td>0.0003</td>
</tr>
<tr>
<td>B: A+ age, sex, hypertension, smoking</td>
<td>-2.07±0.52</td>
<td>0.0002</td>
</tr>
<tr>
<td>C: B+ baseline weight, Δweight</td>
<td>-2.15±0.55</td>
<td>0.0003</td>
</tr>
<tr>
<td>D: C+baseline FMD</td>
<td>-1.35±0.54</td>
<td>0.0151</td>
</tr>
<tr>
<td>D+ΔLDL</td>
<td>-1.33±0.59</td>
<td>0.0277</td>
</tr>
<tr>
<td>D+Δoxidized LDL</td>
<td>-1.61±0.63</td>
<td>0.0141</td>
</tr>
<tr>
<td>D+Δinsulin</td>
<td>-1.53±0.60</td>
<td>0.0143</td>
</tr>
<tr>
<td>D+Δsystolic blood pressure</td>
<td>-1.31±0.55</td>
<td>0.0212</td>
</tr>
<tr>
<td>D+Δadiponectin</td>
<td>-1.59±0.59</td>
<td>0.0101</td>
</tr>
<tr>
<td>D+ΔC-reactive protein</td>
<td>-1.40±0.69</td>
<td>0.0496</td>
</tr>
</tbody>
</table>

*Regression coefficient and standard error.
FMD indicates flow-mediated dilation.
suggested improvement in endothelial function after weight reduction. These investigators observed improved vascular responses to L-arginine and reductions in inflammatory cytokines in 56 obese women after a 1-year multidisciplinary weight reduction program including diet, exercise, and behavioral counseling (average weight reduction: 9.8 kg). In their study, the major factors correlating with the improvement in endothelial function were reductions in pro-inflammatory cytokines and adhesion molecules. Thus, the available studies suggest that weight loss is associated with improvement in endothelial function, although the mechanisms are not fully understood.

Weight reduction induces a wide range of metabolic changes, making it difficult to control for all underlying factors. We did not measure other potential causes for enhanced endothelial function, such as changes in free fatty acids and inflammatory cytokines. We did not study obese controls, but the average FMD of 5.5% in our obese subjects was less than published reference values for healthy adults and of similar magnitude as reported in a previous study of obese adults with similar characteristics and average FMD of 5.7%. Surprisingly, the improvement in FMD was not correlated with weight change. This may indicate that weight reduction per se is not important or that there is a threshold after which no further effect is seen, because all our study subjects lost weight of at least 4 kg. The possibility that the very-low-calorie diet itself contains substances that may improve endothelial cell dysfunction was also addressed. The substances with potential effects on endothelial function in the very-low-calorie diet products included vitamin C (daily dose: 100 mg), vitamin E (daily dose: 24 IU), and folic acid (daily dose: 0.35 mg). These amounts of vitamins reflect the recommended daily intake. Previous studies have demonstrated significant effects on FMD by vitamin C with doses of 500 to 2000 mg/d, vitamin E with doses of 400 to 800 IU/d, and folic acid with doses of 5 mg/d. Therefore, it is unlikely that the amounts of vitamins E and C and folic acid in the VLCD diet could have been high enough to induce the observed improvement in the flow-mediated vasodilation capacity.

Previous studies have shown that an acute increase in plasma glucose concentration is associated with a deterioration in endothelial function. Our data suggest that the lowering of plasma glucose in obese individuals may have an opposite effect. To test this hypothesis, one would need a method to decrease plasma glucose levels without affecting other potentially confounding physiological parameters. Because no such method currently exists, it might be difficult to design a clinical study to directly test this hypothesis. Lastly, our study population was heterogeneous, including men and women, subjects with hypertension, and smokers. Thirty-one women were premenopausal with potential fluctuations in FMD responses caused by menstrual cycle. Nevertheless, a stratified analysis indicated that the effect of weight reduction was similar in each subgroup.

In summary, weight loss with very-low-calorie diet may be an effective non-pharmacological method to improve endothelial function in obese individuals to reduce their risk for atherosclerosis. The beneficial effect of weight loss on endothelial function may be confined to obese subjects who also show a concomitant decrease in fasting plasma glucose concentration.

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