岩手医科大学
審査学位論文
（博　士）

ORIGINAL

Advance Publication
doi: 10.1507/endocrj. EJ13-0524

Effects of changes in adipocyte hormones and visceral adipose tissue and the reduction of obesity-related comorbidities after laparoscopic sleeve gastrectomy in Japanese patients with severe obesity

Akira Umemura[1)], Akira Sasaki[1)], Hiroyuki Nitta[1)], Koki Otsuka[1)], Takayuki Suto[2)] and Go Wakabayashi[1)]

[1)] *Department of Surgery, Iwate Medical University School of Medicine, Morioka 020-8505, Japan*
[2)] *Division of Surgery, Morioka Municipal Hospital, Morioka 020-0866, Japan*

Abstract. The aim of this study was to evaluate the relative contribution of serum adipokines and adipokines from the patient's omentum-derived adipocytes (PODAs) and visceral adipose tissue (VAT) of Japanese patients with severe obesity. Secondarily, we analyzed patients' metabolic changes after laparoscopic sleeve gastrectomy (LSG.). Twenty-three LSG patients and 23 non-obese patients undergoing elective abdominal surgery were enrolled. The levels of adipokines in the serum and the PODAs were measured. The clinical and metabolic data were evaluated at 6 months after LSG. The mean serum leptin levels and the mean serum PAI-1 levels were significantly greater ($p < 0.001$) and the mean adiponectin levels were significantly lower in the LSG group ($p = 0.006$). In the measurements of the PODAs, the mean leptin levels ($p < 0.001$) were significantly greater and the mean adiponectin levels ($p < 0.001$) were significantly lower in the LSG group. The mean BMI (– 12 kg/m^2, $p < 0.001$) and mean VAT (– 135.5 cm^2, $p = 0.001$) were significantly decreased after LSG. In nine patients with type 2 diabetes mellitus, the reduction in VAT correlated with the change in high-sensitivity C-reactive protein ($p = 0.006$) and the homeostasis model of assessment of insulin resistance ($p = 0.001$). After 6 months, LSG markedly improved most obesity-related comorbidities. Our results suggest that LSG may contribute to VAT reduction, improved adipocyte hormone levels, and changes in gut physiology and endocrinology.

Key words: Laparoscopic sleeve gastrectomy, Adiponectin, Leptin, Type 2 diabetes mellitus, Bariatric surgery

SEVERE OBESITY is a major public health problem that contributes significantly to the leading causes of death: cardiovascular disease, type 2 diabetes mellitus (T2DM), and cancer. Bariatric surgery has been shown to be an effective method for achieving sustained weight loss and improvement in comorbidities. In 2011, more than 340,000 bariatric surgeries were performed worldwide [1]. Among such surgeries, laparoscopic sleeve gastrectomy (LSG) was originally intended as the first-stage procedure of biliopancreatic diversion in high-risk patients but has now become a stand-alone operation [2, 3]. In 2012, there was a precipitous increase in the use of LSG (36 %), with a concurrent reduction in the use of laparoscopic Roux-en-Y gastric bypass (LRYGB, 56 %) and a major reduction in laparoscopic adjustable gastric banding (LAGB, 4 %) in the United States [4]. In Japan, there were 228 (54 %) LSG procedures performed in 2011 [5]. Recent studies have shown that LSG is safe and effective, resulting in weight loss somewhere between that of LRYGB and LAGB [6, 7]. Although LSG is a restrictive procedure, removing the gastric fundus, the primary site of ghrelin production, appears to have a hormonal effect, enhancing weight loss by reducing appetite. However, the resolution and improvement rates of obesity-related comorbidities and metabolic changes for Japanese patients with severe obesity after LSG have not yet been established.

Visceral adipose tissue (VAT) can cause metabolic abnormalities by secreting inflammatory adipokines, which induce insulin resistance and T2DM. However, VAT might have beneficial metabolic effects by producing adiponectin. The importance of VAT adipokine production in the pathogenesis of the metabolic abnormalities associated with obesity has not been studied.

Submitted Dec. 10, 2013; Accepted Jan. 15, 2014 as EJ13-0524
Released online in J-STAGE as advance publication Jan. 30, 2014
Correspondence to: Akira Umemura, Iwate Medical University School of Medicine, 19-1 Uchimaru, Morioka, Iwate 020-8505, Japan. E-mail: aumemura@iwate-med.ac.jp

Adipose tissue synthesizes and secretes many peptides that are involved in the regulation of energy homeostasis, insulin action and lipid metabolism. Important adipokines are leptin and adiponectin. Leptin plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism [8]. Adiponectin has insulin-sensitizing, anti-artherogenic, and anti-inflammatory actions [9]. Currently, there is limited evidence of the interaction between leptin and adiponectin systems in humans. VAT is thought to be a more potent contributor to the development of hypertension, insulin resistance, and other obesity-related metabolic abnormalities compared with subcutaneous adipose tissue (SAT) because of its greater metabolic activity and its access to the portal circulation system [10–12]. One of the most important improvements in metabolic status after LRYGB is increased insulin sensitivity or T2DM remission [13–17]. VAT loss and adipokine secretion may be more important as a mediator of improved glucose metabolism after the restrictive procedure of LSG, but this link has not been established.

The primary aim of this study was to evaluate the relative contribution of serum adipokines and adipokines from the patient's omentum-derived adipocytes (PODAs) and adipose tissue of Japanese patients with severe obesity. Secondarily, we analyzed the early metabolic changes and the reduction of obesity-related comorbidities at 6 months after LSG.

Materials and Methods

Patients and study protocol

This study was a single-institution, prospective, data collection and analysis study. Between June 2008 and November 2013, 29 Japanese patients with severe obesity underwent LSG. Eligibility for participation in this study was limited to individuals between the ages of 18 and 65 with a body mass index (BMI) $\geq$ 35 kg/m^2 with comorbid conditions including T2DM, obstructive sleep apnea (OSA), and hypertension according to the 2006 guidelines of the Japanese Society for the Study of Obesity and failure to adhere to regimens of diet and behavior therapy for at least 6 months. All the patients gave written informed consent after receiving detailed explanations of all procedures. All patients were evaluated preoperatively by a multidisciplinary team. The LSG was performed laparoscopically by a single surgeon, and involved a gastric-volume reduction of 75 to 80 % by resecting the stomach alongside a 36-French endoscope beginning 4 cm from the pylorus and ending at the angle of His.

As a preoperative diet, all patients received a low-calorie diet that used MICRODIET (Sunny Health, Tokyo, Japan) in one of three meals (1,200 kcal), in home for the three weeks in home prior to admission, and in hospital during the one–week period before LSG. In the dietary guidelines after bariatric surgery, there was a fluid diet (530–600 kcal/day) in the first two weeks, a soft diet (900 kcal/day) in the subsequent two weeks, and a regular diet in the four weeks following LSG. Dieticians prescribed a balanced diet (1,200–1,500 kcal/day) for four weeks to six months thereafter. All patients also received daily multivitamin and mineral supplementation.

After obtaining approval of the institutional review board at Iwate Medical University, 23 obesity patients who were LSG candidates (BMI > 35 kg/m^2; 11 men and 12 women) and 23 non-obese patients (BMI < 25 kg/m^2; 15 men and 8 women) undergoing elective abdominal surgery (e.g., laparoscopic cholecystectomy, colectomy, or incisional hernia repair) were enrolled in the study. In the bariatric patients, the clinical data and the metabolic effects were evaluated at baseline, 1, and 6 months after LSG.

The metabolic and inflammatory parameters that were quantified included fasting serum glucose, insulin, hemoglobin A1c (HbA1c), ghrelin, leptin, adiponectin, triglycerides, total cholesterol, HDL, LDL, high-sensitivity C-reactive protein (HS-CRP), plasminogen activator inhibitor type-1 (PAI-1), and tumor necrosis factor-α (TNF-α). The homeostasis model of assessment of insulin resistance (HOMA-IR) was used in the calculation of insulin resistance. Glucose, insulin, glucagon-like peptide-1 (GLP-1), and gastric inhibitory polypeptide (GIP) levels during a 75 g oral glucose tolerance test (75 g OGTT) were measured. The highest value obtained at 30 min during the 75 g OGTT was defined as the peak level.

Adipokines from the PODAs

During LSG, omental tissue (10 g) was collected and washed with Hank's balanced salt solution (HBSS). Approximately 0.5 g of the omental tissue was sliced into thin sections, treated with 0.2 % collagenase in HBSS with 1.0 % bovine serum albumin at 37 °C for 40 min, filtered through a 600 μm mesh sieve, diluted with HBSS, and centrifuged at 800 × g for 10 min. The resulting pellet was subjected to three repetitions of the above centrifugation procedure and washing

with HBSS and then resuspended and filtered through a 100 μm mesh sieve. The filtrate was suspended in Visceral Adipocyte Culture Medium ver. 2 (Cosmo Bio Co., Ltd., Tokyo, Japan) up to a concentration of 0.5 × 10^5 cells/cm^2 and cultured for two weeks, with the culture medium being replaced every three days [18]. The insulin concentration in the culture medium was 1 ng/ml. The cell adhesion status was monitored in the presence of various proliferators. At the end of the two weeks culture period, adiponectin, leptin, TNF-α, and PAI-1 levels in the supernatant were measured using the enzyme-linked immunosorbent assay method.

Measurements of VAT and SAT by computed tomography

VAT and SAT were measured using a 64-row computed tomography (CT) (Aquilion™: Toshiba Medical Systems Corporation, Tokyo, Japan; Optima CT660: GE Healthcare, Japan). To measure the abdominal fat, after obtaining a single tomographic slice at the umbilicus level by CT scanning, VAT and SAT were determined using a Hounsfield unit (HU) range for adipose tissue of -150 to 0 HU. The VAT and SAT surface areas were calculated and reported in cm^2. The VAT and SAT of the LSG patients were evaluated at baseline, 1 month, and 6 months after the LSG.

Definition of the resolution or improvement of comorbidities

At each postoperative visit, the patients were required to complete a questionnaire that included their current medications and dosages, and their symptoms related to their comorbidities. Based on their answers and our objective findings, we defined the following categories: Complete resolution was defined as the complete cessation of symptoms or no current pharmacological treatment. Improvement was defined as decreased current pharmacological treatment and decreased severity of the associated symptoms. No effect was defined as no change in pharmacological treatment or the associated symptoms. The patients were considered improved if one or more drugs (but not all) had been discontinued or the dose decreased. T2DM remission was defined as a fasting glucose < 100 mg/dl and HbA1c < 5.8 % for at least 6 months in the absence of antidiabetic medication [19].

Statistical analysis

Data are expressed as mean ± standard deviations (SD). The data were collected and analyzed using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA) statistics software. The Student's *t*-test was used to compare the continuous variable, and the x^2 test was used to compare the categorical variable. The relationship between VAT and the outcome variables was evaluated using linear regression analysis by StatMate IV for Windows (ATMS Co., Ltd, Tokyo, Japan). A p value less than 0.05 was considered significant.

Results

Patient characteristics

Forty-six patients were enrolled in this study, and all the subjects adhered to the protocol. Only the LSG patients were followed up. Detailed patient characteristics are shown in Table 1. In the 23 LSG patients, the mean initial BMI was 44.1 ± 5.8 kg/m^2, and two patients (9 %) in the group were "super obese" (BMI > 50 kg/m^2). VAT ($p < 0.001$) and SAT ($p < 0.001$) were significantly greater in the LSG group than in the control group.

Relationship between levels of adipokines in the serum and the PODAs

In the measurements of the serum adipokines, the mean leptin levels ($p < 0.001$) and the mean PAI-1 levels ($p < 0.001$) were significantly greater in the LSG group than in the control group. In contrast, the mean adiponectin level was significantly lower in the LSG group ($p < 0.001$). No significant differences in TNF-α

Table 1 Patient characteristics

	LSG (n=23)	Control (n=23)	p value
Age (years)	41.9 ± 13.4	65.9 ± 14.4	< 0.001
Male/Female	11/12	15/8	0.234
Initial body weight (kg)	125.0 ± 24.6	56.8 ± 8.0	< 0.001
Initial BMI (kg/m^2)	44.1 ± 5.8	23.3 ± 2.4	< 0.001
VAT (cm^2)	288.3 ± 81.6	83.6 ± 14.4	< 0.001
SAT (cm^2)	539.4 ± 161.7	153.8 ± 48.4	< 0.001

LSG, laparoscopic sleeve gastrectomy; BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue

Table 2 Serum adipokines levels and adipokines levels from the patient's omentum-derived adipocyte

	LSG (n=23)	Control (n=23)	p value
Serum			
Leptin (ng/ml)	28.1 ± 18.4	4.7 ± 3.9	< 0.001
Adiponectin (μg/ml)	2.2 ± 1.7	4.1 ± 2.5	< 0.001
PAI-1 (ng/ml)	50.3 ± 23.2	16.1 ± 8.1	< 0.001
TNF-α (pg/ml)	1.8 ± 0.8	1.8 ± 0.9	0.979
Omentum-derived adipocyte			
Leptin (ng/ml)	2.1 ± 0.7	0.5 ± 0.3	< 0.001
Adiponectin (μg/ml)	0.2 ± 0.1	0.4 ± 0.2	< 0.001
PAI-1 (ng/ml)	55.1 ± 52.0	26.0 ± 23.2	0.020
TNF-α (pg/ml)	1.8 ± 1.3	0.8 ± 0.5	0.003

LSG, laparoscopic sleeve gastrectomy; PAI-1, plasminogen activator inhibitor type-1; TNF-α, tumor necrosis factor-α

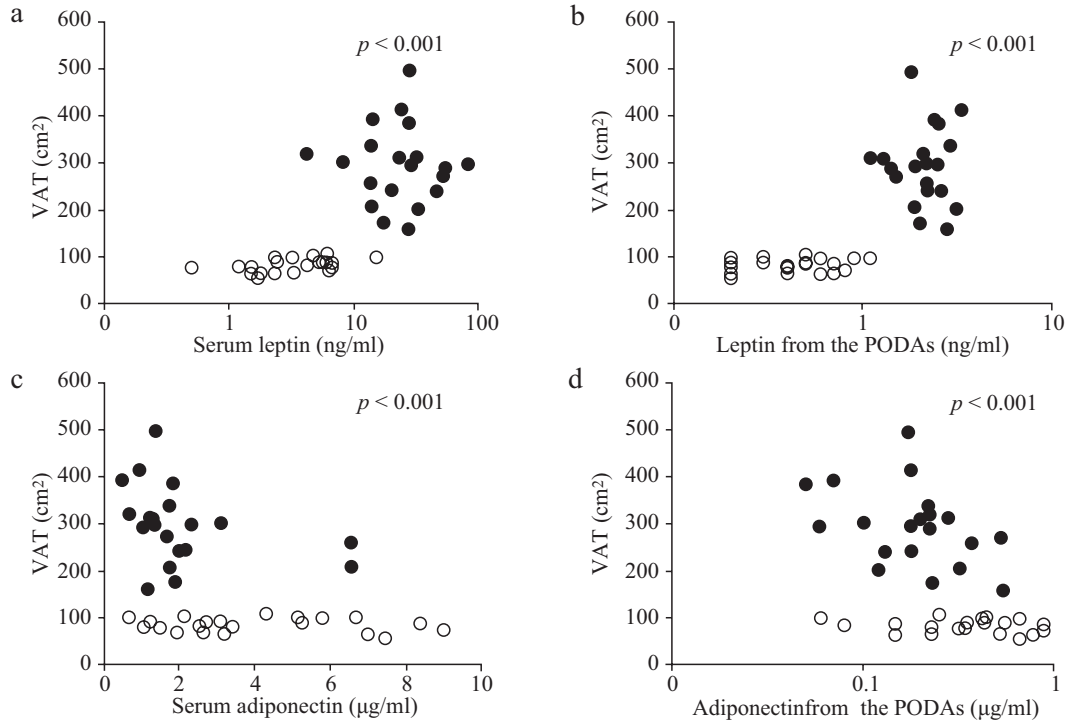

Fig. 1 Correlations between VAT and serum leptin (a), leptin from the patient's omentum-derived adipocyte (b) , serum adiponectin (c), and adiponectin from the patient's omentum-derived adipocyte (d) at baseline in LSG group (close circles) and control group (open circle)

levels were observed between the two groups. In the measurements of the adipokines from the PODAs, the mean leptin levels (p < 0.001), the mean PAI-1 levels (p = 0.020), and the mean TNF-α levels (p = 0.003) were significantly greater. The mean adiponectin levels (p < 0.001) were significantly lower in the LSG group than in the control group (Table 2).

Relationship between adipose tissue volume and adipokine levels

In the LSG group, no significant differences were observed in SAT (588.0 ± 149.5 *vs* 469.1 ± 170.2 cm^2, p = 0.090); in contrast, a significant difference was observed in VAT (322.1 ± 85.8 vs 242.0 ± 54.9 cm^2, p = 0.023) between the male and female patients. The VAT and serum or PODA leptin levels and the VAT and serum or PODA adiponectin levels showed significant differences between the LSG and the control groups (p < 0.001) (Fig. 1). As expected, the mean serum leptin levels (35.8 ± 20.1 *vs* 19.6 ± 9.1 ng/ml, p = 0.029) and the mean PODA leptin levels (2.6 ± 0.6 *vs* 1.5 ± 0.2 ng/ml, p < 0.001) were significantly greater in the LSG

Table 3 Characteristics, metabolic, and inflammatory parameters in LSG patients

	Baseline	1 month	6 months	*p* value [a]
Body weight (kg)	125.0 ± 26.6	104.0 ± 23.9	90.1 ± 22.1	< 0.001
BMI (kg/m^2)	44.1 ± 5.8	36.7 ± 5.2	31.8 ± 4.3	< 0.001
Waist (cm)	121.9 ± 12.0	111.7 ± 12.0	98.8 ± 13.0	0.003
%EWL	–	17.3 ± 5.2	45.6 ± 13.3	0.001 [b]
VAT (cm^2)	288.3 ± 81.6	206.2 ± 46.7	152.8 ± 49.0	0.001
SAT (cm^2)	539.4 ± 161.7	434.2 ± 147.5	293.5 ± 120.9	0.001
TC (mg/dl)	198.3 ± 42.6	173.8 ± 26.4	175.2 ± 29.2	0.100
LDL (mg/dl)	128.4 ± 38.6	108.5 ± 24.6	101.7 ± 29.7	0.049
HDL (mg/dl)	52.0 ± 23.2	39.3 ± 5.5	51.0 ±7.6	0.893
TG (mg/dl)	161.0 ± 104.8	101.7 ± 29.9	89.9 ± 36.6	0.028
Adiponectin (μg/ml)	2.2 ± 1.7	3.4 ± 2.3	3.4 ± 1.7	0.070
Leptin (ng/ml)	28.1 ± 18.4	14.0 ± 10.7	8.6 ± 7.1	0.003
PAI-1 (ng/ml)	50.3 ± 23.3	25.0 ± 12.1	22.8 ± 16.0	< 0.001
TNF-α (pg/ml)	1.8 ± 0.8	1.7 ± 0.5	1.4 ± 0.5	0.127
HS-CRP (ng/ml)	4757 ± 4281	3956 ± 5779	3502 ± 4728	0.510

LSG, laparoscopic sleeve gastrectomy; BMI, body mass index; %EWL, percentage of excess weight loss; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; TC, total cholesterol; TG, triglycerides; PAI-1, plasminogen activator inhibitor type-1; TNF-α, tumor necrosis factor-α; HS-CRP, high sensitivity C-reactive protein

[a], Baseline vs 6 months after LSG; [b],1 month vs 6 months after LSG

group with VAT ≥ 300 cm^2 than in those with VAT < 300 cm^2. No significant differences were observed in the serum and PODA adiponectin levels between the two groups.

Changes in the metabolic and inflammatory parameters in the LSG patients

The demographic, metabolic, and inflammatory parameters in the LSG patients are shown in Table 3. The mean body weight (– 34.9 kg, $p < 0.001$), mean BMI (– 12 kg/m^2, $p < 0.001$), mean percentage of excess weight loss (%EWL) (45.6%, $p = 0.001$), mean VAT (– 135.5 cm^2, $p = 0.001$), and mean SAT (– 245.9 cm^2, $p = 0.001$) were significantly decreased at 6 months after LSG compared to the baseline. LDL cholesterol ($p = 0.049$) and triglycerides ($p = 0.028$) were significantly decreased at 6 months after LSG, and total cholesterol ($p = 0.100$) tended to be lower as well. On the other hand, HDL cholesterol was unchanged.

Six months following LSG, there was a significant decrease in the levels of the proinflammatory parameters leptin ($p = 0.003$) and PAI-1 ($p < 0.001$). At 1 month, the mean serum adiponectin levels of the anti-inflammatory parameter tended to be higher than the baseline levels, and this rise in the adiponectin levels persisted at the 6-month follow-up. The serum TNF-α and HS-CRP levels decreased at 6 months after LSG, but they did not change significantly. Our results show that LSG alone has the potential to produce good results among Japanese patients with severe obesity represented by a BMI of < 50 kg/m^2.

Glucose metabolic changes in LSG patients with T2DM

Six months after LSG, the mean fasting glucose ($p = 0.015$) and the mean HbA1c ($p = 0.006$) improved markedly. There was no significant decrease in hyperinsulinemia ($p = 0.269$) and HOMA-IR ($p = 0.059$). The mean peak GLP-1 levels after OGTT were significantly higher than the baseline levels ($p < 0.001$). No significant differences were observed in the mean peak GIP levels. The mean fasting serum C-peptide levels were significantly lower than the baseline levels ($p = 0.041$). The mean fasting ghrelin levels at one day after LSG were lower than the baseline levels (data not shown), and this change persisted at the 6-month follow-up ($p = 0.002$) (Table 4). LSG patients experienced a markedly reduced appetite throughout the study period.

In the LSG patients with T2DM, variables related to the reduction of VAT from baseline to 1 month after LSG were studied. Using simple linear regressions, the reduction in VAT correlated with the changes in HS-CRP ($r = 0.825$, $p = 0.006$) and HOMA-IR ($r = 0.880$, $p = 0.001$) (Fig. 2).

The mean caloric intake at the initial visit pre-LSG

Table 4 Glucose metabolic changes in LSG patients with T2DM

	Baseline	1 month	6 months	*p* value [a]
Fasting glucose (mg/dl)	138.6 ± 23.2	85.5 ± 8.6	91.3 ± 6.5	0.015
Fasting insulin (ng/ml)	58.0 ± 27.8	11.5 ± 6.2	10.2 ± 5.3	0.269
HOMA-IR	11.5 ± 10.2	2.5 ± 1.4	2.4 ± 1.4	0.059
HbA1c (%)	8.0 ± 1.9	5.5 ± 0.3	5.4 ± 0.3	0.006
GLP-1 (pmol/l) [b]	6.5 ± 4.6	66.6 ± 61.0	33.9 ± 7.5	<0.001
GIP (pg/ml) [b]	373.5 ± 135.7	416.4 ± 186.4	373.3 ± 111.6	0.998
C-peptide (ng/ml)	3.9 ± 2.1	2.5 ± 1.1	2.1 ± 0.9	0.041
Ghrelin (fmol/ml)	99.8 ± 44.0	31.9 ± 12.9	30.5 ± 5.3	0.002

LSG, laparoscopic sleeve gastrectomy; T2DM, type 2 diabetes mellitus; HOMA-IR, homeostasis model of assessment of insulin resistance; HbA1c, hemoglobin A1c; GLP-1, glucagon-like peptide-1; GIP, gastric inhibitory polypeptide

[a], Baseline vs 6 months after LSG; [b], Value at 30 min after 75g oral glucose tolerance test

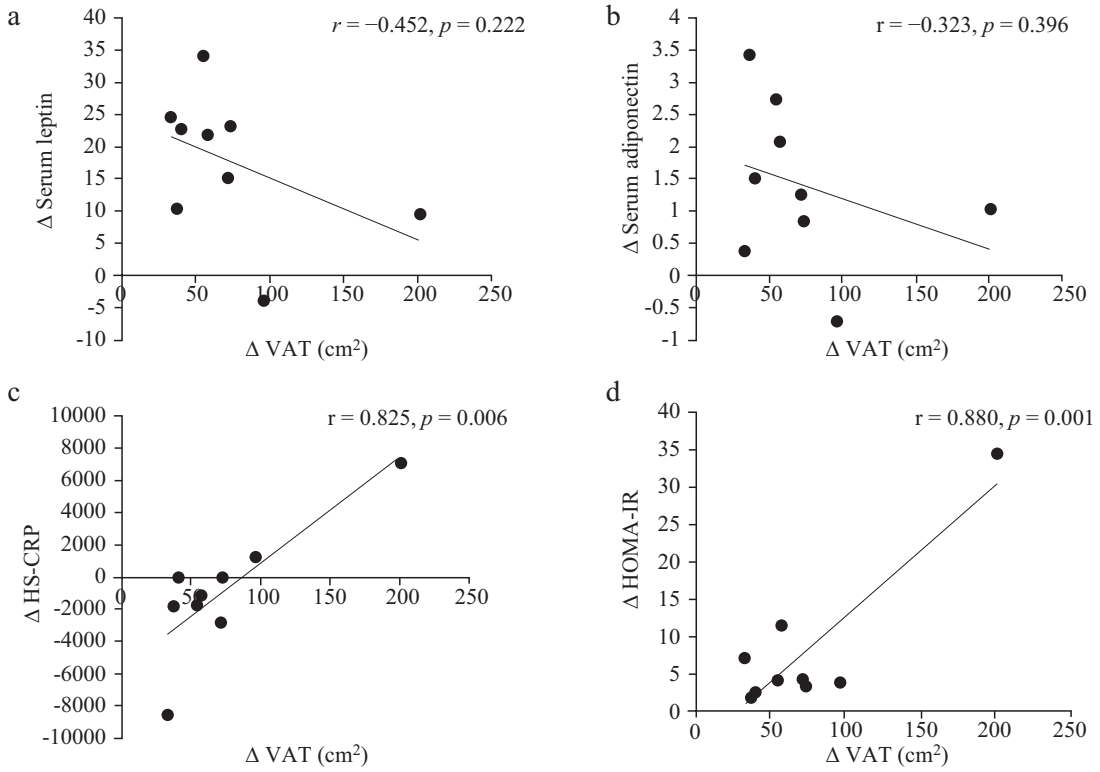

Fig. 2 Linear relationship between the reduction in VAT and reduction in serum leptin (**a**), serum adiponectin (**b**), HS-CRP (**c**), and HOMA-IR (**d**) at 1 month after LSG in T2DM patients

was, surprisingly, 2,655±721 kcal/day (range, 2,250–4,320 kcal/day).

Resolution or improvement of comorbidities

The overall mean number of obesity-related comorbid conditions was 7.0 ± 1.3. Non-alcoholic fatty liver disease (NAFLD), OSA, hypertension, hyperlipidemia, T2DM, and hyperuricemia were present in 23 (100 %), 23 (100 %), 16 (70 %), 15 (65 %), 11 (48 %), and 11 (48 %) patients, respectively. The resolution and improvement rates of comorbidities after 6 months were 100 % for NAFLD, T2DM, hyperlipidemia, and hyperuremia, 91 % for hypertension, and 71 % for OSA. The LSG group not only showed weight loss alone, but also improvements in the prognosis of obesity-related diseases. Changes in medication were analyzed (Fig. 3).

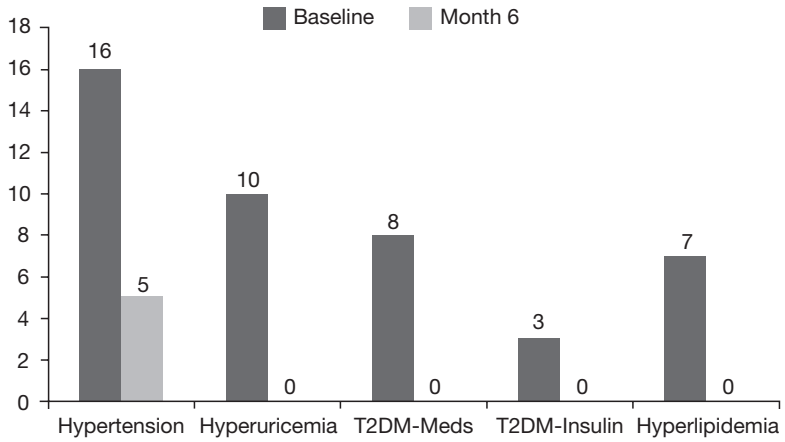

Fig. 3 Number of patients on medications at baseline and 6 months after LSG
T2DM Meds type 2 diabetes mellitus medications, *T2DM Insulin* type 2 diabetes mellitus insulin therapy

At baseline, 16 patients (70 %) were on antihypertensive medication, 10 patients (43 %) were on a uremic acid-lowering agent, 8 patients (35 %) were on hypoglycemic medication, and 7 patients (30 %) were on a lipid-lowering agent. At 6 months after LSG, 22 %, 0 %, 0 %, and 0 % of patients with hypertension, hyperuricemia, T2DM, and hyperlipidemia, respectively, had discontinued their medication. Although 3 (13 %) T2DM patients were using insulin at baseline, none of them were using insulin at 6 months after LSG.

Discussion

Over the last three decades, the mean BMI has increased by 0.4 kg/m^2 per decade worldwide [20]. The problem of obesity in the United States has reached epidemic proportions. It is now estimated that 30 % of American adults are obese, and nearly two-thirds are either overweight or obese [21]. In Japan, BMI values are 30 kg/m^2 or greater in 3 % of the population, 35 kg/m^2 or greater in 0.5 %, and 40 kg/m^2 or greater in 0.2 %, reflecting a lower level of severe obesity than in Europe or the United States [5]. However, a World Health Organization Expert Committee reported that the incidence of health problems stemming from obesity is higher among Asians [22]. In 2011, the International Diabetes Federation (IDF) called for a BMI of 30–35 kg/m^2 to represent a surgical indication for T2DM unresponsive to medical treatment, with priority to be given to a BMI > 35 kg/m^2 [17]. Surprisingly, the IDF also released a statement recommending that this indication criterion be lowered by 2.5 kg/m^2 for Asian individuals. However, it is unknown whether its recommendation is based on genetic considerations or endocrine effects related to transcriptome differences between Asians and all other populations.

Although excess VAT is associated with noninfectious inflammation, it is not clear whether VAT is simply associated with or actually causes metabolic disease in humans. Using PODA obtained from surgical samples, we investigated whether these cells can secrete adipokines. As primary cultures of normal human visceral adipose cells contain impurities such as lipid droplets, it is believed that the cultivation of these cells is very difficult. Nevertheless, the development of a ceiling culture method for obtaining mature adipocytes and recent findings on the pathology of metabolic syndrome have encouraged basic research on obesity-related diseases and bariatric surgery. In the present study of cultured supernatants, adiponectin levels were lower and leptin levels were higher in the supernatants from the LSG group tissues than in the supernatants from the control group tissues, suggesting that obesity might affect the ability of visceral adipose cells to secrete adipokines. It has been found that approximately 40% of patients with *ADIPOQ* gene polymorphisms may have a predisposition toward a low adiponectin level [23], which is probably exacerbated by a high-fat diet and insufficient physical exercise. Single nucleotide polymorphisms and mutations in the obesity gene *LEP*, which encodes leptin, are believed to increase the risk for the onset of severe obesity [24]. Although their serum leptin levels are high, patients with severe obesity show resistance to leptin in general, thereby weakening the

anti-feeding effect of leptin. In the present study, the leptin levels in the PODAs were slightly but significantly increased, suggesting the enhanced ability of the visceral adipose cells to secrete leptin. In the PODAs, the PAI-1 levels were also significantly increased, which probably plays a role in the onset and progression of metabolic syndrome in general. PAI-1 is presumably biosynthesized mainly in the visceral adipose cells and vascular endothelial cells. However, PAI-1 is also deeply involved in the promotion of arteriosclerosis and atherothrombosis formation, and PAI-1 is more likely to be secreted by cells in the circulating blood than by the visceral adipose cells. At present, the basis for the change in adipokine biosynthesis is unclear, and this mechanism needs to be determined in the future. In the present study, the serum leptin levels of patients with > 300 cm^2 of VAT in the LSG group significantly increased, and marked leptin resistance in severe obesity with VAT accumulation probably facilitates the development of obesity and metabolic syndrome. Our results also suggested that the status of the visceral adipose cells in patients with severe obesity receiving LSG might affect these cells' ability to secrete adipokines.

Bariatric surgery is the most effective treatment for severe obesity. Currently, LRYGB is considered the gold standard procedure for severe obesity, and it leads to excellent long-term sustained weight loss and remarkable resolution of comorbidities worldwide [25]. Kasama *et al.* reported that the weight reductions of LRYGB and LSG in Japanese obese patients were 32.0 kg and 27.4 kg 6 months after operation, respectively [26]. They also reported that the BMI changes and %EWL between LRYGB and LSG were -11.8 kg/m^2 and -10.1 kg/m^2, 72.7 % and 49.7 %, respectively [26]. In Japan, LSG is the most frequently performed bariatric surgery, and it accounted for 54 % of such surgeries in 2011 due to its a relatively low rate of complications, less malabsorption, and a shorter learning curve compared to LRYGB [5]. Our results showed a significantly higher %EWL in the LSG group. One of the reasons for this may be that gastrointestinal hormone changes explain some of the decrease in appetite and subsequent weight loss. The greater reduction in hunger after LSG is believed to be due to the resection of the gastric fundus, which produces ghrelin. In the present study, the mean fasting ghrelin levels decreased one day after LSG and this change persisted at the 6-month follow-up. Langer *et al*. [27] reported decreased ghrelin levels 6 months after LSG, in contrast to raised ghrelin levels after LAGB. Consequently, the LSG group had a higher mean %EWL than the LAGB group (61 % *vs* 16 %). However, the mechanism of weight loss after LSG is not clearly understood and there are limited data on the reduction of obesity-related comorbidities after LSG in Japanese patients with severe obesity [5].

The goal of bariatric surgery is not only to achieve satisfactory weight loss but also to obtain an improvement in obesity-related comorbidities, including T2DM, OSA, hyperlipidemia, and hypertension. In general, LRYGB is considered to result in better remission of T2DM compared to LSG. However, the impact of LSG on obesity-related comorbidities for Japanese patients with severe obesity has been reported in very few studies [5]. We found that LSG alone resulted in a 100% remission rate of T2DM at 1 and 6 months after LSG. The high rate of remission of T2DM after LSG has also been reported by many other researchers [28, 29]. Weight loss after LSG is well-documented, and preferential VAT loss is increasingly recognized as a common outcome of LSG. These data document a significant reduction in cardiovascular and metabolic risk factors after LSG and specifically link VAT loss with improvements in the indices of insulin sensitivity. Recent literature has shown that obesity is associated with a low-grade inflammatory state and that proinflammatory cytokines may play an important role in mediating the detrimental effects of obesity on metabolism [30]. In the present study, the T2DM remission and VAT reduction appeared to be more gradual in the LSG patients, and the VAT reduction was associated with changes in HS-CRP and HOMA-IR at 1 month after LSG. Our results show preferential loss of VAT early in the time course of the postoperative weight loss, pointing to the possibility that improved insulin sensitivity after LSG might occur along a time course similar to that after LRYGB.

Marked effects of LSG on ghrelin levels have been reported [31] and have been confirmed by the present study in our small group. Ghrelin infusion is reported to increase insulin resistance, and therefore the removal of the gastric fundus might contribute to improving insulin sensitivity [32]. Although the mechanism of improved glucose tolerance after LSG is unknown, increased secretion of GLP-1 after surgery has been observed, as reported after LRYGB [33]. Basso *et al*. reported a gastric hypothesis that decreased hydrochloric acid production induced by LSG may act on the

innervated antrum to produce a gastrin-releasing peptide responsible for GLP-1 early-phase secretion [34].

Although weight loss itself can change VAT and adipokines, and reduce obesity-related comorbidities, the difference between simple caloric restriction and LSG may have the potential to change GLP-1 and ghrelin levels. In the present study, a mean weight reduction of almost 10 kg was observed during the preoperative diet, and GLP-1 and ghrelin levels remained unchanged from the baseline measurement. Although the mean weight reduction was 11 kg at one month after LSG, the mean GLP-1 levels increased, and mean ghrelin levels decreased significantly. The changes in these hormones may derive from a resection of gastric fundus and hindgut effect with accelerated gastric emptying [5], which would have lead to improvements in obesity-related comorbidities, especially T2DM. In a recent study featuring a randomized, nonblind, single-center trial design, Schauer *et al*. [35] reported the efficacy of intensive medical therapy alone versus medical therapy plus Roux-en Y gastric bypass or sleeve gastrectomy in 150 obese patients with uncontrolled type 2 diabetes. They reported that 12 months of medical therapy plus bariatric surgery achieved glycemic control in significantly more patients than medical therapy alone.

The most important therapy for OSA in obese patients is weight loss, which changes the pharyngeal anatomy and decreases airway collapsibility by increasing the pharyngeal closing pressure [36]. OSA severity is also significantly correlated with fat accumulation in the intra-abdominal region [37]. There is growing evidence that hormonal changes underlie the association between OSA and obesity. The most well-studied adipocyte-derived factor affecting respiratory control is leptin, which is known to play a primary role in binding to receptors in the hypothalamus to reduce satiety and increase metabolism. This phenomenon could perhaps explain the amelioration of OSA in LSG. Hypertension is the most common comorbidity in obese individuals. Critical weight loss is an effective way to control obesity-related hypertension. In the present study, there was a significant reduction in the number or dose of hypertension medications. Although the levels of total, LDL, and HDL cholesterol did not differ significantly after 6 months, there was a significant reduction in the number of medications needed to treat hyperlipidemia.

One limitation of the present study is the selection bias of the surgeon or patients in regard to which procedure was performed. Our study also includes a small sample size from a single institution and short-term follow-up data. Long-term follow-up needs to be conducted to further evaluate the reduction of obesity-related comorbidities after LSG.

We showed *in vivo* and *in vitro* adipokine levels in Japanese obese and non-obese populations. Adipokines were aggressively endocrined in Japanese obese patients proportionately their VAT volumes. In conclusion, LSG markedly improved most obesity-related comorbidities at 6 months after LSG in Japanese patients with severe obesity. These results suggest that LSG may contribute to VAT reduction, improved adipocyte hormone levels, and changes in gut physiology and endocrinology. Further studies are required to determine the complex mechanisms through which adipocyte and gut neurohormonal signals interrelate after LSG.

Acknowledgements

This study was supported by a Keiryokai Collaborative Research Support from Keiryokai Research Foundation (No. 108) and a Grant-in-Aid for Scientific Research (C) 22591440 from the Japan Society for the Promotion of Science.

Disclosure

The authors have no conflicts of interests.

References

1. Buchwald H, Oien DM (2013) Metabolic/bariatric surgery worldwide 2011. *Obes Surg* 23: 427–436.
2. Regan JP, Inabnet WB, Gagner M, Pomp A (2003) Early experience with two-stage laparoscopic Roux-en-Y gastric bypass as an alternative in the super-super obese patients. *Obes Surg* 13: 861–864.
3. Kueper MA, Kramer KM, Kirschniak A, Königsrainer A, Pointner R, *et al.* (2008) Laparoscopic sleeve gastrectomy: standardized technique of a potential standalone bariatric procedure in morbidly obese patients. *World J Surg* 32: 1462–1465.
4. Nguyen NT, Nguyen B, Gebhart A, Hohmann S (2013) Changes in the makeup of bariatric surgery: a national increase in use of laparoscopic sleeve gastrectomy. *J*

Am Coll Surg 216: 252–257.
5. Sasaki A, Wakabayashi G, Yonei Y (2013) Current status of bariatric surgery in Japan and effectiveness in obesity and diabetes. *J Gastroenterol* doi: 10.1007/s00535-013-0802-5
6. Himpens J, Dapri G, Cadière GB (2006) A prospective randomized study between laparoscopic gastric banding and laparoscopic isolated sleeve gastrectomy: results after 1 and 3 years. *Obes Surg* 16: 1450–1456.
7. Hutter MM, Schirmer BD, Jones DB, Ko CY, Cohen ME, *et al.* (2011) First report from the American College of Surgeons Bariatric Surgery Center Network: laparoscopic sleeve gastrectomy has morbidity and effectiveness positioned between the band and the bypass. *Ann Surg* 254: 410–420.
8. Chen J, Pamuklar Z, Spagnoli A, Torquati A (2012) Serum leptin levels are inversely correlated with omental gene expression of adiponectin and markedly decreased after gastric bypass surgery. *Surg Endosc* 26: 1476–1480.
9. Kadowaki T, Yamauchi T (2005) Adiponectin and adiponectin receptors. *Endocr Rev* 26: 439–451.
10. Carroll JF, Franks SF, Smith AB, Phelps DR (2009) Visceral adipose tissue loss and insulin resistance 6 months after laparoscopic gastric banding surgery: a preliminary study. *Obes Surg* 19: 47–55.
11. Kahn BB, Flier JS (2000) Obesity and insulin resistance. *J Clin Invest* 106: 473–481.
12. Arner P (2005) Human fat cell lipolysis: biochemistry, regulation and clinical role. *Best Pract Res Clin Endocrinol Metab* 19: 471–482.
13. Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, *et al.* (2012) Bariatric surgery versus conventional medical therapy for type 2 diabetes. *N Engl J Med* 366: 1577–1585.
14. Jiménez A, Casamitjana R, Flores L, Delgado S, Lacy A, *et al.* (2013) GLP-1 and the long-term outcome of type 2 diabetes mellitus after Roux-en-Y gastric bypass surgery in morbidly obese subjects. *Ann Surg* 257: 894–899.
15. Lee WJ, Chong K, Ser KH, Lee YC, Chen SC, *et al.* (2011) Gastric bypass vs sleeve gastrectomy for type 2 diabetes mellitus: a randomized controlled trial. *Arch Surg* 146: 143–148.
16. Kasama K, Tagaya N, Kanehira E, Oshiro T, Seki Y, *et al.* (2009) Laparoscopic sleeve gastrectomy with duodenojejunal bypass: technique and preliminary results. *Obes Surg* 19: 1341–1345.
17. Dixon JB, Zimmet P, Alberti KG, Rubino F: International Diabetes Federation Taskforce on Epidemiology and Prevention (2011) Bariatric surgery: an IDF statement for obese Type 2 diabetes. *Diabet Med* 28: 628–642.
18. Sugihara H, Yonemitsu N, Miyabara S, Yun K (1986) Primary cultures of unilocular fat cells: characteristics of growth in vitro and changes in differentiation properties. *Differentiation* 31: 42–49.
19. Buse JB, Caprio S, Cefalu WT, Ceriello A, Del Prato S, *et al.* (2009) How do we define cure of diabetes? *Daibetes Care* 32: 2133–2135.
20. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, *et al.* (2011) Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Body Mass Index) National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *Lancet* 377: 557–567.
21. Flegal KM, Carroll MD, Kit BK, Oqden CL (2012) Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA* 307: 491–497.
22. Choo V (2002) WHO reassesses appropriate body-mass index for Asian populations. *Lancet* 360: 235.
23. Bracale R, Labruna G, Finelli C, Daniele A, Sacchetti L, *et al.* (2012) The absence of polymorphisms in ADRB3, UCP1, PPARγ, and ADIPOQ genes protects morbid obese patients toward insulin resistance. *J Endocrinol Invest* 35: 2–4.
24. Boumaiza I, Omezzine A, Rejeb J, Rebhi L, Ouedrani A, *et al.* (2012) Relationship between leptin G2548A and leptin receptor Q223R gene polymorphisms and obesity and metabolic syndrome risk in Tunisian volunteers. *Genet Test Mol Biomarkers* 16: 726–733.
25. Suter M, Donadini A, Romy S, Demartines N, Giusti V (2011) Laparoscopic Roux-en-Y gastric bypass: significant long-term weight loss, improvement of obesity-related comorbidities and quality of life. *Ann Surg* 254: 267–273.
26. Kasama K, Tagaya N, Kanahira E, Umezawa A, Kurosaki T, *et al.* (2008) Has laparoscopic bariatric surgery been accepted in Japan? The experience of a single surgeon. *Obes Surg* 18: 1473-1478.
27. Langer FB, Reza Hoda MA, Bohdjalian A, Felberbauer FX, Zacherl J, *et al.* (2005) Sleeve gastrectomy and gastric banding: effects on plasma ghrelin levels. *Obes Surg* 15: 1024–1029.
28. Boza C, Salinas J, Salgado N, Pérez G, Raddatz A, *et al.* (2012) Laparoscopic sleeve gastrectomy as a standalone procedure for morbid obesity: report of 1,000 cases and 3-year follow-up. *Obes Surg* 22: 866–871.
29. Chopra A, Chao E, Etkin Y, Merklinger L, Lieb J, *et al.* (2012) Laparoscopic sleeve gastrectomy for obesity: can it be considered a definitive procedure? *Surg Endosc* 26: 831–837.
30. Koshy AA, Bobe AM, Brady MJ (2013) Potential mechanisms by which bariatric surgery improves systemic metabolism. *Transl Res* 161: 63–72.
31. Karamanakos SN, Vagenas K, Kalfarentzos F, Alexandrides TK (2008) Weight loss, appetite suppression, and changes in fasting and postprandial ghrelin

and peptide-YY levels after Roux-en-Y gastric bypass and sleeve gastrectomy: a prospective, double blind study. *Ann Surg* 247: 401–407.
32. Vestergaard ET, Djurhuus CB, Gjedsted J, Nielsen S, Møller N, *et al.* (2008) Acute effects of ghrelin administration on glucose and lipid metabolism. *J Clin Endocrinol Metab* 93: 438–444.
33. Zhang N, Maffei A, Cerabona T, Pahuja A, Omana J, *et al.* (2013) Reduction in obesity-related comorbidities: is gastric bypass better than sleeve gastrectomy? *Surg Endosc* 27: 1273–1280.
34. Basso N, Capoccia D, Rizzello M, Abbatini F, Mariani P, *et al.* (2011) First-phase insulin secretion, insulin sensitivity, ghrelin, GLP-1, and PYY changes 72 h after sleeve gastrectomy in obese diabetic patients: the gastric hypothesis. *Surg Endosc* 25: 3540–3550.
35. Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, *et al.* (2012) Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med* 366: 1567–1576.
36. Sutherland K, Lee RW, Phillips CL, Dungan G, Yee BJ, *et al.* (2011) Effect of weight loss on upper airway size and facial fat in men with obstructive sleep apnea. *Thorax* 66: 797–803.
37. Schäfer H, Pauleit D, Sudhop T, Gouni-Berthold I, Ewig S, *et al.* (2002) Body fat distribution, serum leptin, and cardiovascular risk factors in men with obstructive sleep apnea. *Chest* 122: 829–839.