Weight Loss Reduces Plasma Endothelin-1 Concentration in Obese Men

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Obesity is associated with endothelial dysfunction that may contribute to the development of diabetes, hypertension, and atherosclerosis. Endothelin-1 (ET-1), which is produced mostly by vascular endothelial cells, has potent vasoconstrictor and proliferative activity in vascular smooth muscle cells and, therefore, has been implicated in regulation of vascular tonus and the progression of atherosclerosis, suggesting that ET-1 may be important in endothelial dysfunction. We studied whether diet-induced weight loss (i.e., lifestyle modification) affects plasma ET-1 concentration in obese individuals. We measured plasma ET-1 concentration in seven obese men (age: 48 ± 4 years old, body mass index: 27.7 ± 0.5 kg/m^2) before and after a 3-month, diet-induced weight reduction program (i.e., lifestyle modification program). Caloric restriction reduced body weight from 78 ± 3 to 68 ± 2 kg ($P < 0.001$) and resulted in 12.1 ± 1.2% reduction in body mass index (24.3 ± 0.3 kg/m^2, $P < 0.0001$). After the weight reduction program, systolic and diastolic blood pressure significantly decreased (128 ± 7 vs. 115 ± 4 mm Hg, $P < 0.05$ and 88 ± 4 vs. 77 ± 2 mm Hg, $P < 0.01$, respectively). The plasma level of ET-1 significantly decreased after the program (5.1 ± 0.4 vs. 4.0 ± 0.3 pg/ml, $P < 0.05$). The percentage systolic blood pressure reduction and percentage plasma ET-1 concentration reduction was in a linear relationship ($r = 0.86$, $P < 0.05$). Furthermore, the relationship between percentage weight reduction and percentage plasma ET-1 concentration reduction was linear ($r = 0.87$, $P < 0.05$). We conclude that weight loss by low-calorie diet (i.e., lifestyle modification) reduces plasma ET-1 concentration in obese individuals. This reduction may contribute to the improvement of obesity-induced endothelial dysfunction. Exp Biol Med 231:1044–1047, 2006

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Introduction

Obesity is strongly associated with endothelial dysfunction that may play a role in the development of diabetes, hypertension, and atherosclerosis (1, 2). Endothelial dysfunction is a common abnormality in obesity. Damage to the endothelium is an important risk factor for cardiovascular diseases because it leads to structural changes, such as thickening of the intima and media of vessel walls. Clinical and animal studies have confirmed a strong relationship between obesity and cardiovascular disease such as diabetes, hypertension, and atherosclerosis (2–4). However, mechanisms linking obesity with endothelial dysfunction have not yet been fully clarified.

Vascular endothelial cells play a major role in maintaining cardiovascular homeostasis in health and diseases (5, 6). Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by vascular endothelial cells (6, 7). ET-1 has potent vasoconstrictor effect on vascular smooth muscle cells (8). It has also been reported that systemic administration of an endothelin receptor antagonist significantly decreased systemic blood pressure and peripheral vascular resistance in healthy humans, strongly suggesting that endogenous generated ET-1 contributes to basal vascular tonus in humans (9). Furthermore, ET-1 is a pro-mitogen, potentiating the response of other growth factors; therefore, ET-1 has been implicated in the progression of atherosclerosis (6, 10, 11). Thus, ET-1 has been implicated in regulation of vascular tonus and progression of atherosclerosis, suggesting that ET-1 may be important in endothelial dysfunction.

Obesity is strongly associated with endothelial dysfunction. However, the mechanisms underlying obesity-induced endothelial dysfunction are unclear. Endogenous ET-1 may play an important role in endothelial dysfunction because ET-1 has been implicated in regulation of vascular tonus and progression of atherosclerosis. The purpose of the

Table 1. Body Weight, BMI, Blood Pressure, and Plasma Glucose Level in Obese Men Before and After 3-Month Diet-Induced Weight Reduction Program[a]

	Before	After	*P*
Body weight, kg	78 ± 3	68 ± 2	$P < 0.001$
BMI, kg/m^2	27.7 ± 0.5	24.3 ± 0.3	$P < 0.0001$
Blood pressure, mm Hg			
Systolic	128 ± 7	115 ± 4	$P < 0.05$
Diastolic	88 ± 4	77 ± 2	$P < 0.01$
Plasma glucose, mg/dl	100.0 ± 5.1	94.3 ± 3.0	$P < 0.10$

[a] BMI, body mass index. Values are means ± SE.

present study was to examine whether diet-induced weight loss affects plasma ET-1 concentration in obese individuals. It is of great interest and importance to study whether weight loss causes a decrease in plasma ET-1 concentration in obese humans. We hypothesized that weight loss can reduce plasma ET-1 concentration in obese individuals, and that this reduction contributes to the improvement of obesity-induced endothelial dysfunction. In the present study, we measured plasma ET-1 concentration in obese men before and after a 3-month, diet-induced weight reduction program.

Materials and Methods

Subjects. Seven obese men participated in the study (48 ± 4 years old, height: 167.6 ± 2.0 cm, body mass index [BMI]: 27.7 ± 0.5 kg/m^2). None of the participants was taking medication on a regular basis at the time of the study. The obese subjects performed a 3-month, low-calorie diet intervention study.

The study was approved by the Ethical Committee of the Institute of Health and Sport Sciences, the University of Tsukuba. This study conformed with the principles outlined in the Helsinki Declaration, and all subjects gave their written informed consent before inclusion in the study.

Experimental Design. All obese men were studied before and after 3 months of diet-induced weight reduction program (i.e., lifestyle modification program). Body weight, BMI, systolic blood pressure, diastolic blood pressure, venous plasma glucose concentration, and venous plasma ET-1 concentration were measured before and after a 3-month, diet-induced weight reduction program in the obese men. Before they were tested, subjects fasted for 12 hrs. Blood pressure was measured in duplicate, with subjects in the upright sitting position. All measurements were performed at a constant room temperature (25°C).

Dietary Protocol. All subjects were instructed to take meals per day consisting on average of 420 kcal of protein, 840 kcal of carbohydrate, and 420 kcal of fat (total: 1680 kcal/day). Subjects kept daily food diaries during the 3-month intervention period and learned about proper daily nutrition (well-balanced protein, carbohydrates, fat, various amino acids, vitamins, and minerals) through weekly lectures and counseling by skilled dieticians.

Measurement of Plasma ET-1 Concentration by Sandwich-Enzyme Immunoassay. Each blood sample was placed in a chilled tube containing aprotinin (300 KIU/ml) and EDTA (2 mg/ml), and was then centrifuged at 2000 *g* for 15 min at 4°C. The plasma was stored at −80°C until use. Plasma (1 ml) was acidified with 3 ml of 4% acetic acid, and immunoreactive ET-1 was extracted with a Sep-Pak C18 cartridge (Waters, Milford, MA), as previously described in our article (12). The elutes were reconstituted with 0.25 ml of assay buffer and were subjected to sandwich-enzyme immunoassay. The sandwich-enzyme immunoassay for ET-1 was carried out as previously described using immobilized mouse monoclonal antibody AwETN40, which recognizes the NH_2-terminal portion of ET-1, and peroxidase-labeled rabbit anti-ET-1 COOH-terminal peptide (15–25) Fab′ (12). The Fab′ fragment of this rabbit antibody was used as an enzyme-labeled detector antibody after being coupled with horseradish peroxidase. The coefficient of variation of the ET-1 assay for intraassay variation was 11% and coefficient of variation for interassay variation was 13% (13). We previously reported that the lowest detection limit of this assay was 0.4 pg/ml for ET-1 (14). The plasma ET-1 levels in the present study were far beyond the lowest limit of detection with this assay (0.4 pg/ml) in all subjects.

Statistics. Values are means ± SE. To evaluate differences in the levels before and after weight reduction program in obese men, Student's *t* test for paired values was used. $P < 0.05$ was accepted as significant.

Results

All seven obese men completed the 3-month, low-calorie diet intervention study. Table 1 shows the body weight, BMI, blood pressure, and plasma glucose concentration in the obese men before and after 3 months of the weight reduction program. Body weight markedly decreased after the weight reduction program (Table 1). BMI also remarkably decreased after the program (Table 1). After the weight reduction program, systolic blood pressure and diastolic blood pressure significantly decreased (Table 1). There was no significant change in plasma glucose concentration before and after the program (Table 1). The plasma level of ET-1 significantly decreased after the program (5.1 ± 0.4 vs. 4.0 ± 0.3 pg/ml, $P < 0.05$; Fig. 1). Figure 2 shows the relationship between the percentage plasma ET-1 concentration reduction and the percentage systolic blood pressure reduction or percentage weight reduction. The percentage systolic blood pressure reduction and percentage plasma ET-1 concentration reduction was in a linear relationship ($r = 0.86$, $P < 0.05$; Fig. 2A). Furthermore, the relationship between percentage weight reduction and percentage plasma ET-1 concentration reduction was linear ($r = 0.87$, $P < 0.05$; Fig. 2B).

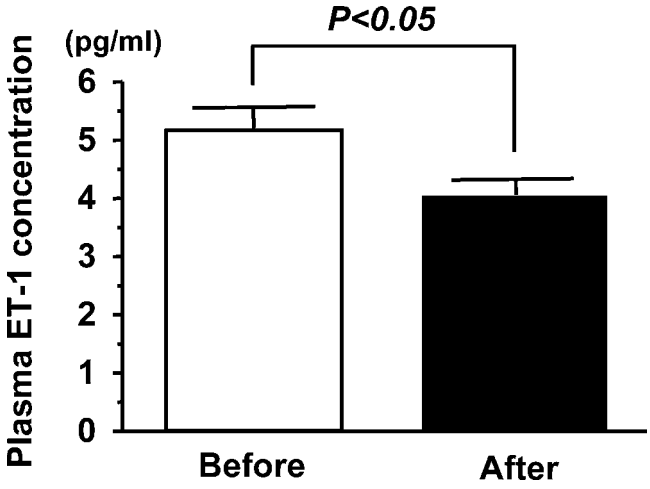

Figure 1. Plasma concentration of ET-1 before and after 3 months of weight reduction program in obese men ($n = 7$). Values are means ± SE.

Discussion

In the present study, we measured plasma ET-1 concentration in obese men before and after a 3-month, diet-induced weight reduction program. After the program, because of which the body weight and BMI markedly decreased, the plasma ET-1 concentration significantly decreased. We also demonstrated that the low-calorie diet in obese men significantly decreased systolic and diastolic blood pressure, suggesting the improvement of endothelial dysfunction. Furthermore, there was a significant positive correlation between the percentage plasma ET-1 concentration reduction and percentage systolic blood pressure or body weight reduction in obese men. Therefore, we suggest that the reduction of ET-1 by weight loss may contribute to the improvement of obesity-induced endothelial dysfunction because ET-1 has been implicated in regulation of vascular tonus and progression of atherosclerosis.

Obesity is strongly associated with cardiovascular disease (1–4). Obese individuals are at increased risk for diabetes, hypertension, atherosclerosis, and other cardiovascular diseases. Obesity is also associated with endothelial dysfunction that may play a role in the development of hypertension and atherosclerosis (1). Endothelial dysfunction is a common abnormality in obesity. Vascular endothelial cells produce ET-1, which is a potent vasoconstrictor peptide and has potent proliferating activity in vascular smooth muscle cells (6, 7, 9–11). Thus ET-1 has been implicated in regulation of vascular tonus and progression of atherosclerosis, suggesting that ET-1 may be important in the endothelial dysfunction. Cardillo and colleagues (15) have shown recently that blockade of endothelin A receptor induces significant vasodilation in overweight and obese humans. Therefore, increased vascular production of ET-1 in hypertensive patients with increased body mass has been suggested as a potential mechanism for endothelial dysfunction. In the present study,

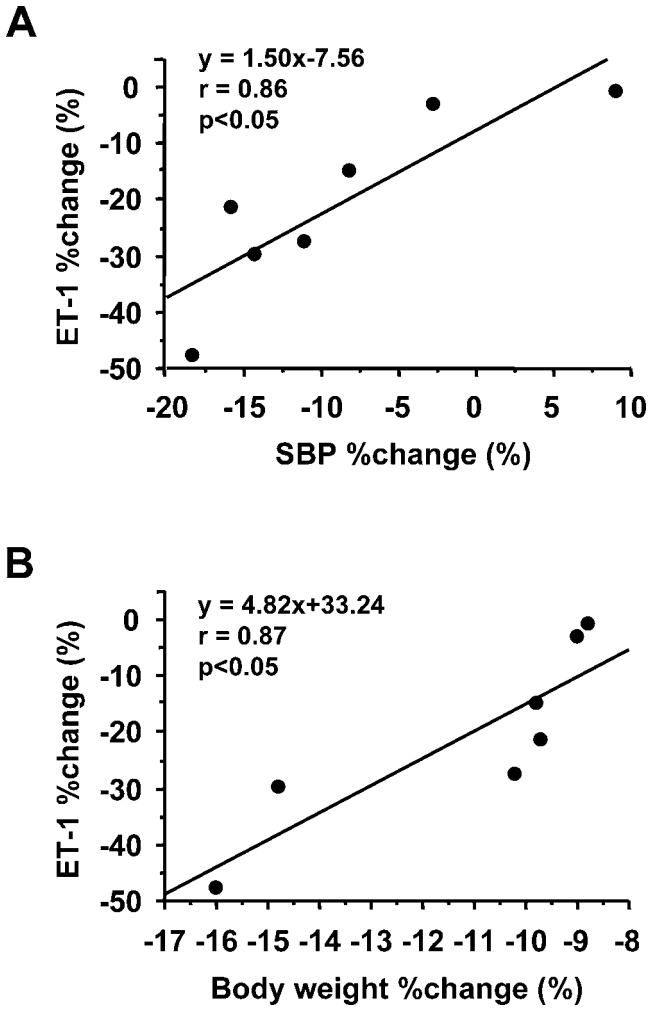

Figure 2. The relationships between the percentage plasma ET-1 concentration reduction and the percentage systolic blood pressure reduction (A) and the percentage weight reduction (B) after 3 months of weight reduction program in obese men.

the plasma ET-1 concentration significantly decreased after the weight reduction program. The present study also demonstrated that blood pressure reduced after the program. We considered that the reduction of ET-1 may participate in the mechanism underlying the improvement of endothelial dysfunction by modest weight loss. Therefore, it is possible that modest weight loss can improve endothelial dysfunction in obese humans through reduction of ET-1 production.

Several reports indicate that weight loss and lifestyle modifications can improve endothelial function. It has been shown that 6 months of weight reduction and exercise improve endothelial function and reduce selective markers of endothelial activation and coagulation in obese individuals (16). Thus lifestyle modification in the form of caloric restriction and moderate intensity physical exercise in obese subjects may be of importance for improvement of endothelial dysfunction. A recent study demonstrated that weight reduction with a very low-calorie diet improves flow-mediated vasodilation in obese subjects (17). It has

been reported that, after 2 weeks of low-calorie intake, a significant improvement in flow-mediated dilatation was observed in obese hypertensive patients (18). Furthermore, healthy premenopausal obese women after 1 year of a weight reduction progam were able to reduce body weight (10%) with an improvement in vascular responses to L-arginine (19, 20). The present study demonstrated that the plasma ET-1 concentration significantly decreased after the weight loss program with reduction of blood pressure. Therefore, we suggest that weight loss (i.e., lifestyle modification) reduces plasma ET-1 concentration in obese men, and this reduction may participate in improvement of endothelial dysfunction, thereby contributing to beneficial effects on the cardiovascular system (i.e., prevention of progression of hypertension and atherosclerosis by endogenous ET-1).

The plasma ET-1 concentration in healthy humans was 1.0–1.5 pg/ml (12), and significantly increased with aging (i.e., plasma ET-1 concentration was markedly higher in healthy older humans than in healthy young or middle-aged humans) (12). Our laboratory previously reported that plasma ET-1 concentration is increased in some human diseases (14, 21) (e.g., chronic heart failure [22] and acute myocardial infarction [21]). In the present study, the plasma ET-1 concentration in obese humans was 5.1 ± 0.4 pg/ml, and was clearly higher in obese humans than in healthy humans. Therefore, the increased plasma ET-1 concentration may be associated with obesity-induced diabetes, hypertension, atherosclerosis, and other cardiovascular diseases.

In conclusion, we demonstrated that weight reduction in obese men significantly decreased plasma ET-1 concentration. Because ET-1 has potent vasoconstrictor and proliferative activity in vascular smooth muscle cells and has been implicated in the regulation of vascular tonus and the progression of atherosclerosis, we propose that the decrease in production of ET-1 by weight loss may be partly involved in the improvement of endothelial dysfunction in obese individuals.

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