ACCUMULATION of ectopic fat, including visceral obesity, has recently been recognized to be a consequence of adipose tissue dysfunction and the inability of subcutaneous adipose tissue to store excess triglycerides as weight is gained [1, 2]. Furthermore, accumulation of ectopic fat to specific organs (e.g., myocardium, liver, and pancreas) and muscle can lead to organ-specific insulin resistance and eventually cause type 2 diabetes mellitus (T2DM) [3]. Various terms, including pancreatic lipomatosis, fatty pancreas, non-alcoholic fatty pancreatic disease, and pancreatic steatosis (PS), have been used to refer to accumulation of fat in the pancreas [1]. In agreement with the findings reported in the well-known study by Smits et al., we believe that PS is the best description of fat accumulation in the pancreas without fat replacement; moreover, PS also allows for the possibility that fat accumulation is a reversible process [4]. To maintain normal glucose homeostasis in severely obese patients, insulin secretion usually increases parallel to insulin resistance. However, fat accumulation in the pancreatic islets leads to decreased insulin secretion, which might explain why insulin-resistant patients cannot meet the higher demands for insulin and, thus, develop T2DM [5, 6]. Therefore, evaluating pancreatic fat in morbidly obese patients may be useful for diagnosing PS and T2DM.

Various imaging modalities, including ultrasonography, magnetic resonance imaging, and computed tomography (CT), have been used to quantify pancreatic fat [7, 8]. Among these imaging modalities, CT may be the most practical and noninvasive, and it also shows fatty infiltration in an organ as a decrease in CT attenuation. Therefore, we chose CT to evaluate PS using pancreatic volumetry and measurements of pancreatic attenuations.
Bariatric surgery has proven to be very successful for severe obesity as well as for obesity-related diseases, especially T2DM. Among various bariatric procedures, laparoscopic sleeve gastrectomy (LSG) has gained worldwide popularity as a standard bariatric surgery due to its simple restrictive mechanism and relatively good weight-loss effects [9]. However, the metabolic effects on T2DM are usually stronger in patients who have undergone malabsorptive procedures, including laparoscopic Roux-en Y gastric bypass (LRYGB), than in patients who have undergone LSG [10]. We previously found that LSG has very good weight-loss and metabolic effects for T2DM in morbidly obese Japanese patients [9]. The primary aim of this present study was to assess the relationship between the metabolic effects after LSG in morbidly obese patients, with or without T2DM, and to assess improvement in PS using pancreatic volumetry and CT measurements of pancreatic attenuations.

Materials and Methods

Inclusion criteria

This study was a single-institution, retrospective, data-collection and analysis study. Between August 2010 and September 2014, 34 Japanese patients with severe obesity underwent LSG at Iwate Medical University Hospital in Morioka, Iwate, Japan. From these patients, 27 patients agreed to participate in this study by thorough informed consent. Eligibility for participation in this study was limited to individuals between the ages of 18 and 65 who had a body mass index (BMI) ≥ 35 kg/m$^2$ and any obesity-related disease. The LSGs involved gastric-volume reductions of 70–80% by resecting the stomach alongside a 36-French endoscope beginning 4 cm from the pylorus and ending at the angle of the His. To assist the weight-loss and improve the metabolic effects of obesity-related diseases, all the patients were evaluated and cared for by a multidisciplinary team from the preoperative stage to postoperative status.

Although this is a retrospective study, our work was approved by the institutional ethics committee (27-47) and undertaken within the ethical principles stipulated in the Declaration of Helsinki. In addition, we obtained informed consent from each of the participants before LSG was performed, and patient anonymity was strictly preserved.

Data collection

For all the enrolled patients, clinical data and data on the metabolic effects were evaluated at baseline and six months after LSG. The metabolic and inflammatory parameters that were quantified included fasting blood sugar (FBS), insulin, hemoglobin A1c (HbA1c), C-peptide, ghrelin, leptin, adiponectin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), liver volume (LV), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels, plasminogen activator inhibitor type-1 (PAI-1), and tumor necrosis factor-α (TNF-α). To calculate insulin resistance, the homeostasis model of assessing insulin resistance (HOMA-IR) was used. Levels of glucose, insulin, glucagon-like peptide-1 (GLP-1), and gastric inhibitory polypeptide (GIP) were measured during a 75-g oral glucose tolerance test (75-g OGTT). Our results showed that the baseline GLP-1 and GIP levels peaked at 30 min during the 75-g OGTT (Fig. 1); hence, those values were defined as the peak levels of GLP-1 and GIP. Ghrelin, GLP-1, and GIP were measured using the enzyme-linked immunosorbent assay method.

Fig. 1 Pancreas volumetry using CT images

Pancreas areas were selected as ROIs in each slice, and the ROI volume was added and calculated using a computer workstation (SYNAPSE VINCENT V4.1; Fujifilm, Tokyo, Japan). Enhanced vessels and visible pancreatic duct were excluded from PV.
In addition, the visceral fat area (VFA) and the subcutaneous fat area (SFA) were measured using a 64-row CT (Aquilion™, Toshiba Medical Systems Corporation; Tokyo, Japan). After obtaining a single tomographic slice at the umbilicus level, VFA and SFA were detected and measured using a Hounsfield unit (HU) range for adipose tissue of -150–0 HU, and then calculated and indicated in cm² [11, 12]. VFA and SFA were evaluated for all the enrolled patients at baseline and six months after LSG.

Pancreas volume (PV), pancreatic attenuation (PA), and splenic attenuation (SA) were measured using a 64-row CT. To measure PV, CT images were downloaded as digital imaging and communication from medical files to a computer workstation (SYNAPSE VINCENT V4.1; Fujifilm; Tokyo, Japan). For each slice, pancreatic parenchyma was selected as the region of interest (ROI), and the ROI volume was calculated (Fig. 2) [13]. CT attenuation of the pancreas was measured in three ROIs, with areas of 1.0 cm² in the pancreatic head, body, and tail, and the mean CT attenuation of the three ROIs was used as the PA [14]. CT attenuation of the spleen was measured in two ROIs, with areas of 1.0 cm² in the upper and lower splenic poles, and the mean attenuation of the two ROIs was used as the SA [14].

The ABCD Diabetic Surgery Score has been reported previously by Lee et al. [15]. This scoring system consists of four variables that are independent predictors of T2DM remission: patient age, BMI, C-peptide, and duration of T2DM. A 4-point score ranging from 0-3 was assigned to all the variables except patient age. The points for each variable were added to obtain a total ABCD score ranging from 0–10 points. In this study, T2DM evaluation was defined as follows: complete remission was defined as an HbA1c < 6% without use of oral hypoglycemic agents or insulin treatment; partial remission was defined as an HbA1c < 6.5%; and improvement was defined as an HbA1c < 7%, six months after LSG [16].

**Statistical analysis**

Data are described as mean ± standard deviations. The Student’s t-test was used to compare the continuous variable, the χ² test was used to compare the categorical variable, and the relationships between PV, PA-SA, PA/SA, and the outcome variables were evaluated using single and multiple regression analysis and by StateMate V for Windows (ATMS CO., Ltd; Tokyo, Japan). A p value of less than 0.05 in a two-tailed test was considered significant.

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**Figure 2**

Changes of plasma glucose, insulin, GLP-1 and GIP during the 75-g OGTT in all enrolled patients

Graphic chart of the 75-g OGTT at baseline (A), and at 6 months after LSG (B). The mean and standard error of each parameters are denoted.
Results

Patient characteristics

All 27 patients were enrolled in this study between August 2010 and September 2014, and all the patients adhered to the study protocol. Table 1 shows detailed information about the patient characteristics. For the 27 enrolled patients, the mean initial body weight and BMI were 125.1 kg and 43.4 kg/m², respectively. As an obesity-related disease, 14 of the subjects had T2DM.

Changes in the clinical parameters

Table 2 shows the demographic clinical parameters of the enrolled patients at baseline and six months after LSG. The mean body-weight loss and BMI loss were -34.4 kg ($p < 0.001$) and -11.0 kg/m² ($p < 0.001$), respectively. In addition, the mean percentage of excess weight loss (%EWL) was 43.7%. The mean VFA (-191.2 cm², $p < 0.001$) and SFA (-339.2 cm², $p < 0.001$) also decreased significantly six months after LSG compared to the values at baseline. Liver dysfunction improved significantly, with shrinkage of the mean LV (-636 mL, $p < 0.001$). According to the serum lipid markers, TC (-25.1 mg/dL, $p = 0.005$), TG (-60.9 mg/dL, $p = 0.006$), and LDL (-25.1 mg/dL, $p = 0.005$) decreased significantly. In contrast, HDL (5.1 mg/dL, $p = 0.017$) increased significantly six months after LSG compared to the HDL value at baseline. There were significant decreases in the blood levels of leptin (-19.4 ng/mL, $p < 0.001$) and PAI-1 (-24.2 ng/mL, $p < 0.001$).

Table 1  Characteristics of enrolled patients

| Characteristic                          | LSG (n = 27) |
|-----------------------------------------|--------------|
| Age (years)                             | 43.6 ± 13.5  |
| Sex (n)                                 |              |
| Male                                    | 14           |
| Female                                  | 13           |
| Initial body weight (kg)                | 125.1 ± 20.4 |
| Initial BMI (kg/m²)                     | 43.4 ± 5.5   |
| Number of obesity-related disease (n)   | 6.7 ± 1.1    |
| Obesity-related disease (n) *           |              |
| Hypertension                            | 19           |
| Hyperlipidemia                          | 18           |
| Hyperuricemia                           | 12           |
| T2DM                                    | 14           |
| Sleep apnea syndrome                    | 26           |
| NAFLD                                   | 27           |

Abbreviations: LSG, laparoscopic sleeve gastrectomy; BMI, body mass index; T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease. * The every total number was overlapped.

Table 2  Clinical parameters of the patients at baseline and 6 months after LSG

| Variables                  | Baseline     | 6 months after LSG | $p$ value |
|----------------------------|--------------|--------------------|-----------|
| Body weight (kg)           | 125.1 ± 20.4 | 90.7 ± 18.7        | $< 0.001$ |
| %EWL (%)                   | –            | 43.7 ± 15.9        | –         |
| BMI (kg/m²)                | 43.4 ± 5.5   | 32.4 ± 13.8        | $< 0.001$ |
| Waistline (cm)             | 121.0 ± 10.4 | 102.9 ± 9.7        | 0.008     |
| VFA (cm²)                  | 310.3 ± 161.9| 119.1 ± 109.8      | $< 0.001$ |
| SFA (cm²)                  | 509.7 ± 329.0| 170.5 ± 225.8      | $< 0.001$ |
| VFA/SFA (no unit)          | 0.65 ± 0.24  | 0.56 ± 0.29        | 0.001     |
| AST (U/L)                  | 37.4 ± 21.6  | 16.4 ± 4.7         | $< 0.001$ |
| ALT (U/L)                  | 59.6 ± 49.8  | 14.9 ± 5.7         | $< 0.001$ |
| Liver volume (mL)          | 2401.9 ± 722.3| 1765.9 ± 1062.0    | $< 0.001$ |
| TC (mg/dL)                 | 199.4 ± 46.9 | 174.3 ± 120.5      | 0.005     |
| TG (mg/dL)                 | 185.1 ± 95.6 | 124.2 ± 72.3       | 0.006     |
| HDL (mg/dL)                | 48.3 ± 8.6   | 53.4 ± 39.8        | 0.017     |
| LDL (mg/dL)                | 123.6 ± 32.8 | 104.1 ± 77.9       | 0.002     |
| Adiponectin (µg/mL)        | 2.0 ± 1.6    | 4.0 ± 3.7          | 0.497     |
| Leptin (ng/mL)             | 31.6 ± 22.2  | 12.2 ± 10.7        | $< 0.001$ |
| PAI-1 (ng/mL)              | 48.5 ± 38.2  | 24.3 ± 15.7        | $< 0.001$ |
| TNF-α (pg/mL)              | 1.8 ± 1.1    | 2.2 ± 1.3          | 0.451     |

Abbreviations: LSG, laparoscopic sleeve gastrectomy; %EWL, percentage of excess weight loss; BMI, body mass index; VFA, visceral fat area; SFA, subcutaneous fat area; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor type-1; TNF-α, tumor necrosis factor-α.
The resolution and improvement rates for obesity-related comorbidities six months after LSG were 89.4% for hypertension (17/19), 100% for hyperlipidemia (18/18), 100% for hyperuricemia (12/12), 73.1% for sleep apnea syndrome (19/26), and 100% for non-alcoholic fatty liver disease (NAFLD) (27/27). Changes in medication for obesity-related comorbidities, except for T2DM, are shown in Fig. 3. At baseline, 17 patients (89.4%) were on anti-hypertensive drugs, 15 patients (83.3%) were on anti-hyperlipidemic drugs, and 12 patients were also on anti-hyperuricemic drugs (100%). Six months after LSG, 52.6%, 100%, and 100% of patients with hypertension, hyperlipidemia, and hyperuricemia, respectively, had discontinued their medication.

**Glucose metabolic changes in enrolled patients with T2DM**

Table 3 shows glucose metabolic changes in enrolled patients with T2DM. Fig. 1 also presents the results of 75-g OGTT at baseline and six months after LSG in patients with T2DM. In the 14 patients with T2DM, the mean FBS (-33.4 mg/dL, \( p = 0.009 \)) and HbA1c (-1.7%, \( p = 0.008 \)) improved dramatically six months after LSG. Hyperinsulinemia improved markedly (-30.9 ng/mL, \( p = 0.010 \)), and HOMA-IR decreased (-5.7, \( p < 0.001 \)). Six months after LSG, the mean peak GLP-1 levels after the 75-g OGTT were significantly higher than the baseline levels (13.6 pmol/L vs. 33.0 pmol/L, \( p = 0.025 \)), and the mean fasting serum ghrelin levels were significantly lower than the baseline levels (84.8 fmol/mL vs. 32.3 fmol/mL, \( p < 0.001 \)).

### Table 3 Glucose metabolic changes in LSG patients with T2DM (n = 14)

| Variables                  | Baseline     | 6 months after LSG | \( p \) value |
|----------------------------|--------------|--------------------|---------------|
| Insulin (ng/mL)            | 38.0 ± 42.2  | 7.1 ± 3.4          | 0.010         |
| Peak Insulin (ng/mL)       | 90.2 ± 65.8  | 89.6 ± 51.8        | –             |
| Time during 75-g OGTT (min)| 90           | 30                 |               |
| FBS (mg/dL)                | 116.0 ± 43.6 | 82.6 ± 8.6         | 0.009         |
| HbA1c (%)                  | 7.0 ± 2.0    | 5.3 ± 0.8          | 0.008         |
| C-peptide (ng/mL)          | 3.3 ± 1.8    | 2.1 ± 1.1          | 0.006         |
| HOMA-IR (no unit)          | 8.0 ± 7.4    | 2.3 ± 1.3          | < 0.001       |
| Peak GLP-1 (pmol/L)        | 13.6 ± 14.5  | 33.0 ± 22.0        | 0.025         |
| Peak GIP (pmol/L)          | 411.9 ± 178.6| 435.0 ± 204.0      | 0.828         |
| Ghrelin (fmol/mL)          | 84.8 ± 28.7  | 32.3 ± 5.3         | < 0.001       |
| Anti-diabetic drugs (n) *  |              |                    |               |
| Number of drugs            | 1.9 ± 1.0    | 0.2 ± 0.4          | < 0.001       |
| Insulin                    | 2            | 0                  | 0.714         |
| GLP-1 analogue             | 3            | 1                  |               |
| DPP-4 inhibitor            | 7            | 0                  |               |
| Sulfonylurea               | 4            | 0                  |               |
| α-glucosidase inhibitor    | 2            | 0                  |               |
| Biguanide                  | 9            | 2                  |               |

Abbreviations: LSG, laparoscopic sleeve gastrectomy; T2DM, type 2 diabetes mellitus; 75-g OGTT, 75-g oral glucose tolerance test; FBS, fasting blood sugar; HOMA-IR, homeostasis model assessment of insulin resistance; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinoetric polypeptide; DPP-4, dipeptidyl peptidase-4. * The every total number was overlapped.
Based on insulin secretion during 75-g OGTT, the peak level of insulin at baseline was observed at 90 min, but the response time was shortened to 30 min six months after LSG (Fig. 1).

All the enrolled patients with T2DM were treated with more than one anti-diabetic drug. Biguanide was the most prescribed drug preoperatively, followed by a dipeptidyl peptidase-4 inhibitor. The mean number of prescribed anti-diabetic drugs significantly decreased six months after LSG (1.9 vs. 0.2, p < 0.001).

**Changes in PV and CT attenuations**

Table 4 shows changes in PV and the CT attenuations between the baseline findings and the findings obtained six months after LSG. At baseline, the mean PV was 96.7 mL; six months after LSG it had decreased markedly (-16.3mL, p < 0.001). The CT attenuations show that the mean PA significantly increased six months after LSG (9.5 HU, p < 0.001); however, the mean SA did not change. To validate the PS, the PA-SA and PA/SA were calculated at baseline and six months after LSG. Both PA-SA (-23.2 HU vs. -13.3 HU, p = 0.003) and PA/SA (0.54 vs. 0.73, p < 0.001) increased significantly six months after LSG.

Table 5 compares the initial characteristics of the glucose metabolic parameters, the PV and CT attenuations between T2DM patients (n = 14) and non-T2DM patients (n = 13). No significant difference in age, sex, initial body weight and BMI was observed between the two groups. Insulin (38.0 ng/mL vs. 21.6 ng/mL, p = 0.043), HbA1c (7.0 % vs. 5.6%, p < 0.001), and HOMA-IR (8.0 vs. 2.8, p < 0.001) were, naturally, significantly higher in the T2DM patients than in the non-T2DM patients. PV, PA, PA-SA, and PA/SA did not change significantly in the T2DM patients in comparison to the non-T2DM patients.

| Variables | Baseline | 6 months after LSG | p value |
|-----------|----------|--------------------|---------|
| PV (mL)   | 96.7 ± 57.8 | 80.4 ± 47.2 | < 0.001 |
| PA (HU)   | 25.8 ± 22.1 | 35.3 ± 21.8 | < 0.001 |
| SA (HU)   | 49.0 ± 27.5 | 48.6 ± 27.4 | 0.838 |
| PA-SA (HU) | -23.2 ± 22.6 | -13.3 ± 12.2 | 0.003 |
| PA/SA (no unit) | 0.54 ± 0.42 | 0.73 ± 0.24 | < 0.001 |

Abbreviations: PV, pancreatic volume; CT, computed tomography; LSG, laparoscopic sleeve gastrectomy; PA, pancreatic attenuation; SA, splenic attenuation.

| Variables | T2DM (n = 14) | Non-T2DM (n = 13) | p value |
|-----------|---------------|-------------------|---------|
| Age (years) | 45.5 ± 11.8 | 40.6 ± 15.1 | 0.356 |
| Sex        |               |                   | 0.700 |
| Male       | 7             | 7                 |        |
| Female     | 7             | 6                 |        |
| PV at baseline (mL) | 96.8 ± 33.9 | 97.2 ± 33.7 | 0.975 |
| PV at 6 months after LSG (mL) | 83.6 ± 20.8 | 76.5 ± 21.2 | 0.394 |
| Shrinkage of PV (mL) | 13.3 ± 4.6 | 20.7 ± 6.9 | 0.361 |
| PA at baseline (HU) | 26.8 ± 20.4 | 24.6 ± 21.4 | 0.789 |
| PA at 6 months after LSG (HU) | 35.6 ± 2.7 | 34.9 ± 3.9 | 0.838 |
| PA-SA at baseline (HU) | -22.4 ± 23.2 | -24.1 ± 22.8 | 0.852 |
| PA-SA at 6 months after LSG (HU) | -13.4 ± 8.7 | -13.2 ± 15.9 | 0.970 |
| PA/SA at baseline (no unit) | 0.57 ± 0.42 | 0.51 ± 0.44 | 0.769 |
| PA/SA at 6 months after LSG (no unit) | 0.72 ± 0.19 | 0.75 ± 0.30 | 0.792 |

Abbreviations: PV, pancreatic volume; CT, computed tomography; T2DM, type 2 diabetes mellitus; PA, pancreatic attenuation; SA, splenic attenuation.
Lee et al. [15] reported the original data of T2DM remission stratified by ABCD score, and all the patients whose ABCD score ranged from 8–10 achieved complete remission in this data; hence we made cutoff line at 7 points to divide the T2DM patients into two groups. From prior reason, we divided the T2DM patients into two groups: T2DM patients whose ABCD score ranged from 0–7 (the low group, n = 6) and T2DM patients whose ABCD score ranged from 8–10 (the high group, n = 8). Table 6 shows a comparison of the results of the metabolic effect after LSG and the PV and CT attenuations of the low and high groups. Based on the ABCD scores for both the low and high groups, the initial BMI was significantly higher in the high group than in the low group (44.8 kg/m² vs. 39.1 kg/m², p < 0.008). On the other hand, the duration of T2DM was significantly longer in the low group (104.7 months vs. 39.1 months, p < 0.008). FBS decreased markedly in the high group in comparison to the low group (78.1 mg/dL vs. 87.5 mg/dL, respectively; p < 0.001); similarly, HbA1c also decreased markedly between the high group and the low group (5.0% vs. 5.7%, respectively; p = 0.012). All the patients in the high group achieved complete remission of T2DM six months after LSG. At baseline and six months after LSG, no significant differences were observed for the mean PV. However, the mean reduction in PV was significantly higher in the high group than the low group (24.1 mL vs. 0.9 mL, respectively; p = 0.007). In addition, six months after LSG, the mean PA (p = 0.027), PA-SA (p = 0.020), and PA/SA (p = 0.020) were significantly higher in the low group than in the high group.

| Variables                              | Low group (n = 6) | High group (n = 8) | p value |
|----------------------------------------|-------------------|--------------------|---------|
| ABCD score (points)                    | 5.6 ± 1.3         | 8.6 ± 0.5          | -       |
| Age (years)                            | 46.0 ± 15.9       | 45.1 ± 7.8         | 0.892   |
| Initial BMI (kg/m²)                    | 39.1 ± 2.4        | 44.9 ± 4.2         | 0.008   |
| C-peptide at baseline (ng/mL)          | 2.6 ± 1.4         | 4.0 ± 2.0          | 0.145   |
| Duration of T2DM (months)              | 104.7 ± 73.6      | 35.3 ± 29.1        | < 0.001 |
| Peak insulin at baseline (ng/mL)       | 104.6 ± 85.3      | 122.9 ± 88.7       | -       |
| Peak time during 75-g OGTT (min, n)    | 30, 1             | 30, 0              |         |
|                                          | 60, 2             | 60, 4              |         |
|                                          | 90, 1             | 90, 4              |         |
| FBS at 6 months after LSG (mg/dL)       | 87.5 ± 3.1        | 78.1 ± 2.6         | < 0.001 |
| HbA1c at 6 months after LSG (%)         | 5.7 ± 0.6         | 5.0 ± 0.2          | 0.012   |
| HOMA-IR at 6 months after LSG (no unit) | 2.6 ± 0.3         | 2.0 ± 0.7          | 0.055   |
| Evaluation of T2DM (n)                 | 0.078             | -                  |         |
| Complete remission                     | 3                 | 8                  |         |
| Partial remission                      | 2                 | 0                  |         |
| Improved                               | 1                 | 0                  |         |
| PV at baseline (mL)                    | 80.2 ± 20.0       | 111.3 ± 38.0       | 0.975   |
| PV at 6 months after LSG (mL)           | 79.4 ± 16.0       | 87.2 ± 24.8        | 0.486   |
| ΔPV (mL)                               | -0.9 ± 9.0        | -24.1 ± 17.0       | 0.007   |
| PA at baseline (HU)                     | 36.2 ± 11.8       | 18.6 ± 23.4        | 0.096   |
| PA at 6 months after LSG (HU)           | 41.7 ± 5.3        | 30.3 ± 11.2        | 0.027   |
| ΔPA (HU)                               | 5.5 ± 7.7         | 11.7 ± 15.4        | 0.353   |
| PA-SA at baseline (HU)                  | 47.7 ± 5.7        | 50.5 ± 6.7         | 0.404   |
| PA-SA at 6 months after LSG (HU)        | 0.8 ± 0.1         | 0.6 ± 0.2          | 0.020   |
| PA/SA at baseline (no unit)             | 0.76 ± 0.23       | 0.39 ± 0.48        | 0.097   |
| PA/SA at 6 months after LSG (no unit)   | 0.84 ± 0.07       | 0.61 ± 0.20        | 0.020   |

Abbreviations: LSG, laparoscopic sleeve gastrectomy; PV, pancreatic volume; CT, computed tomography; BMI, body mass index; T2DM, type 2 diabetes mellitus; 75-g OGTT, 75-g oral glucose tolerance test; FBS, fasting blood sugar; HOMA-IR, homeostasis model assessment of insulin resistance; PA, pancreatic attenuation; SA, splenic attenuation.
Relationship between metabolic effect and PS after LSG

For all the enrolled patients, simple linear regressions were used to correlate the reduction in PV with %EWL ($r = 0.462$, $p = 0.015$) (Fig. 4). In addition, in the T2DM patients, decreased PV was correlated with decreased FBS ($r = 0.709$, $p = 0.004$), decreased insulin ($r = 0.896$, $p < 0.001$), and reduced LV ($r = 0.720$, $p = 0.004$) (Fig. 4, Table 7). Decreased FBS, decreased LV, increased GLP-1 and decreased HOMA-IR were

![Graphs A, B, C, D]

Fig. 4  Linear regression analyses between the change of PV and metabolic parameters

Linear relationship between the reduction of PV and %EWL in all enrolled patients (A), the reduction in FBS (B), insulin (C), and liver volume (D) in T2DM patients.

Table 7  Single regression analyses between PS and metabolic effects after LSG

| Correlation coefficient ($r$) | ΔPV | ΔFBS | Δinsulin | Δpeak insulin | ΔLV | ΔHbA1c | ΔGLP-I | ΔHOMA-IR |
|-----------------------------|-----|------|----------|---------------|-----|--------|--------|----------|
| $ΔPV$                      | −   | 0.709| 0.896    | 0.334         | 0.720| -0.298 | 0.778  | -0.637    |
| $ΔFBS$                     | 0.709| −    | 0.858    | -0.886        | 0.890| -0.261 | 0.720  | -0.439    |
| $Δinsulin$                 | 0.896| 0.858| −        | -0.486        | 0.812| -0.266 | 0.793  | -0.614    |
| $Δpeak insulin$            | 0.334| -0.886| −        | -0.723        | -0.723| 0.186  | -0.453 | 0.148     |
| $ΔLV$                      | 0.720| 0.890| 0.812    | -0.723        | -0.723| 0.186  | -0.453 | 0.148     |
| $ΔHbA1c$                   | -0.298| -0.261| −        | 0.186         | -0.111| −      | 0.151  | 0.106     |
| $ΔGLP-I$                   | 0.778| 0.720| 0.793    | -0.453        | 0.745| -0.151 | 0.745  | -0.801    |
| $ΔHOMA-IR$                 | -0.637| -0.439| −        | 0.148         | -0.545| 0.106  | -0.801 | −        |

$p$ value

| Correlation coefficient ($r$) | ΔPV | ΔFBS | Δinsulin | Δpeak insulin | ΔLV | ΔHbA1c | ΔGLP-I | ΔHOMA-IR |
|-----------------------------|-----|------|----------|---------------|-----|--------|--------|----------|
| $ΔPV$                      | −   | 0.004| < 0.001  | 0.243         | 0.004| 0.301  | 0.007  | 0.001    |
| $ΔFBS$                     | 0.004| −    | < 0.001  | < 0.001       | < 0.001| 0.367  | 0.004  | 0.117    |
| $Δinsulin$                 | < 0.001| < 0.001| −        | 0.078         | < 0.001| 0.359  | < 0.001| 0.020    |
| $Δpeak insulin$            | 0.243| < 0.001| 0.078    | −             | 0.003| 0.525  | 0.104  | 0.613    |
| $ΔLV$                      | 0.004| < 0.001| < 0.001  | 0.003         | 0.007| −      | 0.002  | 0.044    |
| $ΔHbA1c$                   | 0.301| 0.367| 0.359    | 0.525         | 0.705| −      | 0.606  | 0.719    |
| $ΔGLP-I$                   | 0.001| 0.004| < 0.001  | 0.104         | 0.002| 0.606  | < 0.001| −        |
| $ΔHOMA-IR$                 | 0.014| 0.117| 0.020    | 0.613         | 0.044| 0.719  | < 0.001| −        |

Abbreviations: PS, pancreatic steatosis; LSG, laparoscopic sleeve gastrectomy; PV, pancreatic volume; FBS, fasting blood sugar; LV, liver volume; GLP-1, glucagon-like peptide-1; HOMA-IR, homeostasis model assessment of insulin resistance.
highly correlated with almost other decreased parameters except for decreased HbA1c, decreased peak insulin was also correlated with decreased FBS ($r = -0.886$, $p < 0.001$) and decreased LV ($r = -0.723$, $p = 0.003$) (Table 7). Multiple regression analyses revealed that decreased insulin was the factor with the highest correlation to decreased PV ($\beta = 0.753$, $p$ value = 0.016, 95% confident interval: 0.168 – 1.236) (Table 8). Although, the same process was conducted to determine changes in PA-SA and PA/SA, no significant differences were observed between changes in the CT attenuations and metabolic effects in the T2DM patients.

### Discussion

It is clear that obesity is a major risk factor for T2DM, and it appears to drive tissue insulin resistance via ectopic fat gain, with the best-studied organ being the liver [1-3]. Excess liver fat, and its related condition of NAFLD, have been recognized as playing an important role in the T2DM-onset mechanism [3, 17]. However, pancreatic ectopic fat, which we previously defined as PS, may also impair pancreatic β-cell function. In particular, pancreatic ectopic fat is primarily stored as large lipid droplets within exocrine and endocrine cells and interstitial spaces. In contrast to exocrine cells, pancreatic β-cells have less capacity to store surplus TG, and this fact may lead to activation of apoptotic pathways and decreased insulin secretory capacity [18, 19]. In addition, a greater proportion of PS is associated with increased insulin levels in morbidly obese patients without T2DM, due to their strong insulin resistance. Therefore, it may take the toxic effect of PS a long time to manifest as impaired β-cell function, and it has been assessed that pancreatic β-cell damage is present for more than a decade before T2DM is diagnosed [1, 2]. Therefore, early detection of PS in morbidly obese patients without T2DM can be an important indication for performing bariatric surgery to avoid the onset of T2DM. Our study showed that PV significantly decreased and PA/SA and PA-SA significantly increased, all because of improved PS.

To date, few studies have defined the standard for PV and few have validated the changes in PV before and after T2DM onset. Saisho et al. reported that PV increased in a linear fashion from birth to age 20; it then reached a plateau and gradually declined after age 60 due to pancreatic parenchymal atrophy and fat infiltration of the pancreas [20]. In addition, Saisho et al. examined the effect of BMI on PV in adults and reported that, in both genders, PV increased in response to obesity due to PS [20]. Anatomical, histological, and imaging studies have reported pancreas atrophy in patients with type 1 diabetes mellitus [21]. In addition, when compared with nondiabetic controls in a large, case-matched, cohort study, PV decreased slightly, but significantly, in patients with T2DM [20, 22]. These findings indicate that PV in adults does not change if body weight is not gained and if insulin resistance is not acquired. However, as body weight is gained, PV increases. After T2DM onset, PV decreases due to atrophy of the pancreatic islets. After LSG, PV decreases as body weight is lost in high group (-24.1 mL), indicating that PS and T2DM are reversible. Therefore, T2DM patients have the possibility of not only improving their condition but also of inducing complete remission after LSG. In contrast to the high group, patients in the low group had a tendency of lower PV, significant low change of PV and PA after LSG, and significant high change of PA-SA after LSG. In patients with low ABCD score, atrophy of pancreatic islets had already progressed due to long duration of T2DM and deterioration of β-cell [15, 20, 22]. In addition, metabolic effect to pancreas has become weaker in patients with low ABCD score due to deficient weight loss after

#### Table 8 Multiple regression analyses between PS and metabolic effects after LSG

| Parameters | $\beta$ | $p$ value | 95% CI     |
|------------|--------|-----------|------------|
| ΔFBS       | -0.311 | 0.256     | -0.917 – 0.295 |
| Δinsulin   | 0.753  | 0.016     | 0.168 – 1.236 |
| Δpeak insulin | 0.059  | 0.452     | -0.121 – 0.241 |
| ΔLV        | -18.705| 0.067     | -39.153 – 1.743 |
| ΔHbA1c     | -4.055 | 0.333     | -13.485 – 5.374 |
| ΔGLP-1     | 0.249  | 0.416     | -0.449 – 0.947 |
| ΔHOMA-IR   | 0.245  | 0.634     | -0.953 – 1.444 |

Abbreviations: PS, pancreatic steatosis; LSG, laparoscopic sleeve gastrectomy; CI, confident interval; PV, pancreatic volume; FBS, fasting blood sugar; LV, liver volume; GLP-1, glucagon-like peptide-1; HOMA-IR, homeostasis model assessment of insulin resistance.
LSG compared to in patients with high ABCD score. Therefore, PS did not improved enough for T2DM to be lead remission. From these mechanism, the mean PA remained higher and PA-SA and PA/SA were also higher in the low group.

Although CT evaluation for NAFLD has already been thoroughly investigated, and the reference value of CT attenuations has been used to diagnose NAFLD [17, 23], the reference value of PS/SA and PA-SA as diagnostic criteria for PS, insulin resistance, and T2DM have not been established due to the lack of studies. Utilizing CT parameters, Sakata et al. used density—the arterial (A) and portal (P) phase density of the pancreas and A/P ratio—to assess endocrine function; they found that high arterial density of the pancreas and A/P ratio—to assess endocrine function; they found that high arterial density of the pancreas ($r^2 = 0.12, p = 0.004$) and high A/P ratios ($r^2 = 0.11, p = 0.006$) correlated well with endocrine function, suggesting that well-established vascularization in the pancreas correlates with good pancreatic islets [13]. Kim et al. reported that PA/SA and PA-SA were good tools for evaluating pancreatic β-cell function, and they noted that both were well-correlated with histological PS and significantly associated with impaired glucose metabolism [14]. In addition, Kim et al. calculated the cutoff values that provide balanced sensitivity and specificity for predicting impaired glucose metabolism [14]. These cutoff values for PA/SA and PA-SA were 0.9 (sensitivity: 69.0%, specificity: 57.6%) and -1.9 (sensitivity: 79.3%, specificity: 42.2%), respectively. In the present study, none of the patients satisfied these cutoff values before LSG; therefore, even morbidly obese patients who do not have T2DM have potentially impaired glucose metabolism because of PS. However, none of the patients who successfully induced complete remission of T2DM (n = 11) satisfied these cutoff values. Therefore, further large-scale studies are warranted to validate more accurate PA/SA and PA-SA cutoff values for estimating the clinical conditions of PS.

In general, the role of PS in impaired insulin secretion remains controversial. Some studies have concluded that PS is a strong determinant of impaired secretion [24, 25], and Macauley et al. established a significant relationship between PV and HOMA-β in both T2DM and non-T2DM populations [22]. The present study detected a relationship between decreased PV and %EWL in morbidly obese patients. This data may indicate that weight loss after bariatric surgery could have an equal effect on ectopic fat, including fat in the pancreas, and visceral fat. However, Honka et al. reported no relationship between decreased pancreatic fat and the degree of change in body weight and VFA [26]. Therefore, reduced pancreatic fat after bariatric surgery may be mediated by other factors, including incretin hormones, in addition to the usual effect of weight loss [26]. To clarify the relationship between improved PS and the secretion of incretin hormones, further basic and clinical research is warranted. However, in the present study, linear regression analyses of the data suggested a relationship between decreased PV and improved T2DM, especially reduced FBS and insulin levels. Although we also investigated the correlation between decreased PV and changes in the peak insulin level, no significant correlation was observed; the reason for this unexpected result may be explained by the increase in the level of GLP-1 [9, 10]. These results indicate that improved PS after bariatric surgery seems to play a major role in improving impaired glucose metabolism. In addition, the present study showed that, after LSG, a normal positive correlation between decreased PV and decreased LV was observed in T2DM patients. According to the current hypothesis of the development of NAFLD, obesity and insulin resistance increase the release of free fatty acids from visceral adipocytes and hepatic insulin resistance and hepatic steatosis precede the development and progression of T2DM [23]. These results indicate that the intercept of de novo lipogenesis due to PS and NAFLD has a significant potential to improve T2DM [22, 23]. However, further studies are required to investigate this potential.

Bariatric surgery leads to rapid and sustained weight loss, and it results in a nearly 80% remission rate in T2DM patients. 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 obesity-related diseases, worldwide [10]. However, in Japan, although LSG is a well-tolerated, effective weight-reduction surgery for morbidly obese patients, it is a restrictive procedure and often does not result in adequate weight-loss during long-term follow-up [9]. Kasama et al. reported LSG with duodenojejunal bypass (LSG/DJB) as an original bariatric procedure for morbidly obese Japanese patients [27], and Seki et al. also reported that LSG/DJB yielded excellent body-weight loss and high remission rates in T2DM patients who had low ABCD scores [28]. Based on these results,
LSG/DJB involving standardized procedures with malabsorptive effects is a safe, feasible procedure for patients at risk of gastric cancer, and it provides outcomes that are similar to LRYGB outcomes in short-term follow-up. In the near future, LSG/DJB may prove to be another standard bariatric procedure for morbidly obese Asian patients [10, 27, 28]. Therefore, further studies investigating changes in PV, PA-SA, and PA/SA in patients who undergo LSG/DJB can provide more data on PS.

The present study has some limitations. One limitation is the selection bias of the surgeons or patients in regard to choosing LSG. The study also includes a small sample size from a single institution and short-term follow-up. We were unable to completely match the location of ROIs for the CT attenuation measurements; hence, heterogeneous PS findings between the baseline and six months after LSG were not strictly evaluated. Long-term follow-up data on PV and changes in PS, including CT attenuations, are needed to further investigate the relationship between PS and T2DM, and, if possible, histological findings of pancreatic biopsy specimens should be added to clarify the crucial role of PS and the sequential mechanism from PS to T2DM onset.

In summary, the present study showed that PV significantly decreased after LSG in morbidly obese Japanese patients, and decreased PV improved PS. Therefore, decreased PV was well-correlated with %EWL and the improvement of impaired glucose metabolism. Noninvasive, unenhanced CT provides very useful CT attenuation parameters that play a potential role in predicting glucose homeostasis. Further studies are warranted to validate the clinical implications of PS evaluation using PV and CT attenuations, not only in LSG patients, but also in patients who have undergone LSG/DJB.

Disclosure

Akira Umemura and all the co-authors have no conflict of interest.

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