Leptin, Superoxide Dismutase, and Weight Loss: Initial Leptin Predicts Weight Loss

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Abstract

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Objective: Our goal was to study how plasma leptin concentration, superoxide dismutase (SOD) activity, and weight loss are related in obese adults.

Research Methods and Procedures: Serum leptin concentration, SOD activities, general biochemical data, and body composition measurements were obtained for 62 overweight and obese subjects before and after an 8-week body weight reduction (BWR) regimen. The subjects were on dietary control, performed moderate aerobic and strength training exercises, and attended educational lectures.

Results: The measurement results indicated that the following criteria were significantly reduced: body weight [84.4 ± 17.0 vs. 79.3 ± 16.1 (standard error) kg, $p < 0.001$]; BMI (31.5 ± 4.3 vs. 29.4 ± 4.2 kg/m^2, $p < 0.001$), and fat mass (33.3 ± 10.0 vs. 29.8 ± 10.4 kg, $p < 0.001$). Plasma leptin levels also significantly decreased from 31.5 ± 17.6 to 26.5 ± 17.2 ng/mL ($p < 0.001$). Additionally, SOD activity was significantly increased from 261.4 ± 66.0 to 302.7 ± 30.9 U/mL ($p < 0.001$). Based on linear regression analysis results, a 3.78- to 8.13-kg reduction in weight can be expected after the 8-week BWR regimen when initial leptin concentration was 5 to 30 ng/mL.

Discussion: We found that an 8-week exercise and diet program was effective in reducing weight and fat mass and, notably, had further beneficial effects on leptin resistance and SOD activity. Additionally, this study demonstrated that initial plasma leptin concentration may be used as a predictor for weight loss outcome.

Key words: weight reduction, fat mass, BMI, leptin, superoxide dismutase activity

Introduction

Obesity endangers human health. Leptin, a protein secreted from adipose tissue and released into circulation, has been strongly correlated with obesity and weight loss (1). Leptin is produced by the *ob* gene and provides a feedback signal that regulates energy homeostasis (2). A mutant mouse with the *ob* gene (i.e., *ob/ob* mouse) typically is extremely obese and lacks leptin expression, which is in contrast to the increased leptin concentrations common in obese patients (3,4). The *db/db* mutant mouse has a leptin receptor mutation that results in a high leptin concentration, a finding similar to that found in clinical studies of obese patients. A positive correlation exists between body weight and *ob* mRNA in adipose tissue (4). A positive correlation has been shown between serum leptin concentrations and BMI and body fat percentage (1,3,5–9).

The antioxidant defense system for living organisms uses scavenger free radicals produced during normal metabolic processes. Several oxidative enzymes, such as glutathione peroxidase (GSH-Px),[1] superoxide dismutase (SOD), catalase, glutathione reductase, some trace elements, and vitamins A, E, and C, are involved in limiting oxidative damage (10). Among these enzymes, SOD is considered the first enzyme in the defense against oxidative stress produced by normal metabolism (11).

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[1] Nonstandard abbreviations: GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; RBC, red blood cell; BWR, body weight reduction; WFH, Wan-Fang Hospital; FM, fat mass; SE, standard error; Mn, manganese; sOB-R, soluble leptin receptor.

A previous animal study of antioxidative enzymes in *ob/ob* mice showed that copper-zinc SOD and hepatic GSH-Px activity were 30% lower than in control mice (12). Additionally, erythrocyte SOD activity in obese individuals was significantly lower than in a normal-weight population (13). In erythrocytes, aerobic (and not anaerobic) training in humans increased SOD activities (14). Alterations in GSH-Px in *ob/ob* mice can be restored by leptin treatment; this finding supports leptin's putative role in modulating antioxidant enzymes (15). Plasma concentrations of nitrite/nitrate in plasma increased in rats injected with leptin (16). In a mouse alcoholic fatty liver model, leptin concentration was markedly elevated (17,18). Administration of alcohol and leptin to Swiss mice reduced body weight and SOD activities (19). These findings indicate that leptin may likely increase body oxidative stress through inducing interaction between superoxide and NO, resulting in the damage of antioxidative enzyme activities, such as SOD and GSH-Px (20).

Despite considerable interest in these issues, limited data exist regarding the effect of exercise on plasma leptin concentration and red blood cell (RBC)-SOD activity in overweight and obese Taiwanese adults. The specific goal of this study was to confirm the effects of an 8-week integrated, hospital-based body weight reduction (BWR) regimen on plasma leptin concentration and erythrocyte SOD activity.

Research Methods and Procedures

Subjects

Sixty-two patients were enrolled from those seeking obesity treatment at the Obesity Research Center of Taipei Municipal Wan-Fang Hospital (WFH). All subjects met the following criteria: apparently healthy; BMI $\geq$27 kg/m^2 (the cut-off point of obesity in Taiwan) (21); and no history of a recent weight reduction program (stable weight for the previous 4 months). Individuals previously diagnosed with diabetes, thyroid disease, renal insufficiency, cardiovascular disease, pulmonary dysfunction, severe hypertension, or severe osteoarthritis of the knees and those taking medications that can affect metabolic variables were excluded. All subjects completed the 8-week BWR regimen. Blood samples were obtained before and after the BWR regimen. This study was approved by the Institutional Human Subject Review Board of WFH, Taiwan. The purpose and objectives of this study were explained to each subject; each subject provided written informed consent.

BWR Regimen

Energy-restricted Diet. Each subject received individual diet counseling by a registered dietitian. Recommended daily caloric intake was 1200 to 1600 kcal/d. Calorie goals were determined by multiplying baseline weight in kilograms by 25 (to obtain an estimate of current caloric consumption) and subtracting 500 kcal to promote a weight loss of roughly 0.50 to 1 kg/wk (22). Participants were advised to consume two commercial meal replacements for lunch and dinner, which were reconstituted to a semiliquid form (Soupal; KAHO Co., Taipei, Taiwan) containing 9 grams of protein, 37 grams of carbohydrate, 4 grams of fat, and 10 grams of dietary fiber (220 kcal in meal). The meal replacement supplied micronutrients according to the recommended dietary allowance in Taiwan. A low-fat breakfast comprising typical breakfast foods, at least five servings of fruits and vegetables, and 2000 mL of water per day were recommended. Subjects were requested to maintain a daily food diary. Dietitians reviewed these food logs weekly and provided additional advice when needed.

Nutritional Education. To optimize cognitive behavior modification, weekly lectures, demonstrations, and group discussions (with and without a supervisor) focused on nutritional topics. Small symposia, given by clinical physicians, were held monthly on topics such as strategies for losing weight and health improvements.

Physical Counseling. The low-impact exercises consisted of two to three sessions (1 h/session) weekly led by a clinical physician.

Body Composition

Technicians measuring experimental variables were blinded to group assignment. Height and weight measurements were obtained while subjects wore clothing and no shoes. Body weight was measured to the nearest 0.10 kg on a calibrated clinical balance scale. Subjects were weighed at baseline and post-BWR with the same scale. Body height was measured to the nearest 0.10 cm on a standardized wall-mounted height board. BMI was calculated as weight in kilograms divided by height in meters squared. Body compositions were assessed using a foot-foot bioimpedance analysis (TBF 410GS; Tanita Corp., Tokyo, Japan) (23). A 3-hour fast was required before body fat measurement using bioimpedance. Fat mass (FM) was calculated by multiplying percentage of body fat by body mass (kilograms). Waist and hip circumferences were measured to assess changes in central obesity. Waist circumference was measured at the narrowest point of the trunk, using a standard spring-loaded measuring tape. Hip circumference was determined at the site of the greatest gluteal protrusion. Each patient was admitted to the hospital for body composition assessment once per week.

Blood Sampling

Blood samples were collected from an antecubital vein into vacuum tubes (Becton Dickinson VACUTAINER Systems; Becton Dickinson and Company, Franklin Lakes, NJ) containing heparin. Blood samples were obtained from all subjects at 8:00 AM to 10:00 AM, after a 12-hour overnight fast, before (at baseline) and after the BWR program. Sam-

Table 1. Body composition and biochemical data of subjects before an 8-week program

	Both sexes ($n = 62$)	Men ($n = 19$)	Women ($n = 43$)	p
Age (years)	35.9 (10.7)	31.1 (9.5)	38.0 (10.6)*	0.017
Body height (cm)	163.6 (8.3)	173.3 (6.2)	159.3 (4.6)‡	0.000
Body weight (kg)	84.4 (17.0)	100.3 (17.6)	77.4 (11.1)‡	0.000
BMI (kg/m^2)	31.5 (4.3)	33.2 (4.5)	30.7 (4.0)*	0.029
Body fat mass (kg)	33.3 (10.0)	33.9 (11.1)	33.1 (9.6)	0.761
Waist (cm)	98.6 (11.8)	108.0 (12.1)	94.4 (9.0)‡	0.000
Hips (cm)	111.7 (8.1)	114.6 (9.1)	110.3 (7.3)	0.068
Glutamate-pyruvate-transaminase (U/L)	48.8 (42.0)	69.0 (53.6)	39.8 (32.2)*	0.038
Total cholesterol (mg/dL)	186.5 (31.0)	173.1 (29.4)	192.5 (30.0)*	0.022
Fasting plasma glucose (mg/dL)	95.4 (17.0)	89.0 (7.8)	98.2 (19.0)†	0.009
Triglyceride (mg/dL)	115.3 (75.3)	129.1 (112.4)	110.0 (52.3)	0.342
Uric acid (mg/dL)	6.8 (1.8)	8.0 (1.8)	6.3 (1.5)‡	0.000

Data are shown as mean (standard error),
p values from Student's t tests between both sexes: * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$.

ples were separated by centrifugation (3000 rpm for 10 minutes), and plasma was transferred to a clean microcentrifuge tube and stored at −70 °C. The Buffy coat was removed, and RBC was transferred to a 15-mL conical tube and washed three times with normal saline. The resulting aliquot was put into microcentrifuge tubes and stored at −70 °C. Plasma leptin levels and RBC-SOD activity were measured using commercial ELISA kits (Diagnostic Systems Laboratories and Randox, respectively; Diagnostic Systems Laboratories, Inc., Webster, TX; Randox Laboratories Ltd., Crumlin, UK). Biochemical data were collected in cooperation with the Laboratory of Medicine at WFH.

Statistical Analysis

Statistical analyses were performed using SPSS 11.0 for Windows XP system (SPSS, Chicago, IL). Data are presented as means [standard error (SE)]. Experimental data taken at baseline and at the end of the 8-week BWR program in the same group were compared using the paired-samples t tests. The Student's t test was used to analyze data between groups. A general linear regression was applied to show relationships between variables. A value of $p < 0.05$ was considered statistically significant.

Results

Baseline Description of Body Composition and Biochemical Data

Study subjects were 17 to 59 years old. Mean age was 31.1 ± 9.5 (SE) years for the men and 38.0 ± 10.6 (SE) for the women. Before the BWR regimen, body weight and BMI were significantly lower and waist circumference was significantly smaller in women than in men (women: 77.43 ± 11.11 kg, 30.7 ± 4.0 kg/m^2, and 94.4 ± 9.0 cm, respectively; men: 100.3 ± 17.6 kg, 33.2 ± 4.5 kg/m^2, and 108.0 ± 12.1 cm, respectively; $p < 0.05$). Total cholesterol and fasting plasma glucose were significantly higher in women than in men at baseline (women: 192.5 ± 30.0 and 98.2 ± 19.0 mg/dL, respectively; men: 173.1 ± 29.4 and 89.0 ± 7.8 mg/dL, respectively). The glutamate-pyruvate-transaminase and uric acid levels were significantly lower in women than in men (women: 39.8 ± 32.2 U/L and 6.3 ± 1.5 mg/dL, respectively; men: 69.0 ± 53.6 U/L and 8.0 ± 1.8 mg/dL, respectively; Table 1).

Changes in Body Composition after the BWR Regimen

The BWR regimen resulted in a significant decrease in body weight and BMI in both men and women (men: 7.6 ± 4.3 kg and 2.6 ± 1.5 kg/m^2, respectively; women: 4.0 ± 2.5 kg and 1.8 ± 1.6 kg/m^2, respectively; $p < 0.001$) and significant changes in body composition variables such as body FM and waist and hip circumferences. For both genders combined, body weight, BMI, FM, and waist and hip circumferences decreased significantly (body weight: 84.4 ± 17.0 vs. 79.3 ± 16.1 kg, $p < 0.001$; BMI: 31.5 ± 4.3 vs. 29.4 ± 4.2 kg/m^2, $p < 0.001$; body FM: 33.3 ± 10.0 vs. 29.8 ± 10.4 kg, $p < 0.001$; waist: 98.6 ± 11.8 vs. 92.7 ± 11.6 cm, $p < 0.001$; hips: 111.7 ± 8.1 vs. 105.2 ± 15.2 cm, $p < 0.001$). After the 8-week BWR regimen, changes to body composition were similar for both genders (Table 2).

Table 2. Body composition of subjects before and after an 8-week intervention period

	Both sexes (n = 62)	Men (n = 19)	Women (n = 43)
Body weight (kg)			
Before	84.4 (17.0)	100.3 (17.6)	77.4 (11.1)
After	79.3 (16.1)*	92.7 (18.2)*	73.4 (10.9)*
BMI (kg/m^2)			
Before	31.5 (4.3)	33.2 (4.5)	30.7 (4.0)
After	29.4 (4.2)*	30.7 (4.7)*	28.9 (3.9)*
Body fat mass (kg)			
Before	33.3 (10.0)	33.9 (11.1)	33.1 (9.6)
After	29.8 (10.4)*	30.0 (13.1)*	29.7 (9.1)*
Waist (cm)			
Before	98.6 (11.8)	108.0 (12.1)	94.4 (9.0)
After	92.7 (11.6)*	101.4 (12.0)*	88.7 (9.0)*
Hips (cm)			
Before	111.7 (8.1)	114.6 (9.1)	110.3 (7.3)
After	105.2 (15.2)*	110.2 (9.2)*	102.9 (16.8)*

Data are shown as mean (standard error).
* $p < 0.001$ compared with baseline values.

Plasma Leptin Concentrations and RBC-SOD Activities

Before the BWR regimen, leptin concentrations and RBC-SOD activities were significantly higher in women than in men (leptin: 36.9 ± 17.1 ng/mL in women and 19.36 ± 12.0 ng/mL in men, $p < 0.001$; RBC-SOD: 274.0 ± 55.6 U/mL in women and 232.6 ± 79.4 U/mL in men, $p < 0.05$). After the BWR program, leptin concentration decreased significantly, by 5.8 ± 12.4 and 3.2 ± 9.2 ng/mL ($p < 0.01$) in women and men, respectively (Figure 1A). The RBC-SOD activities increased significantly, by

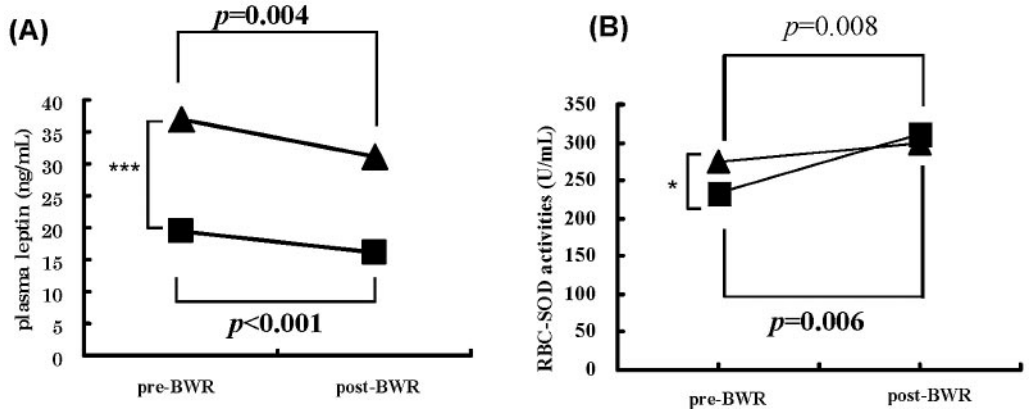

Figure 1: (A) Changes in plasma leptin concentration in male (■) and female (▲) groups from pre- to post-BWR. *** $p < 0.001$ (women compared with men at baseline). (B) Changes in RBC-SOD activities in male (■) and female (▲) groups from pre- to post-BWR. * $p < 0.05$ (women compared with men at baseline).

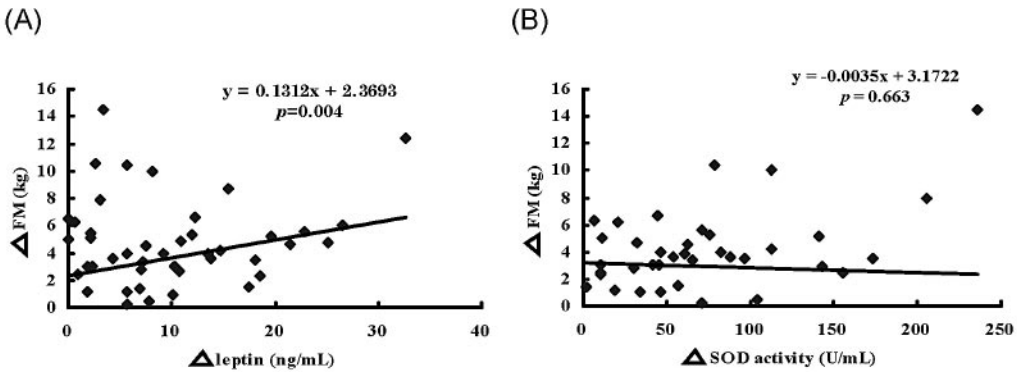

Figure 2: Relationship of Δleptin (A) and ΔSOD (B) with fat mass loss (ΔFM).

24.8 ± 58.8 ($p = 0.008$) and 78.8 ± 100.5 U/mL ($p = 0.006$) in women and men, respectively, after the BWR regimen (Figure 1B).

Relationship Among Body Composition, Leptin Concentration, and RBC-SOD Activity

After the BWR regimen, changes in FM (ΔFM) were significantly and positively correlated (Figure 2A) with changes in leptin concentration (Δleptin; $p = 0.004$), but not RBC-SOD activity ($p = 0.663$; Figure 2B). Reduction in leptin/BMI ratio was significant in women ($p = 0.042$) but not in men; no significant reduction was noted in leptin/FM in men or women (Figure 3). The SOD/leptin ratio ($p < 0.01$ and $p < 0.001$, respectively) in men and women increased significantly after the BWR regimen (Figure 4). Weight reduction after the BWR regimen was negatively correlated with initial leptin ($p < 0.001$; Figure 5A) but not with SOD ($p = 0.122$; Figure 5B). The group with log [initial leptin] <1.477, which represents the 52% of lower initial leptin concentration population, showed significant correlation between initial leptin concentration and weight loss ($p < 0.01$; Figure 5C). We developed a linear regression equation, $y = -9.118x + 17.244$, that can predict a weight loss of 3.78 to 8.13 kg with an initial leptin concentration of 5 to 30 ng/mL after an 8-week BWR program (Figure 5D).

Discussion

This study assessed the effects of an 8-week integrated, hospital-based BWR regimen on plasma leptin concentration and erythrocyte SOD activities. Experimental results indicated that leptin levels were significantly reduced, and SOD activity was significantly increased in both men and women after the BWR regimen. Additionally, at baseline, leptin concentration was significantly higher in women than in men, a finding in agreement with that obtained by Lonnqvist et al. (24), who noted that obese women had 2-fold higher leptin levels than men. In this study, leptin levels of obese subjects were significantly higher in women than in men at baseline and after the BWR regimen. In studies by Giannopoulou et al, Thong et al., and Thompson et al. (25–27), leptin concentration was significantly reduced through diet control

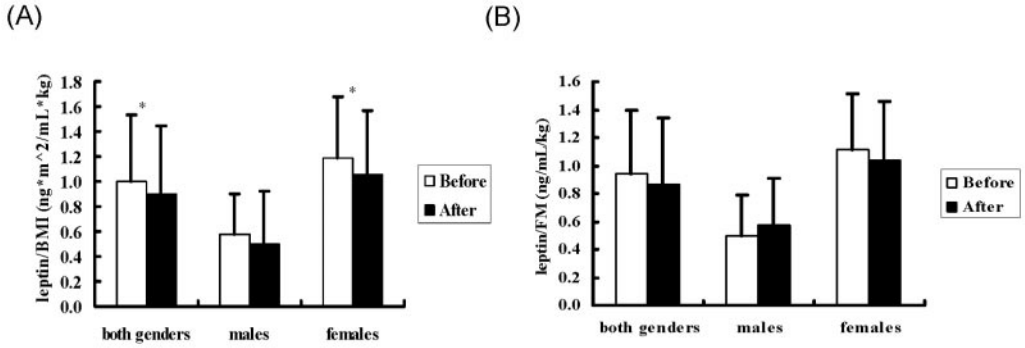

Figure 3: Mean values (SE) of leptin/BMI (A) and leptin/FM (B) ratio observed before (□) and after (■) an 8-week integrated BWR program in 62 obese patients (both genders). * $p < 0.05$.

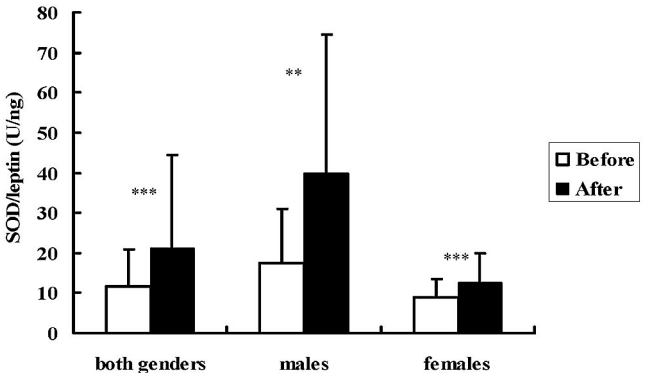

Figure 4: Mean SOD/leptin ratio observed before (□) and after (■) an 8-week integrated BWR program in 62 obese patients (both genders, males, and females). ** $p < 0.01$; *** $p < 0.001$.

or exercise for 14 weeks. In this study, leptin levels in men and women were also significantly reduced after the BWR regimen. Lazzer et al. (28) found that body fat, low-density lipoprotein concentration, and leptin concentration were reduced after weight loss in extremely obese children. Reinehr et al. (29) also shows that significant correlations existed between variation in leptin/BMI ratio and body fat after weight loss. In this study, body weight, BMI, body fat, and waist and hip circumferences in men and women were also significantly reduced after the BWR regimen. Leptin/BMI ratio was significantly reduced in both sexes after the BWR regimen.

In the study by Tungtrongchitr et al. (30), serum zinc concentration and SOD activity were lower in the overweight group than in a control group. Chang et al. (31)

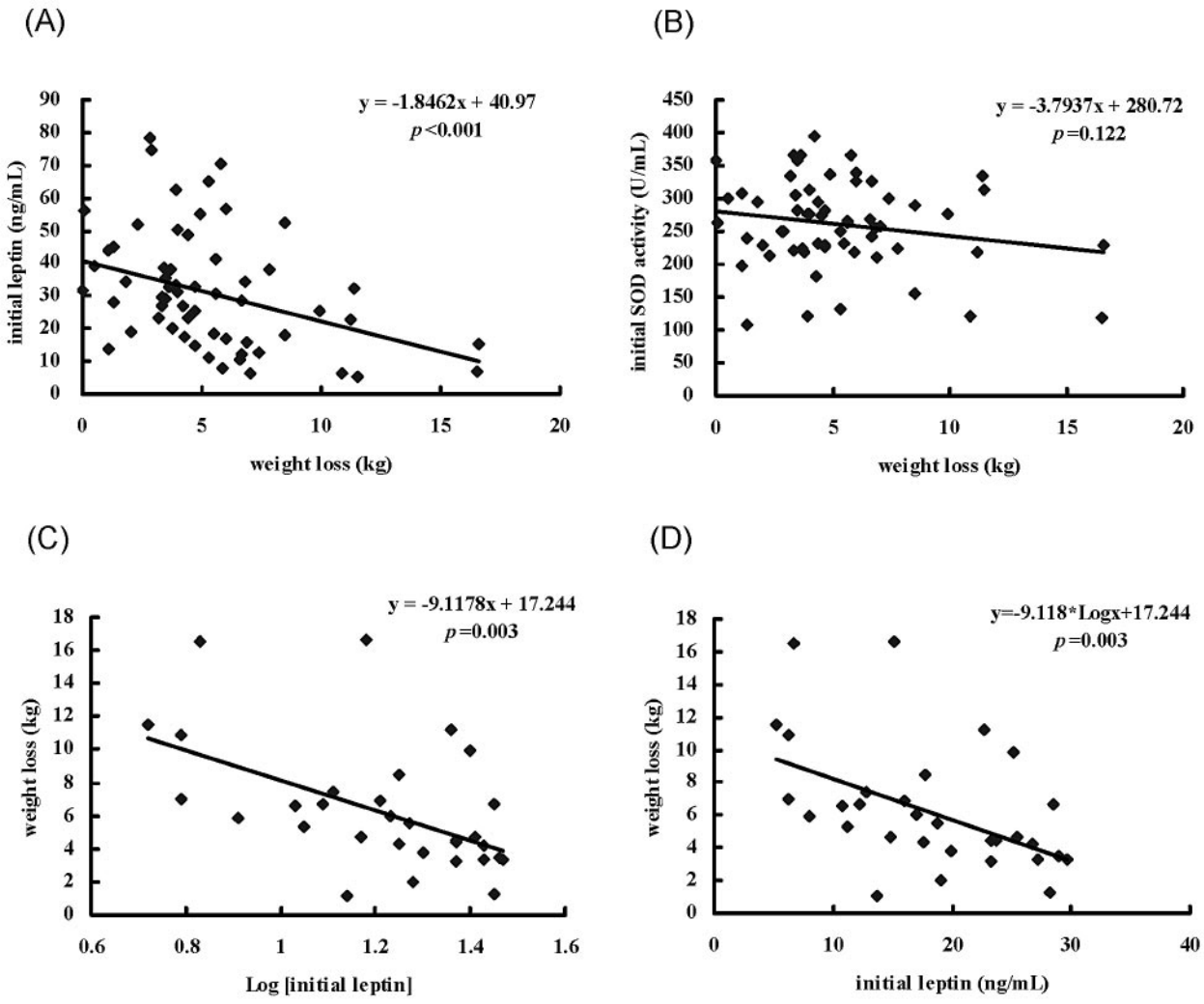

Figure 5: Relationship of initial leptin (A) and initial SOD (B) with weight loss (Δweight). Relationship between log[initial leptin] and weight loss in the 52% of the study subjects with lower initial leptin concentration (C), and the prediction of weight loss according to initial leptin concentration (D).

showed that a significant reduction in mRNA levels and protein content of hepatic manganese (Mn)-SOD and GSH-Px enzymes was found in non-exercising obese groups; however, mRNA and protein levels for these enzymes were substantially increased after exercise training. Regular moderate exercise can improve antioxidant defense function of Mn-SOD, GSH-Px, and GSH in obese Zucker rats. Nakatani et al. (32) found that SOD activity in the diaphragm and kidney increased in rats that habitually exercised. Tauler et al. (33) also observed that SOD activity increased by 25% after a submaximal test of professional athletes, but not amateur athletes. According to a study by Parise et al. (34), exercise training significantly increased copper-zinc–SOD in older adults. This study showed that RBC-SOD activity was clearly elevated in both sexes after the BWR regimen. Parise et al. observed that unilateral resistance exercise was an adequate stimulus for increasing antioxidant enzyme activity and may have suppressed increases in reactive oxygen species and protein carbonyl levels (34). There are three possible explanations that could account for the increase in SOD activity: 1) oxidative stress produced by exercise during the BWR regimen; 2) enhanced antioxidative ability after losing weight; and 3) oxidative stress produced by plasma leptin is strongly correlated with SOD, as indicated by the fact that RBC-SOD activity increased as plasma leptin levels reduced. Furthermore, an increase in RBC-SOD activity is significantly related to reduction of leptin, as indicated by the fact that ΔSOD/leptin increased in women and men. After conversion of $O_2^{-\cdot}$ into H_2O_2 by SOD, catalase and GSH-Px take over for the subsequent reaction of converting H_2O_2 into H_2O. There are several enzymatic and non-enzymatic biomarkers that can be used for monitoring antioxidative status (35). In the study of Chang et al. (31), increased Mn-SOD, GSH-Px, and GSH mRNA and protein levels were found in the obese Zucker rat after exercise. We focused on the first-line defense enzyme, SOD, in this study, but consideration should also be made regarding the other biomarkers.

A positive correlation was noted between Δleptin and ΔFM ($p = 0.004$), despite no significant differences in leptin/BMI ratio in men or leptin/FM ratio in men and women. The leptin variation is significantly positively correlated with FM variation but not with SOD variation. This indicates that FM has direct effects on leptin concentration, because leptin is produced by fat cells; in contrast, FM has no obvious effects on SOD activity variation.

Di Stefano et al. (36), who studied a substantial number of prepubertal and pubertal obese children, observed that high baseline leptin levels were related to increased BMI reductions after a long-term, education-based weight reduction program. Conversely, Sartorio et al. (37) showed that a high baseline leptin concentration can negatively affect weight loss in severely obese patients after a 3-week BWR program. This study also indicated that individuals with low initial plasma leptin concentrations achieved substantial weight loss. That is, individuals with high initial leptin concentrations may achieve less weight loss than those with low initial leptin concentrations. Leptin overproduction likely plays a negative role in weight loss because of leptin resistance in obese subjects. A control group with BMI <27 kg/m^2 is needed to elucidate the effect of leptin resistance in obese subjects. This study compared weight loss variables before and after the integrated BWR program. Leptin regulates energy expenditure and homeostasis (38). A recent study by Rosenbaum et al. (39) showed that low-dose leptin administration reverses the effects of weight gain after weight loss. Circulating leptin in humans is either in free-form or bound-form with its receptor, OB-R (40). A soluble form of OB-R (sOB-R) is the major receptor of leptin protein in circulation. In the study by Laimer et al. (41), the authors concluded that sOB-R may negatively regulate leptin, because circulating leptin was decreased, whereas sOB-R and bound-leptin were increased, in a group of obese women undergoing weight loss induced by surgical intervention. This study used an 8-week BWR regimen; the effects of leptin on weight loss during a long-term BWR and weight regain can be assessed in a future study.

We found that in the group with log [initial leptin] <1.477, which represented 52% of the subjects studied, significant correlation was found between initial leptin concentration and weight loss. According to the linear regression equation, $y = -9.118x + 17.244$, we can predict a weight loss of 3.78 to 8.13 kg with the initial leptin concentration of 5 to 30 ng/mL after an 8-week BWR program.

In conclusion, this large-scale study examined the relationship between leptin and SOD activities in obese subjects before and after an 8-week BWR regimen. This study indicated that plasma leptin concentration was significantly reduced and RBC-SOD activity was significantly increased after the BWR regimen. There is a complicated mechanism among obesity, leptin concentration, and activity of antioxidative enzymes. For the first time, to our knowledge, using a linear regression model, we demonstrated that an initial plasma leptin concentration can be utilized as a predictor for BWR outcome.

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