

# Lowering of LDL Cholesterol Rather Than Moderate Weight Loss Improves Endothelium-Dependent Vasodilatation in Obese Women With Previous Gestational Diabetes

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**OBJECTIVE** — Effects of weight loss on vascular function are unknown. We compared, in the face of similar weight loss over 3–6 months, effects of orlistat (120 mg t.i.d.,  $n = 23$ ) and placebo ( $n = 24$ ) on in vivo endothelial function in a high-risk group of obese (BMI  $32.1 \pm 0.4$  kg/m<sup>2</sup>) premenopausal nondiabetic women with a history of gestational diabetes.

**RESEARCH DESIGN AND METHODS** — Forearm blood flow responses to intra-arterial infusions of acetylcholine (ACh) and sodium nitroprusside (SNP), body composition, and serum lipids were determined before and after weight loss.

**RESULTS** — Weight loss averaged  $7.3 \pm 0.2$  kg ( $8.3 \pm 0.1\%$ ) and  $7.4 \pm 0.2$  kg ( $8.2 \pm 0.1\%$ ) of initial body weight in the orlistat and placebo groups, respectively. Forearm and body compositions changed similarly in both groups. Responses to ACh increased by 41% to the low dose ( $5.9 \pm 0.6$  vs.  $8.3 \pm 0.3$  for flow in the experimental/control arm,  $P < 0.01$ ) and by 33% to the high dose ( $7.6 \pm 0.8$  vs.  $10.1 \pm 0.6$ ,  $P < 0.001$ ) in the orlistat group, but they remained unchanged in the placebo group. The blood flow responses to SNP did not differ significantly between the groups. LDL cholesterol decreased significantly in the orlistat group from  $3.5 \pm 0.2$  to  $3.0 \pm 0.1$  mmol/l ( $P < 0.01$ ) but remained unchanged in the placebo group. Within the orlistat group, the decrease in LDL cholesterol correlated significantly with the improvement in the blood flow response to ACh ( $r = -0.44$ ,  $P < 0.05$ ).

**CONCLUSIONS** — Orlistat but not moderate (8%) weight loss per se improves endothelial function in women with previous gestational diabetes. This improvement is associated with a lowering of LDL cholesterol by orlistat.

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Endothelial dysfunction, defined as an impairment of endothelium-dependent vasorelaxation caused by a loss of nitric oxide (NO) bioactivity in the vessel wall, is considered an early functional change preceding atherosclerosis (1). Obesity is associated with endothelial dysfunction (2–7). In these studies, both

obesity (2) and associated metabolic abnormalities such as dyslipidemia (4), hypertension (5), insulin resistance (4), and accumulation of fat in intra-abdominal rather than subcutaneous depots (3,6,7) have correlated with altered vascular function. The exact cause of endothelial dysfunction in obesity is, however, unclear.

Weight loss has beneficial effects on multiple markers of cardiovascular risk (8). Their magnitude of improvement is proportional to the amount of weight loss (8). Modest weight loss (5–10%) may not always result in significant long-term changes in risk factors. Effects of weight loss of any magnitude on endothelium-dependent and -independent vasodilatory responses have not been studied.

Orlistat is a lipase inhibitor that prevents fat absorption, facilitates weight loss, and prevents weight regain (9). Although most effects of orlistat are attributable to weight loss, it lowers LDL cholesterol more than expected from weight loss alone (10). The latter may be beneficial for vascular function because lowering of LDL cholesterol with statins improves endothelial function (11).

In the present study, we compared the effects of moderate weight loss with or without simultaneous lowering of fat absorption and LDL cholesterol on endothelial function in obese women. For this purpose, we designed a study in which the goal was to reduce body weight by 8% both in a group of women receiving orlistat and in another group receiving placebo.

## RESEARCH DESIGN AND METHODS

### Subjects and study design

This was an investigator-initiated, double-blind, placebo-controlled study with a parallel group design. The participants were recruited among women who had

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**Abbreviations:** ACh, acetylcholine; GDM, gestational diabetes mellitus; MRI, magnetic resonance imaging; SNP, sodium nitroprusside.

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

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been treated during the years 1987–1999 at the Department of Obstetrics and Gynecology at the Helsinki University Central Hospital because of gestational diabetes mellitus (GDM). The women had to fulfill, based on available hospital records, the following inclusion criteria: 1) previous GDM, 2) current age 20–50 years, 3) current BMI between 28 and 35 kg/m<sup>2</sup>, and 4) no known acute or chronic disease. All eligible women ( $n = 70$ ) were invited to a screening visit, which included a history and physical examination and standard laboratory tests. A 2-h 75-g oral glucose tolerance test was performed to exclude women with diabetes, using World Health Organization criteria. Other exclusion criteria included clinical or biochemical evidence of any disease, use of any drugs that may alter glucose tolerance, lack of reliable contraception, and smoking >10 cigarettes per day. A total of 57 women fulfilled both the inclusion and exclusion criteria. These women participated in the following tests before and after weight loss: 1) assessment of in vivo endothelial function, 2) measurement of forearm composition by magnetic resonance imaging (MRI), and 3) measurement of circulating markers of glucose and lipid metabolism. After baseline measurements, the subjects were randomized to receive, in a double-blind fashion, in addition to a hypocaloric diet, either orlistat 120 mg t.i.d. or an identical-looking placebo. The weight loss target was to achieve 8% weight loss over 3–6 months. The subjects were withdrawn from the study if they had not lost 5% of their body weight during the first 3 months. Once the subjects had lost 8% of body weight, they were placed on a weight-maintaining diet for at least 2 weeks (range 2–4) before the baseline measurements were repeated. A total of 47 women (23 in the orlistat and 24 in the placebo group) achieved the weight loss goal and completed the study. The body weight of these women at the time of delivery was  $92 \pm 2$  kg in the orlistat and  $94 \pm 2$  kg in the placebo group. The women in the orlistat and placebo groups were studied  $3.7 \pm 0.6$  and  $4.1 \pm 0.6$  years after delivery, respectively. The assessments of endothelial function were performed on comparable days of the menstrual cycle: days  $9 \pm 2$  and  $9 \pm 2$  before and after weight loss, respectively, in the orlistat group and days  $10 \pm 2$  and  $12 \pm 2$ , respectively, in the placebo group.

The nature and potential risks of the study were explained to all subjects before obtaining their written informed consent. The ethical committee of the Helsinki University Central Hospital approved the experimental protocol.

## Methods

**Weight loss program.** During baseline assessments, the subjects were prescribed a caloric level based on an estimate of their initial maintenance energy needs. For the weight loss period, an individual hypocaloric diet was calculated. The study diet was a fat-restricted hypocaloric diet in which 30% of energy was derived from fat (10% from saturated, 10% from unsaturated, and 10% from polyunsaturated fat), 50% from carbohydrates, and 20% from protein. Cholesterol intake was limited to 300 mg/day and alcohol intake to 150 g/week.

After baseline measurements, the subjects met with a dietitian every 2 weeks. During these visits, body weight, blood pressure, and heart rate were measured and changes in eating habits and possible adverse effects were recorded.

**In vivo endothelial function.** Vascular function was assessed in forearm resistance vessels by measuring forearm blood flow responses to intrabrachial artery infusions of endothelium-dependent (acetylcholine [ACh]) and -independent (sodium nitroprusside [SNP]) vasodilators, as previously described in detail (12). Blood flow measurements were performed simultaneously in the infused (experimental) and control arm. Blood flow results during infusion of the vasodilators are reported as a ratio of blood flow in the experimental arm divided by blood flow in the control arm. This method of data expression is not influenced by weight loss-induced changes in forearm composition. However, to enable analysis of blood flow responses also relative to actual muscle mass at the site of the blood flow measurement before and after weight loss, forearm MRI scans were performed in all women. This allowed calculation of blood flow responses per muscle mass in the experimental arm. The means of the final five blood flow measurements of each recording period were used for analysis.

**Quantitation of forearm composition.** Resting forearm blood flow is closely related with relative muscularity of the forearm, which varies between 20 and

80% in normal men and women (13). Because weight loss changes body and forearm composition, we measured forearm composition by MRI using a 1.5-T whole-body system (Siemens Magnetom Vision, Erlangen, Germany). The extremity transmitting and receiving coil (knee) was used for the determination of subcutaneous fat of the arm, and the elbow was placed in the center. A series of T1-weighted transaxial scans (15 slices) were acquired with a 150-mm field of view, a matrix of  $224 \times 512$ , four acquisitions, a 5-mm slice thickness, a repetition time of 570 ms, and an echo time of 14 ms. Subcutaneous fat and forearm muscle areas were quantified using an image analysis program (Alice 3.0; Parexel, Waltham, MA). The muscle volume of the slice, which corresponded to the site of blood flow measurement (vide supra), was used as the denominator when normalizing blood flow to muscle mass.

**Other measurements.** Whole-body fat was measured by a single-frequency bioelectrical impedance device (model BIA-101A; Bio-Electrical Impedance Analyzer System, Mt. Clemens, MI). Waist circumference was measured midway between the spina iliaca superior and the lower rib margin, and hip circumference was measured at the level of the greater trochanters.

**Analytical procedures.** Plasma glucose concentrations were measured in duplicate with the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Serum free insulin concentrations were measured by radioimmunoassay (Phadeseeph Insulin RIA; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) after precipitation with polyethylene glycol. Serum free fatty acids were measured using a fluorometric method (14). HbA<sub>1c</sub> was measured by high-pressure liquid chromatography using the fully automated Glycosylated Hemoglobin Analyzer System (BioRad, Richmond, CA). Serum total and HDL cholesterol and triglyceride concentrations were measured with respective enzymatic kits from Roche Diagnostics using an autoanalyzer (Roche Diagnostics Hitachi 917; Hitachi, Tokyo). LDL cholesterol concentration was calculated using the formula of Friedewald.

**Statistical analyses.** The unpaired *t* test was used to compare single measurements between subjects in the orlistat and placebo groups. Single measurements before and after therapy were compared us-

Table 1—Clinical and biochemical characteristics of the study groups

	Orlistat before weight loss	Change	Placebo before weight loss	Change
<i>n</i>	23	—	24	—
Age (years)	39 ± 1	—	39 ± 1	—
Weight (kg)	88.8 ± 1.5	−7.3 ± 0.2*	90.6 ± 1.4	−7.4 ± 0.2*
BMI (kg/m <sup>2</sup> )	32.3 ± 0.4	−2.7 ± 0.1*	32.3 ± 0.4	−2.7 ± 0.1*
Waist-to-hip ratio	0.94 ± 0.02	0.023 ± 0.01†	0.95 ± 0.01	0.029 ± 0.01‡
Fat (%)	35.6 ± 0.4	−1.7 ± 0.3*	36.8 ± 0.5	−2.4 ± 0.4*
Fasting plasma glucose (mmol/l)	5.7 ± 0.1	−0.03 ± 0.1	5.6 ± 0.1	0.04 ± 0.1
HbA <sub>1c</sub> (%)	5.5 ± 0.1	−0.08 ± 0.04	5.5 ± 0.1	0.01 ± 0.05
Fasting serum insulin (mU/l)	9.8 ± 1.1	−3.2 ± 0.8*	9.7 ± 1.1	−3.2 ± 0.8*
Systolic blood pressure (mmHg)	126 ± 3	−0.1 ± 1.8	123 ± 2	−2 ± 1.2
Diastolic blood pressure (mmHg)	85 ± 2	−2 ± 1	80 ± 2	−2 ± 1
Serum HDL cholesterol (mmol/l)	1.31 ± 0.05	−0.02 ± 0.03	1.28 ± 0.06	0.07 ± 0.03
Serum LDL cholesterol (mmol/l)	3.53 ± 0.16	−0.48 ± 0.15‡	3.14 ± 0.11	−0.17 ± 0.09
Serum triglycerides (mmol/l)	1.45 ± 0.15	−0.07 ± 0.17	1.37 ± 0.13	−0.27 ± 0.09‡
Serum free fatty acids (μmol/l)	726 ± 35	−56 ± 41	704 ± 34	−40 ± 46

\* $P < 0.001$ , † $P < 0.05$ , and ‡ $P < 0.01$  for change by weight loss.

ing the paired *t* test. Comparison of blood flow responses to the two doses of vasoactive drugs was performed using ANOVA for repeated measures. Correlation analyses were calculated using Spearman's nonparametric rank correlation coefficient. A *P* value of  $<0.05$  was considered statistically significant. All data are shown as means ± SE.

## RESULTS

### Baseline characteristics

Clinical and biochemical characteristics of the study groups at baseline are shown in Table 1. The two groups were comparable with respect to age; all measures of body weight and composition; and serum glucose, insulin, and lipid concentrations.

### Effects of weight loss on body composition and metabolic parameters

Weight loss averaged  $7.3 \pm 0.2$  kg ( $8.3 \pm 0.1\%$ ) and  $7.4 \pm 0.2$  kg ( $8.2 \pm 0.1\%$ ) of initial body weight in orlistat and placebo groups, respectively (Table 1). The mean time to achieve weight loss averaged  $20 \pm 1$  weeks in the orlistat group and  $18 \pm 1$  weeks in the placebo group (NS). The percent whole-body fat decreased similarly in both groups (Table 1). Forearm subcutaneous fat volumes (Fig. 1) decreased similarly in both study groups. The percent forearm muscle increased from  $53.2 \pm 1.2$  to  $53.9 \pm 1.2\%$  ( $P < 0.01$ ) in the orlistat group and from

$52.1 \pm 1.1$  to  $52.8 \pm 1.0\%$  ( $P < 0.05$ ) in the placebo group.

Fasting serum insulin concentrations decreased significantly and by  $\sim 30\%$  in both groups (Table 1). Systolic and diastolic blood pressures remained unchanged (Table 1). Serum LDL cholesterol decreased significantly in the orlistat group (by 11% or  $-0.48 \pm 0.15$  mmol/l,  $P < 0.01$ ) but not in the placebo group ( $-0.17 \pm 0.09$  mmol/l, NS) (Table 1). Serum triglycerides decreased in the pla-

cebo but not in the orlistat group (Table 1). Concentrations of fasting plasma glucose and HbA<sub>1c</sub> remained unchanged.

The orlistat group had significantly more gastrointestinal but not other side effects compared with the placebo group (total 93 vs. 60%,  $P < 0.01$ ; fatty stools 81 vs. 17%,  $P < 0.005$ ; soft stools 37 vs. 17%,  $P < 0.001$ ).

### Endothelial function

Before weight loss, both groups had similar basal flows ( $3.5 \pm 0.2$  vs.  $3.4 \pm 0.2$  ml/dl muscle · min, orlistat versus placebo) and similar flow responses to SNP and ACh (Figs. 2 and 3). After weight loss, basal flows were unchanged ( $3.4 \pm 0.3$  vs.  $3.2 \pm 0.3$  ml/dl muscle · min), but responses to ACh had increased significantly in the orlistat group by 41% to the low dose (flow in the experimental/control arm:  $5.9 \pm 0.6$  vs.  $8.3 \pm 0.3$  for before vs. after weight loss,  $P < 0.01$ ) and by 33% to the high dose ( $7.6 \pm 0.8$  vs.  $10.1 \pm 0.6$ ,  $P < 0.001$ ). Blood flow responses to ACh remained unchanged in the placebo group (Figs. 2 and 3). The ACh response increased significantly more in the orlistat compared with the placebo group ( $P = 0.005$ ). When blood flow during ACh was expressed per muscle mass, endothelium-dependent vasodilatation was also increased significantly by orlistat but not placebo (Fig. 3).

In the orlistat group, endothelium-independent blood flow responses to the low dose of SNP ( $3 \mu\text{g}/\text{min}$ ) increased by

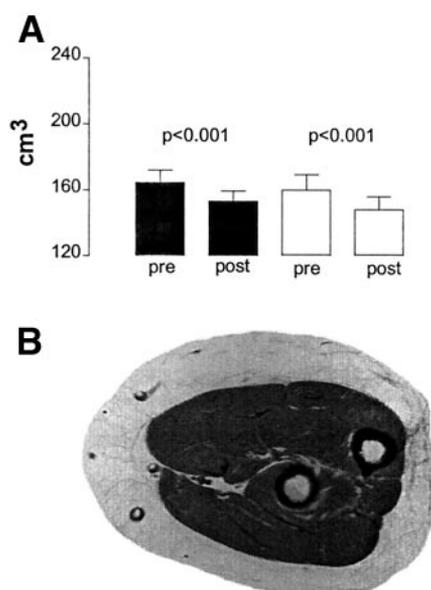
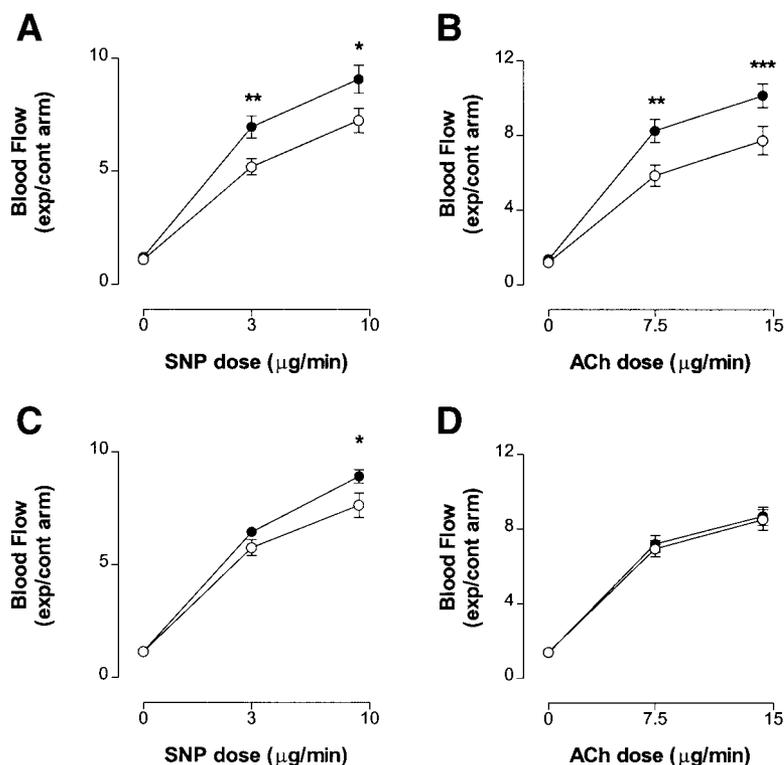


Figure 1—A: Forearm subcutaneous fat before and after weight loss. ■, Orlistat; □, placebo. B: Forearm magnetic resonance image.



**Figure 2**—Forearm blood flow responses to SNP and ACh before (○) and after (●) weight loss expressed as experimental/control arm. A: SNP and orlistat. B: ACh and orlistat. C: SNP and placebo. D: ACh and placebo. \* $P < 0.05$ ; \*\* $P < 0.01$ ; and \*\*\* $P < 0.001$ .

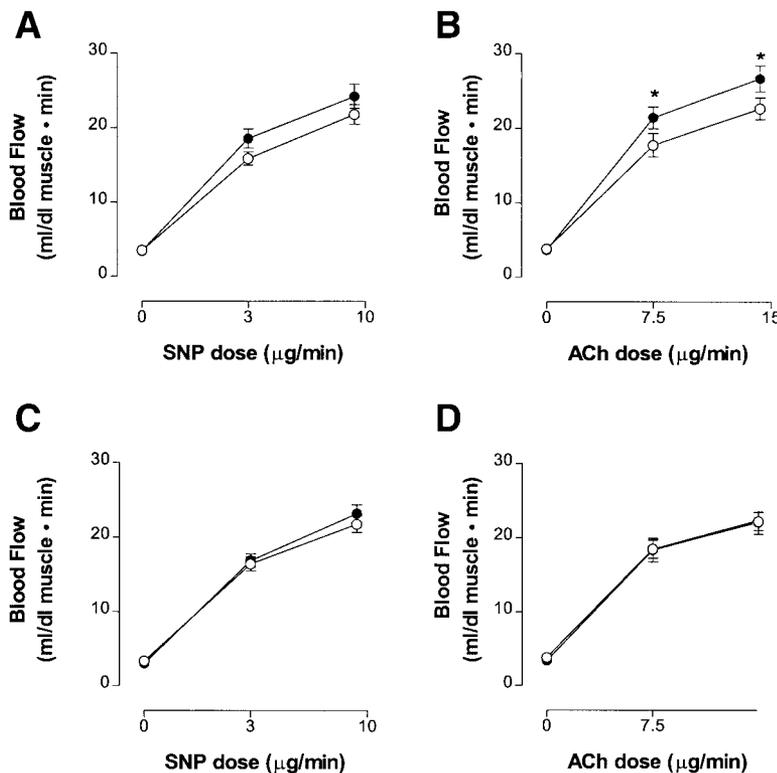
35% ( $5.2 \pm 0.4$  vs.  $7.0 \pm 0.5$  for before vs. after weight loss for flow in the experimental/control arm,  $P < 0.01$ ), and responses to the high dose ( $10 \mu\text{g}/\text{min}$ ) increased by 25% ( $7.3 \pm 0.5$  vs.  $9.1 \pm 0.6$ ,  $P < 0.02$ ). In the placebo group, there was a marginally significant (ANOVA  $P = 0.05$ ) increase in the blood flow response to SNP (Fig. 2). The changes in blood flow at the two doses of SNP between the groups were not significantly different ( $P = 0.31$ ). When blood flow during SNP infusions was expressed per muscle mass in the forearm, it did not change significantly in either the orlistat or the placebo group (Fig. 3).

**Interrelationships between measures of body composition, metabolic characteristics, and endothelial function**

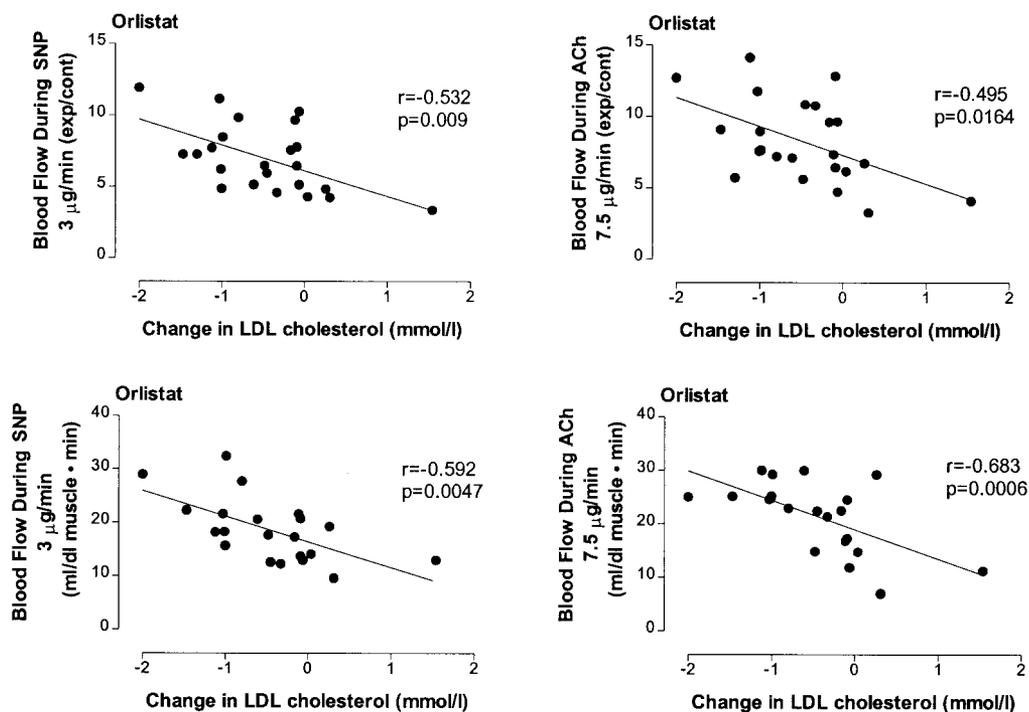
The change in LDL cholesterol within the orlistat group also correlated closely with blood flow responses to the low dose of SNP ( $r = -0.53$ ,  $P = 0.009$ ), the low dose of ACh ( $r = -0.50$ ,  $P = 0.016$ ) (Fig. 4), the high dose of SNP ( $r = -0.37$ ,  $P = 0.08$ ), and the high dose of ACh ( $r = -0.48$ ,  $P = 0.023$ ) after weight loss.

When the changes in blood flow responses were compared between the groups, the change in the response to ACh was significantly greater in the orlistat compared with the placebo group, even when baseline LDL cholesterol (which was slightly although not significantly higher in the orlistat compared with the placebo group) was included as a covariate ( $P = 0.012$ ). The relationships were essentially similar when blood flow was expressed per muscle mass (data not shown), although there was also a marginally significant correlation among all women before weight loss, between serum LDL cholesterol and mean blood flow (expressed as  $\text{ml}/\text{dl muscle} \cdot \text{min}$ ) during infusions of SNP (mean blood flow during low and high doses) ( $r = 0.41$ ,  $P = 0.08$ ), and between LDL cholesterol and mean blood flow during ACh infusions ( $r = 0.48$ ,  $P < 0.002$ ).

**CONCLUSIONS**— In this study, we found that moderate weight loss achieved by combining a hypocaloric diet with orlistat, compared with identical weight loss achieved by a hypocaloric diet and placebo, improves endothelium-dependent



**Figure 3**—Forearm blood flow responses to SNP and ACh before (○) and after (●) weight loss expressed per muscle mass. A: SNP and orlistat. B: ACh and orlistat. C: SNP and placebo. D: ACh and placebo. \* $P < 0.05$ ; \*\* $P < 0.01$ ; and \*\*\* $P < 0.001$ .



**Figure 4**—Interrelationships between change in LDL cholesterol and blood flow responses to SNP and ACh after weight loss.

vasodilatation in previously gestational diabetic obese women. Although there was some increase in endothelium-independent vasodilatory responses, the changes in responses to SNP by weight loss were not significant between the two groups, and when expressed per muscle mass, there was no change in endothelium-independent blood flow responses. Within the orlistat group, the change in the blood flow response to ACh by weight loss and ACh-stimulated blood flow after weight loss correlated with the change in LDL cholesterol (Fig. 4), but not with changes in body composition or other metabolic parameters. The latter data suggest that even modest decreases in LDL cholesterol may favorably influence endothelial function in obese women, whereas modest weight loss is ineffective in this respect.

The lack of significant changes in vascular function in the placebo group should not be interpreted to imply that it is not possible to enhance vascular function by weight loss. If one assumes that changes in vascular function are at least in part mediated via changes in markers of cardiovascular risk, these markers changed little by the 8% (7.4 kg) weight loss. The concentration of LDL cholesterol decreased by 0.17 mmol/l in the placebo group. This small change is in line with predictions from a meta-analysis

(15) that concluded that every kilogram of weight loss decreases LDL cholesterol by 0.02 mmol/l, i.e., 0.15 mmol/l for the observed 7.4 kg loss of body weight in the placebo group. Serum triglycerides decreased by 0.27 mmol/l in the placebo group, which is consistent with previous studies in obese women who have lost moderate amounts of weight (5–10%) (16). The small change in serum triglycerides is unlikely to have any significant impact on LDL size, which may influence endothelial function independent of LDL cholesterol concentrations (17). HDL cholesterol remained unchanged, in keeping with the suggestion that modest short term weight loss does not increase HDL cholesterol (15). Taken together, the changes in lipid parameters or insulin sensitivity were not sufficient to influence endothelial function.

In the orlistat group, serum LDL cholesterol concentrations decreased significantly more than in the placebo group, despite identical weight loss. This is in agreement with other studies showing that orlistat reduces LDL cholesterol more than can be explained by weight loss per se, as well as with the magnitude of LDL cholesterol lowering reported in other studies in patients losing moderate amounts of weight (10).

Analysis of dietary records based on a 3-day food intake diary (data not shown)

revealed that the orlistat and placebo groups were similar before weight loss. During weight loss, we did not request repeat food diaries, but there is no reason to believe that orlistat was not reducing fat absorption in an expected fashion. In studies where the dose of orlistat was similar to the doses in the present study, the 120-mg t.i.d. dose was shown to induce a ~25% decrease in cholesterol absorption and a 30% decrease in fat absorption (18,19). Presumably, had we recorded dietary intake, it would have been greater in the orlistat compared with the placebo group. The composition of the diet should not have differed because both groups were given similar advice.

The decrease in LDL cholesterol but not other parameters (data not shown) correlated with the improvement in endothelium-dependent vasodilatation in the orlistat group. Previous studies have documented that lowering of LDL cholesterol by statins improves the blood flow response to ACh but not to SNP (20). Except for the study of O'Driscoll et al. (21), the magnitude of improvement in endothelial function in the present study was similar to that observed in statin trials, although LDL cholesterol decreased much less than with statins. These data raise the possibility that factors other than LDL cholesterol contributed to improved endothelial function in the orlistat group.

Data are contradictory regarding the possibility that changes in fatty acid composition, which is altered during orlistat treatment (22), influence endothelial function.

In conclusion, moderate weight loss (8%) in obese women, which has minor effects on lipids and blood pressure, does not alter blood flow responses to endothelium-dependent or -independent vasodilatory agents. Although obesity with its accompanying metabolic alterations clearly is a cardiovascular risk factor, there are few studies to show that weight loss protects against cardiovascular death (23). This contrasts with the growing number of intervention studies, such as the Heart Protection Study, confirming that LDL cholesterol lowering is cardioprotective irrespective of baseline cholesterol concentrations (24). In the present study, weight loss combined with significant LDL cholesterol lowering by orlistat improved endothelial function, and the changes in LDL cholesterol and endothelial function were significantly interrelated. Whether inhibition of fat absorption with orlistat has effects beyond simple LDL cholesterol lowering, which improves endothelial function, warrants further investigation. Even so, the present data suggest that weight loss has beneficial effects on endothelial function, if accompanied by a decrease in LDL cholesterol.

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