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# Effects of bariatric weight loss surgery on glucose metabolism, inflammatory cytokines, and serum tartrate-resistant acid phosphatase 5a in obese Chinese adults

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ABSTRACT

*Background:* We determined effects of bariatric weight loss surgery on serum tartrate-resistant acid phosphatase 5a (TRACP 5a), inflammatory cytokines and glucose homeostasis in severely obese Chinese adults.
*Methods:* Severely obese adults undergoing bariatric surgery were recruited. Anthropometry, insulin resistance (IR), inflammatory markers and serum TRACP 5a were measured at baseline and 3, 6 and 12 months postoperatively.
*Results:* Data of 93 patients, including 69 non-diabetic (non-DM group) and 24 diabetic (DM group), were analyzed. Anthropometry decreased significantly at 3 months postoperatively in both groups; low-density lipoprotein cholesterol decreased obviously at 3, 6 and 12 months in non-DM group, while improving significantly at 6 and 12 months in DM group. Homeostasis model assessment for IR (HOMA-IR) improved significantly at 3, 6 and 12 months in non-DM group and 12 months in DM group. In DM group, C-reactive protein (CRP) decreased significantly at 3 months postoperatively and inflammatory markers interleukin-6 (IL-6) and TRACP 5a improved at 6 months postoperatively; in non-DM group, serum TRACP 5a decreased obviously at 12 months postoperatively without significant changes in CRP and IL-6.
*Conclusion:* Weight reduction by bariatric surgery decreases anthropometry, IR, lipids and inflammatory markers in severely obese Chinese adults.



## 1. Introduction

Obesity is a chronic multifactorial disease that has reached epidemic proportions throughout the world [1,2], as well as being a relevant risk factor for other chronic diseases, including type 2 diabetes mellitus, hypertension, dyslipidemia, metabolic syndrome, coronary heart disease, ischemic stroke, nonalcoholic steatohepatitis, polycystic ovarian syndrome, respiratory disease (sleep apnea and obesity-hypoventilation syndrome), gallbladder disease, musculoskeletal disease, and certain cancers [3]. In Taiwan, the prevalence of obesity and associated chronic diseases has increased rapidly [4]. Data from three consecutive Nutrition and Health surveys in Taiwan showed that, since 1993–1996, the prevalence of obesity has tripled for boys and doubled for girls in elementary school. Defined by body mass index (BMI) ≧27 kg/m$^2$, the prevalence of obesity in adults increased from 10.5% for men and 13.2% for women in the 1993–1996 survey, to around 17% in 2005 [4].

Numerous molecules derived from adipose tissue have been shown to influence glucose homeostasis, vascular biology, tumor development, and lipoprotein metabolism and on the inflammatory process [5,6]. The cytokines produced lead to a chronic inflammatory state within the organism that subsequently results in local and systemic alterations, leading to pathologies associated with increased body fat [7]. The link between adiposity and insulin sensitivity may be mediated through certain adipokines and other inflammatory mediators. Insulin resistance (IR) is associated with high levels of serum interleukin-6 (IL-6) and C-reactive protein (CRP) [8,9]. Obesity is associated with increased macrophage infiltration into adipose tissue, which leads to a chronic inflammatory state and contributes to IR [10,11]. Measurement of these

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cytokines and acute phase reactants may be used to assess the severity of chronic inflammation, disease risk and treatment response. Some studies have suggested that bariatric surgery and/or weight loss may result in decreased IL-6 and CRP levels in adults, and these changes have been associated with improved insulin sensitivity [12,13].

Tartrate-resistant acid phosphatase (TRACP) is a marker of differentiation and activation of monocyte-derived cells [14]. In human blood, TRACP circulates as two isoforms, TRACP 5a and 5b, each with unique properties and clinical significance [15]. TRACP 5b is a proteolytically processed isoform released by bone resorbing osteoclasts [16]; it reflects systemic osteoclast numbers [17] and correlates closely with bone resorption [18]. TRACP 5a is an intact polypeptide secreted selectively by macrophages and dendritic cells in vitro [19], and represents the vast majority of circulating TRACP protein in humans [20]. Immunohistochemical studies show that macrophages and dendritic cells that are associated with inflammatory pathology contain abundant TRACP 5a, which could be the source of serum TRACP 5a [21,22]. Based on our previous studies on rheumatoid arthritis [23], end-stage renal disease [24], childhood obesity [25], cardiovascular disease [26] and sarcoidosis [27], we propose that serum TRACP 5a signifies the extent of the systemic macrophage burden or the severity of chronic inflammatory diseases.

Recently, TRACP messenger RNA and a monomeric TRACP protein, equivalent to serum TRACP 5a, were shown to be more abundantly expressed in adipose-derived macrophages from obese adults than in those of lean individuals [28]. A TRACP transgenic mouse subline was developed that preferentially expressed monomeric TRACP 5a-like protein in adipose tissue macrophages and displayed spontaneous insulin-sensitive obesity [28]. We hypothesized that elevated serum TRACP 5a may be an indicator of advanced or systemic inflammatory disease and treatment reducing adiposity may be able to reduce its concentration and lessen the inflammatory burden.

## 2. Subjects and methods

### 2.1. Subjects

A total of 93 severely obese adult patients who had received weight loss treatment at Min-Sheng Bariatrics by undergoing laparoscopic roux-en-y gastric bypass surgery or laparoscopic vertical banded gastric partition were enrolled. The study had a longitudinal design in which each subject served as their own control. All subjects met the inclusion criteria, including that they had not participated in a weight loss program for at least 6 months prior to the study, and were nonsmokers. Subjects were eligible for surgery after evaluation (clinical, psychological, and nutritional) at the time they were recruited for the study. Exclusion criteria were (1) age $<18$ y or $>65$ y, (2) BMI $<30$ kg/m$^2$, and (3) the presence of heart disease, renal disease, secondary obesity, or underlying genetic syndromes. The use of all medication, including that used to treat metabolic syndrome and diabetes, was monitored throughout the study. Recent diet history, using 3-day food records, was collected at each study visit. The Taipei Veteran General Hospital Ethics Committee and Min-Sheng Healthcare Institution Review Board approved the study, and all patients gave informed consent before they were enrolled. After fulfilling the inclusion criteria, all subjects were asked to maintain their eating habits and to record a baseline diet log before initiation of the study. However, diet-related lifestyle changes were not monitored.

### 2.2. Protocol design

Measurements of anthropometry, lipids, IR, and serum inflammation markers were obtained at baseline and at 3, 6 and 12 months after surgery. The measures were obtained during the week before the baseline and 3, 6 and 12 months after surgery. Subjects were weight stable ($\pm 1$ kg) and placed on a diet containing sufficient carbohydrates ($\geqq 150$ g) to allow for optimal blood testing. Subjects were analyzed between 8:00 and 8:30 AM after a 12-h fast. Subjects filled out a short questionnaire to verify that the guidelines regarding maintenance of their dietary habits and activity levels were followed. Specifically, subjects were asked to follow a prescribed weight-maintaining high-carbohydrate diet and to refrain from hard exercise 3 days before the baseline anthropomorphic analysis. After each participant emptied his/her bladder, body height (BH), body weight (BW), waist circumference (WC) and hip circumference (HC) were measured by trained staff. Waist–hip ratio (WHR) was calculated as WC divided by HC. BW was measured with subjects in light clothing, in the fasting state, and immediately after voiding in the morning. WC was measured at the midway point between the inferior margin of the last rib and the crest of the ilium in a horizontal plane and measured to the nearest 0.1 cm, whereas HC was determined as the maximum value over the buttocks. Blood pressure (BP) and heart rate were also recorded. Biochemical markers and lipids (triglyceride, TG; total cholesterol, TC; low-density lipoprotein cholesterol, LDL-c; and high density lipoprotein cholesterol, HDL-c) were also assessed in the laboratory of Tri-Service General Hospital. An electrocardiogram was used to evaluate cardiac function in all subjects, and blood samples were taken from the antecubital vein of the arm at 9:00 AM. These specific measurements were also determined in the obese subjects at 3, 6 and 12 months after surgery.

### 2.3. Laboratory measurements

A venous blood sample was taken after a 12-h fast for the measurement of biochemical markers, lipids, plasma glucose (PG), serum insulin, CRP, IL-6, and TRACP 5a. All sera were stored at $-80$ °C and thawed at room temperature immediately before biochemical markers were measured. Serum lipids were measured using commercial assay kits (Roche Diagnostics) in an automated blood chemistry analyzer (Roche-Hitachi 7180, Roche). Serum HDL-c was determined by a polyethylene glycol-modified enzymatic cholesterol assay after detran sulfate precipitation. PG was detected using the glucose oxidase method (Model 2300 Stat, Yellow Springs Instrument). Serum insulin was determined by a microparticle enzyme immunoassay using the Axsym system from Abbott Diagnostics (Abbott Laboratories). Homeostasis model assessment for IR (HOMA-IR), or the IR index, was applied to estimate the degree of IR [HOMA-IR $=$ SI $\times$ PG/22.5, where insulin is expressed in μunit/ml and glucose in mmol/l] [29].

### 2.4. Biochemical markers

Serum TRACP 5a protein was determined by two-site immunoassay as previously described [23,30]. Briefly, streptavidin wells (Pierce Chemical Co.) were coated with 1 μg biotinlylated mab220 specific for serum TRACP 5a. After a brief wash, 10 μl of serum samples were added with 90 μl dilution buffer in duplicate and incubated overnight at 4 °C. Wells were washed and 100 μl of a 1:1000 dilution of horseradish peroxidase (HRP)-conjugated anti-TRACP mab162 were added. After 1 h incubation wells were washed again and HRP was detected with a solution of *o*-phenylene diamine and $H_2O_2$ at pH 5.0. After 15 min, the reaction was stopped with 50 μl 2 mol/l $H_2SO_4$. TRACP 5a assay was calibrated with 2-fold serial dilutions of partially purified serum adjusted to concentrations from 5 μg/l to 0.08 μg TRACP 5a/l. Serum CRP was estimated by an in-house, high-sensitivity two-site immunoassay, similar to that for TRACP 5a, constructed from purified and HRP-conjugated polyclonal antibodies to human CRP and purified human CRP as standard (Dako Denmark) [23]. Serum IL-6 was determined using a commercial immunoassay kit (RayBiotech).

### 2.5. Statistical analyses

The differences between baseline measures and measures at 3, 6 and 12 months after surgery were analyzed using the Wilcoxon signed ranks test, while differences between DM and non-DM groups were