Obesity Facts

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Research Article

Liver-Expressed Antimicrobial Peptide 2 is a Hepatokine that Predicts Weight Loss and Complete Remission of Type 2 Diabetes Mellitus After Vertical Sleeve Gastrectomy in Japanese Individuals

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Short Title: LEAP2 predicts weight loss and remission of diabetes after VSG

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Abstract

Introduction: Vertical sleeve gastrectomy (VSG) is considered one of the most effective treatments for sustained weight loss and complete remission of type 2 diabetes mellitus (CR-T2DM). Liver-expressed antimicrobial peptide 2 (LEAP2), a ghrelin receptor antagonist peptide, is a metabolic hormone regulated by VSG. However, it is unknown whether LEAP2 can be used to predict the outcomes of VSG. This study aimed to evaluate LEAP2 as a predictive factor for weight loss and CR-T2DM after VSG.

Methods: This retrospective study included 39 Japanese participants with obesity who underwent VSG. Serum LEAP2, des-acyl ghrelin (DAG), and other metabolic and anthropometric parameters were studied before and at 12 months after VSG. Receiver operating characteristics (ROC) curve was generated to evaluate predictive score for weight loss with cut-off value of > 50 percent excess weight loss (%EWL). ROC curve was also generated to assess CR-T2DM.

Results: Serum LEAP2 levels were significantly higher in participants with body mass index (BMI) 32–50 kg/m^2 than in those with normal weight. Participants with BMI > 50 kg/m^2 had lower serum LEAP2 concentrations than those with BMI 32–50 kg/m^2. VSG caused a significant reduction in serum DAG concentrations, but it did not affect serum LEAP2 concentrations in either male or female participants. Preoperative serum LEAP2 concentration of 2.88 pmol/mL was the optimal cutoff value for predicting weight loss after VSG, with sensitivity of 80.0% and specificity of 75.9%. Preoperative serum LEAP2 level higher than 4.67 pmol/mL predicted CR-T2DM after VSG with sensitivity of 100% and specificity of 58.8%,

Discussion/Conclusion: Preoperative serum LEAP2 could predict weight loss and CR-T2DM as outcomes of VSG.

Introduction

Obesity has become a global epidemic and the World Health Organization reported that around 1.9 billion adults worldwide were obese in 2016, more than triple the prevalence since 1975 [1]. In addition to the loss of quality of life because of weight gain itself, obesity causes several chronic diseases such as diabetes, cardiovascular disease, osteoarthritis, and psychosis which put a great burden on the healthcare system [2].

Bariatric surgery such as vertical sleeve gastrectomy (VSG), a surgical procedure to reduce the size of the stomach, is the most effective and durable treatment for class III obesity [3, 4]. Roux-en-Y gastric bypass (RYGB) was the most common bariatric surgery procedure but recently VSG has become the most frequently performed bariatric surgical intervention in the world [5]. In Japan, VSG is the only bariatric surgery procedure covered by the national health insurance system [6]. Successful weight loss after bariatric surgery is defined as excess weight loss (%EWL) greater than 50% [7]. Bariatric surgery treats diabetes, dyslipidemia, hypertension, and other obesity-related comorbidities [3, 6]. The precise mechanisms by which bariatric surgery cures these disorders remain unidentified; however, its effectiveness is due to alterations in gastrointestinal peptides, bile acids, adipokines, hepatokines, and microbiome in addition to weight loss [3].

Some scoring systems such as ABCD (age, body mass index (BMI), C-peptide level, and duration of diabetes), DiaRem (age, glycosylated hemoglobin (HbA1c), anti-diabetic medication use, and insulin use), and Individualized Metabolic Surgery Score (IMS; number of anti-diabetic medications, insulin use, duration of diabetes, and HbA1c) have been used to predict complete remission of type 2 diabetes mellitus (CR-T2DM) after bariatric surgery [4, 8, 9]. Because these predictive scores do not include gastrointestinal peptides, we studied ghrelin and liver-expressed antimicrobial peptide 2 (LEAP2), which regulate feeding and energy homeostasis.

Ghrelin is a 28-amino-acid peptide with a middle chain fatty acid modification at serine 3. It is primarily produced in the stomach. It is involved in the regulation of energy homeostasis by enhancing feeding, adiposity, blood glucose levels, and growth hormone (GH) secretion [10, 11]. Ghrelin binds to the growth hormone secretagogue receptor (GHSR), a G protein–coupled receptor, and transmits signals to the hypothalamus [10–15]. Ghrelin-producing cells account for 20–25% of endocrine cells in the gastric corpus [13]. Des-acyl ghrelin (DAG), which lacks the fatty acid modification, is the major molecular form and accounts for approximately 80–90% of the ghrelin moiety in the blood [13]. LEAP2 is a 40-amino-acid hepatokine that was originally isolated from human hemofiltration fluid [16]. LEAP2 has biological function as an antimicrobial agent; it is a part of the innate immune system [17, 18]. Ge et al*.* identified endogenous GHSR antagonism as a novel role for LEAP2 [19]. They found that *Leap2* expression increases in the stomach and decreases in the duodenum after VSG in mice with obesity induced by a high-fat diet (HFD). LEAP2 inhibits ghrelin's actions *in vivo* such as ghrelin-induced hyperphagia, GH secretion, blood glucose elevation, and body temperature reduction in rodents [19–21].

Plasma LEAP2 and ghrelin in humans and rodents have opposing actions based on energy status [19, 20, 22]. Plasma LEAP2 levels increase in individuals with obesity and HFD-induced obese mice and decrease in mice during fasting or calorie restriction [19, 21, 22]. *Leap2* knockout (KO) mice have enhanced ghrelin-induced food intake and GH secretion; HFD-fed female *Leap2* KO mice eat more and gain more body weight (BW) their littermates [23].

The present study aimed to study the relationship between serum LEAP2 concentrations and blood metabolic parameters before and after VSG in Japanese individuals with obesity. We also explored the ability of LEAP2 to act as a preoperative biomarker for predicting weight loss and T2DM remission after VSG.

Methods

Study design and subjects

We retrospectively reviewed clinical data from 39 individuals with obesity who underwent VSG obtained from August 2012 to June 2019 at Toho University Sakura Medical Center and followed for 12 months postoperatively. The prevalence of T2DM, hypertension, and dyslipidemia before surgery was 66.7%, 69.2%, and 87.2%, respectively (Table 1). In Japan, obesity is defined as BMI ≥ 25.0 kg/m^2. The guidelines of the Japanese Society for the Treatment of Obesity state that the indications for bariatric surgery are 1) BMI ≥ 32 kg/m^2 with at least one obesity-related comorbidity such as T2DM, hypertension, or dyslipidemia or 2) BMI ≥ 35 kg/m^2 [24]. Healthy individuals with normal BW (4 men and 4 women) were also enrolled at the University of Miyazaki. Their mean age was 38.5 ± 7.2 years, ranging from 31 to 53 years, and their BMI was 22.0 ± 1.9 kg/m^2, ranging from 18.5 to 24.9 kg/m^2. Their serum concentrations of LEAP2 and DAG were determined (Fig. 1A).

Anthropometric analysis

We evaluated the following anthropometric parameters before and 12 months after VSG: BW, body fat mass, visceral fat area (VFA), and subcutaneous fat area (SFA). VFA was determined using computed tomography at the level of the umbilicus, with the participant resting in the supine position. SFA was calculated by subtracting VFA from total fat area. Radiologists quantified fat area using Ziostation2 software version 2.9.7.1 (Ziosoft, Tokyo, Japan) [6]. The outcome at 12 months after VSG was evaluated as %EWL, which was calculated as (preoperative BW − postoperative BW) / (preoperative BW − BW corresponding to BMI of 25 kg/m^2 in each participant) × 100 [25]. CR-T2DM evaluated 12 months after the surgery was defined as HbA1c < 6.0% without use of any anti-diabetic medications [26].

Biochemical analysis

Blood parameters listed in Table 1 were determined. Blood was collected in the morning after an overnight 12 h fast. Immediately after blood collection, serum or plasma was separated by centrifugation at 3,000 rpm for 10 min. Serum was used to measure FBG, C-peptide, lipids, albumin, AST, ALT, γGTP, creatinine, uric acid, GH, IGF-1, and CRP as described elsewhere [24]. For HbA1c measurements, blood was collected in tubes containing ethylenediaminetetraacetic acid and measured using high-pressure liquid chromatography and an HLC-732G11 analyzer (Tosoh Bioscience, Yamaguchi, Japan) [24].

For LEAP2 measurement, 100-µL serum samples that were kept at −80°C were loaded onto a Sep-Pak Vac C18 cartridge (Waters Corporation, Milford, MA, USA) prepared as described elsewhere [27]. Peptides were eluted with 60% acetonitrile containing 0.1% trifluoroacetic acid. Next, the eluate was lyophilized. Serum LEAP2 concentration was measured with a LEAP2 EIA kit (Phoenix Pharmaceuticals, Burlingame, CA, USA). The detection range of the LEAP2 EIA kit is 0–100 ng/mL; the respective intra- and inter-assay variations were < 3% and < 5% [21]. Serum DAG levels were measured on an AIA-600II immunoassay analyzer (Tosoh, Tokyo, Japan) as described elsewhere [20]. Intra- and inter-assay variation for DAG was each < 3% [21].

Statistical analysis

Results are expressed as means ± SD. Comparison of parameters between the two groups was performed using the Wilcoxon signed-rank test. Spearman's correlation coefficient analysis was performed to evaluate correlations between LEAP2 concentrations and each parameter. Sensitivity and specificity for %EWL and CR-T2DM were analyzed using receiver operating characteristic (ROC) curves. $P < 0.05$ was considered significant. Statistical analysis was performed using GraphPad Prism software (GraphPad Prism 7; San Diego, CA, USA).

Results

Alternations in metabolic parameters

Table 1 shows postoperative reductions in BMI and body fat mass, including VFA and SFA (Table 1). Mean %EWL was 71.5 ± 29.1%, ranging from 17.8% to 144.9%. The number of patients who had T2DM, hypertension, and dyslipidemia decreased after VSG (Table 1). The number of patients taking oral anti-diabetes medications, insulin, antihypertensive medications, or lipid-lowering drugs also decreased after VSG (Supplementary Table 1). SBP, FBG, HbA1c, TG, AST, ALT, γGTP, uric acid, and CRP decreased and HDL-C increased (Table 1).

Serum LEAP2 and DAG concentrations

Participants with BMI 32–50 kg/m^2 had higher serum LEAP2 concentrations than participants with normal weight (Fig. 1A) whereas participants with BMI >50 kg/m^2 had lower serum LEAP2 concentrations than participants with BMI 32–50 kg/m^2 (Fig. 1A). Serum LEAP2 concentrations were not correlated with BMI in the range 18.5–77.8 kg/m^2, whereas serum DAG concentrations were negatively correlated with BMI (Fig. 1B, C). Serum LEAP2 concentrations did not change after VSG, but serum DAG concentrations decreased after VSG (Fig. 2A, B). A previous study demonstrated that plasma LEAP2/acyl ghrelin molar ratio is positively correlated with body weight, implying that a higher LEAP2/acyl ghrelin molar ratio exists in the state of obesity [22]. However, LEAP2/DAG molar ratio increased after VSG despite weight loss (Fig. 2C). This discrepancy might be due to lower DAG levels after surgical intervention. These results were observed in both genders (Fig. 2D–I).

Correlations between LEAP2 and clinical parameters after VSG

We defined %LEAP2, %BMI, %VFA, %TG, and %HbA1c as the ratios of these parameters' post-VSG values to their pre-VSG values. %LEAP2 was positively correlated with %BMI, %VFA, and %TG and not correlated with %HbA1c (Fig. 3A–D). %LEAP2 was negatively correlated with %EWL (Fig. 3E).

Preoperative serum LEAP2 levels predict weight loss and CR-T2DM after VSG

We conducted ROC analysis to investigate whether preoperative LEAP2 levels could predict weight loss and CR-T2DM after VSG (Fig. 4A and B). The optimal preoperative serum LEAP2 cutoff value for predicting weight loss was 2.88 pmol/mL (sensitivity 80.0%, specificity 75.9%) (Fig. 4A). We compared preoperative serum LEAP2 levels with ABCD and IMS scores. Participants with CR-T2DM had higher preoperative serum LEAP2 levels than those without CR-T2DM (Supplementary Fig. 1A). Both ABCD and IMS scores were insignificant in these groups (Supplementary Fig. 1B and C). The optimal preoperative serum LEAP2 cutoff value for predicting CR-T2DM was 4.67 pmol/mL (sensitivity 100.0%, specificity 55.6%) (Fig. 4B).

Discussion

In this study, VSG significantly improved BMI and clinical variables such as FBG, HbA1c, TG, body fat mass, and liver function. VSG reduced serum DAG concentrations and increased GH concentrations as a result of gastric resection, but it did not alter serum LEAP2 or IGF-1 concentrations.

A previous study demonstrated that LEAP2 levels are positively correlated with BMI among individuals with normal weight, overweight, and obesity [22]. In the present study, serum LEAP2 levels were higher in participants with BMI 32–50 kg/m^2 than those in normal-weight participants. However, LEAP2 levels in participants with BMI > 50 kg/m^2 were lower than those in participants with BMI of 32–50 kg/m^2. We have previously shown that blood LEAP2 levels depend on hepatic *Leap2* expression [20]. Serum albumin levels were lower, while serum AST and ALT levels were higher in participants with BMI > 50 kg/m^2 compared with those in participants with BMI of 32–50 kg/m^2 (Table S2). These findings suggest that protein synthesis in the liver of participants with BMI > 50 kg/m^2 was impaired. Thus, we speculate that the lower LEAP2 levels in participants with BMI > 50 kg/m^2 might be explained by liver dysfunction. LEAP2 administration lowered postprandial glucose excursions and suppressed food intake in healthy men [28]. We speculate that participants who gained weight but had low LEAP2 might have been more likely to gain weight because there was less compensation for LEAP2's effect on feeding suppression and weight loss. Mani et al. showed that RYGB significantly reduced plasma LEAP2 levels at 24 months after surgery [22]. They also showed that VSG did not alter plasma LEAP2 levels at 12–18 months after surgery. In the present study, VSG did not alter serum LEAP2 levels measured at 12 months after surgery. Ghrelin reduced LEAP2 mRNA expression in the mouse liver [20]. Ghrelin-induced LEAP2 suppression after VSG could be attenuated, while weight loss via VSG might downregulate LEAP2 expression, which leads to no change in LEAP2 concentrations.

In individuals with obesity, IGF-1 levels are increased and GH secretion is suppressed. In this study, IGF-1 levels were unchanged after VSG while GH levels increased. Previous studies have shown mixed results: GH levels have increased after VSG in some studies, while IGF-1 levels increased or remained unchanged after bariatric surgery in other studies. Juiz-Valiña et al. insisted that the chronic inflammation caused by obesity decreases after surgery and GH and IGF-1 insufficiency improve [29]. On the other hand, Al-Regaiey et al. insisted that improved postoperative insulin sensitivity enhances the signaling of IGF-1, which shares a receptor with insulin so that postoperative IGF-1 levels remain unchanged [30]. In this study, the prognosis for VSG with respect to weight loss and glucose tolerance was good. Thus, IGF-1 levels did not change due to improved insulin signaling.

Bariatric surgery is the most effective and sustainable weight-loss treatment. However, insufficient postoperative weight loss has often been documented. Failure to achieve successful weight loss is likely multi-factorial, including technical factors, preoperative patient education, and factors related to patient gender, age, hormonal factors, and metabolic factors [31]. The present study suggests that high preoperative serum LEAP2 levels might predict successful weight loss after VSG. Scores such as the ABCD, DiaRem, and IMS scores have been proposed to predict which patients will achieve CR-T2DM after bariatric surgery [4, 8, 9]. We have found that preoperative LEAP2 concentration > 4.67 pmol/mL could be the cutoff value for predicting CR-T2DM in patients undergoing VSG. Higher preoperative LEAP2 levels suggest improvement in BW and glucose metabolism. We compared LEAP2 with ABCD and IMS scores. We could not analyze the DiaRem score because of the lack of data about drug therapy in this study. The CR-T2DM group had higher preoperative LEAP2 levels than the group without CR-T2DM. There were no differences in ABCD or IMS scores between the two groups. In the present study, the patients had a lower ABCD score because they were aged (42.7 ± 9.5 years) and had a long duration of diabetes (8.0 ± 8.1 years). Patients with high preoperative LEAP2 levels are more likely to reach CR-T2DM after VSG even with low ABCD or IMS scores. We propose LEAP2 can be used as a factor to predict CR-T2DM after VSG in addition to ABCD and IMS scores.

This study had two limitations. First, this study was a single-center retrospective study. Second, there were only 39 study participants. A multicenter prospective study is desired to verify the relationship between LEAP2 concentrations and the effects of VSG.

In conclusion, our study demonstrates that LEAP2 is a novel metabolic factor that could predict the outcomes of VSG.

Statement of Ethics

Data in the present study were collected at the University of Miyazaki and Toho University Sakura Medical Center. The protocol of the study from individuals who underwent VSG was prepared in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Toho University Sakura Medical Center (approval

date: April 23, 2020; Approval No. S19079). Our institute waived the need to obtain written informed consent due to the retrospective study design.
The protocol of the study from healthy individuals was prepared in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Miyazaki (approval date: April 23, 2017; Approval No. O-136). All subjects received explanations about the objectives and procedures of the study. Those who consented to participate and satisfied the inclusion and exclusion criteria provided written informed consent.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

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Author Contributions
Hiroki Nabekura: Conceptualization, Data curation, Formal analysis, Methodology, Investigation, and Writing – original draft. Md Nurul Islam: Conceptualization, Data curation, Formal analysis, Methodology, Investigation, Writing – original draft, and Writing – review and editing. Hideyuki Sakoda: Conceptualization, Supervision, Funding acquisition, and Writing – review and editing. Takashi Yamaguchi: Methodology, Investigation, and Resources. Atsuhito Saiki: Methodology, Investigation, and Resources. Taiki Nabekura: Methodology, Investigation, and Resources. Takashi Oshiro: Methodology, Investigation, and Resources. Yuri Tanaka: Data curation. Shinya Murayama: Data curation. Weidong Zhang: Data curation and Methodology. Ichiro Tatsuno: Methodology, Investigation, and Resources. Masamitsu Nakazato: Conceptualization, Supervision, Funding acquisition, and Writing – review and editing. All authors approved the final version of the manuscript.

Data Availability Statement
All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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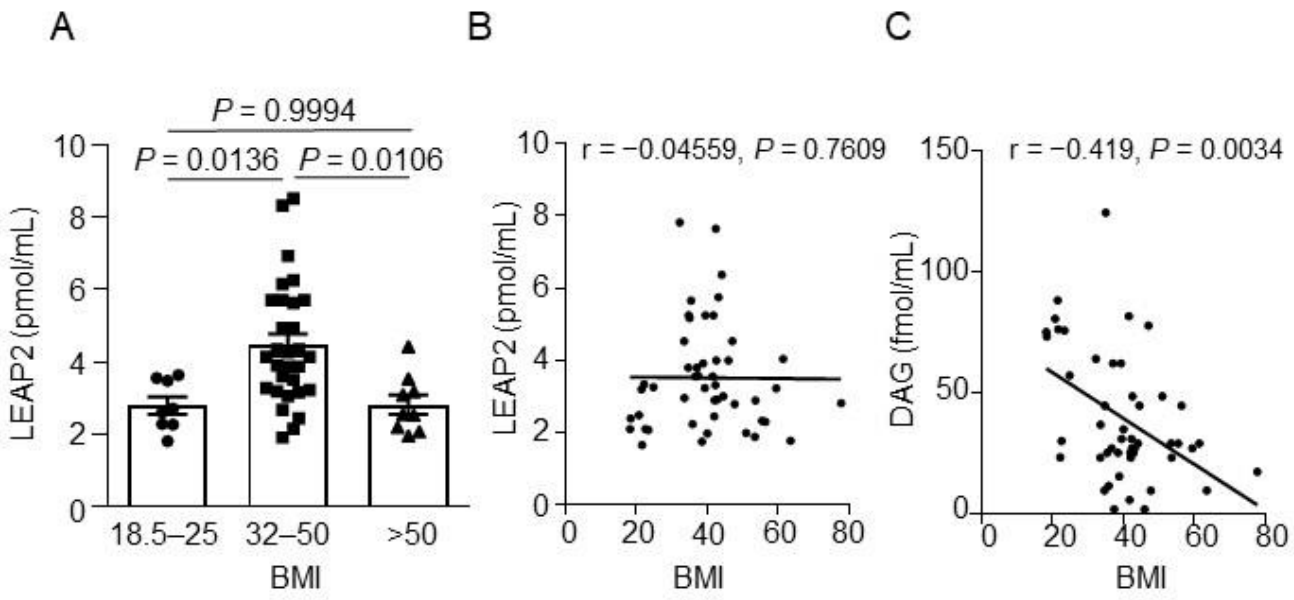
Figure legends

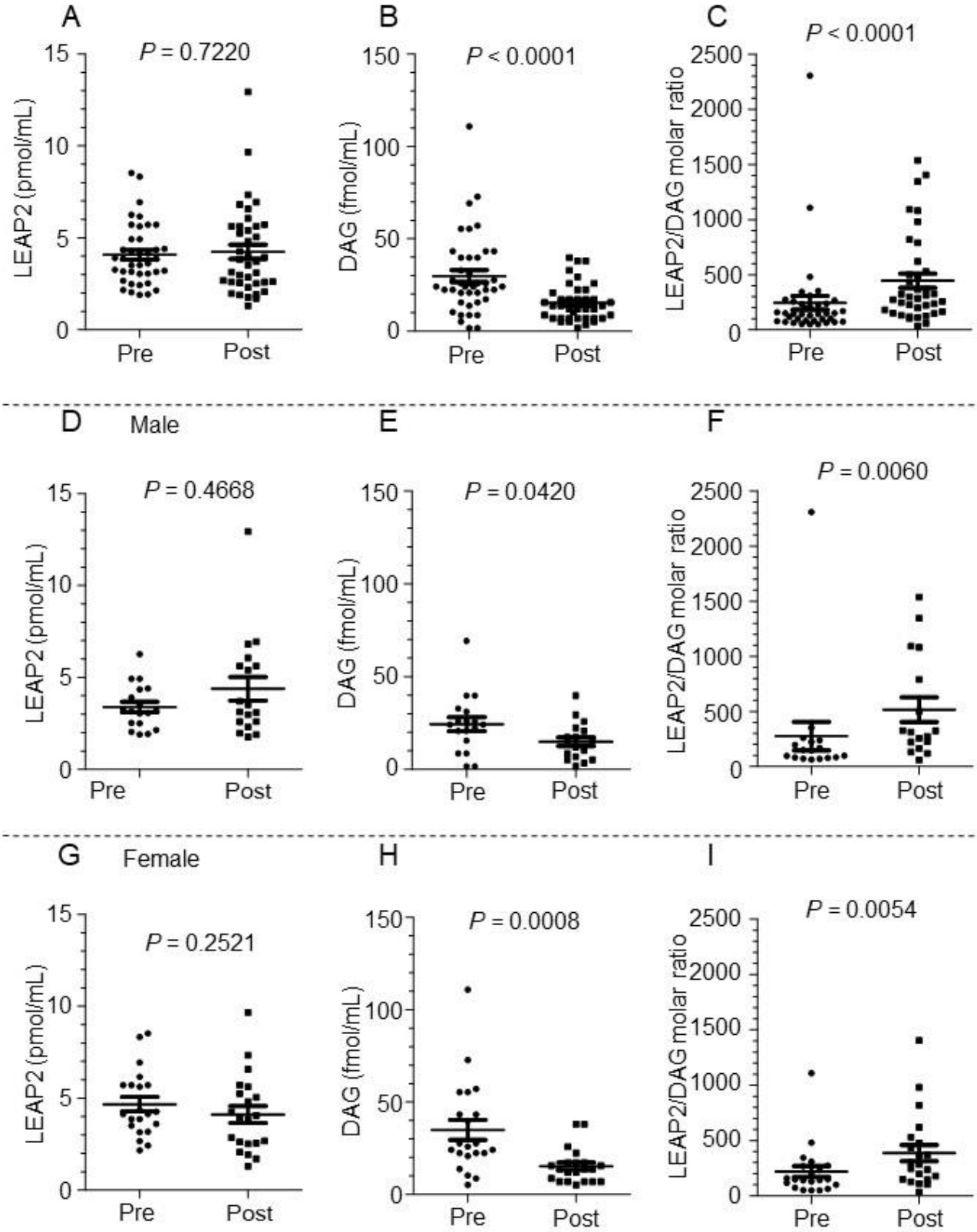
Fig. 1. Correlation of serum LEAP2 and DAG with BMI and age. Serum LEAP2 (A) among different BMI groups. (BMI < 25, n = 8, BMI 32–50, n = 30; BMI > 50, n = 9). Correlation of serum LEAP2 (B) or DAG (C) with BMI (n = 47). Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test (A), or Pearson's correlation coefficient (B, C).

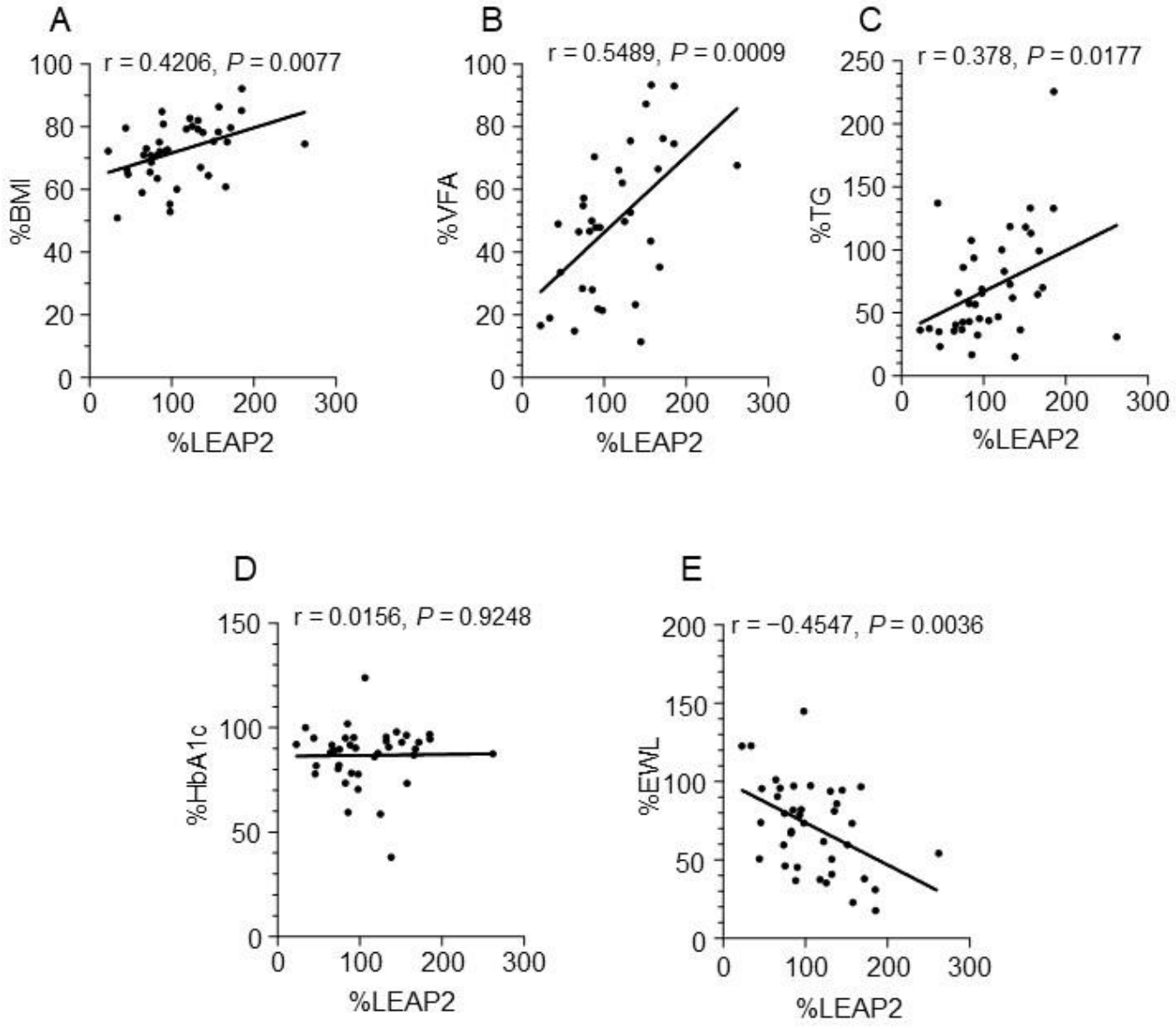
Fig. 2. Alternations of serum LEAP2 and DAG after VSG. Serum LEAP2 (A), DAG (B), and LEAP2/DAG molar ratio (C) in subjects with obesity before VSG (Pre) and 12 months after VSG (Post). Serum LEAP2, DAG, and LEAP2/DAG molar ratio in Pre and Post of male (D–F) and female (G–I) subjects. Male, n = 18; female, n = 21. Data were analyzed by Wilcoxon signed-rank test (A–I).

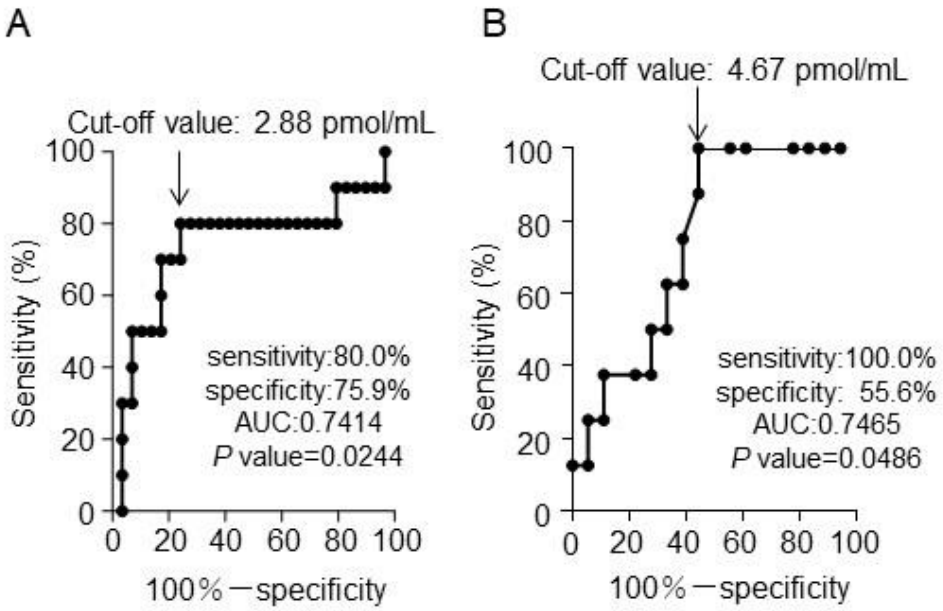
Fig. 3. Relationship of ratios of alternations between LEAP2 and clinical parameters after VSG. Relationship between %LEAP2 and %BMI (A), %VFA (B), %TG (C), %HbA1c (D), and %EWL (E). BMI, body mass index; VFA, visceral fat area; TG, triglycerides; EWL, excess weight loss; HbA1c, glycated hemoglobin or hemoglobin A1c. Data were analyzed by Pearson's correlation coefficient (A–E).

Fig. 4. Preoperative serum LEAP2 predicts postoperative %EWL and CR-T2DM. ROC curve of preopetative serum LEAP2 at an optimal level of 50% EWL (n = 39) (A) and CR-T2DM (n = 26) (B).









Parameter	Pre-VSG (n = 39)	12 months after VSG (n = 39)	*P* value
Age	42.7 ± 9.5	—	
Gender (men/women)	18/21	—	
%EWL	—	71.5 ± 29.1	
Diabetes mellitus	26	8	
Hypertension	27	13	
Dyslipidemia	34	8	
BMI (kg/m^2)	44.2 ± 9.8	32.0 ± 8.2	＜0.0001
Body fat mass (kg)	55.4 ± 20.3	32.5 ± 17.7	＜0.0001
VFA (cm^2)	189.1 ± 88.1	88.2 ± 47.2	＜0.0001
SFA (cm^2)	560.8 ± 250.7	367.8 ± 183.7	＜0.0001
SBP (mmHg)	139.9 ± 19.6	132.7 ± 17.1	0.0092
DBP (mmHg)	83.2 ± 13.4	80.6 ± 13.2	0.3544
FBG (mg/dL)	109.9 ± 23.9	97.6 ± 18.0	0.008
Fasting C-peptide (ng/mL)	2.68 ± 0.9	2.6 ± 2.2	0.112
HbA1c (%)	6.7 ± 1.5	5.6 ± 0.7	＜0.0001
TC (mg/dL)	185.9 ± 46.6	187.9 ± 31.6	0.1928
TG (mg/dL)	161.3 ± 99.2	93.8 ± 57.6	＜0.0001
LDL-C (mg/dL)	115.7 ± 34.2	105.5 ± 27.2	0.0868
HDL-C (mg/dL)	44.3 ± 14.2	62.6 ± 13.2	＜0.0001
Albumin (g/dL)	4.3 ± 0.4	4.3 ± 0.5	0.89
AST (IU/L)	27.6 ± 12.1	21.2 ± 11.9	0.0018
ALT(IU/L)	32.7 ± 18.1	26.3 ± 54.0	＜0.0001
γGTP (IU/L)	35.6 ± 18.4	26.1 ± 20.3	0.0002
Creatinine (mg/dL)	0.7 ± 0.3	0.7 ± 0.3	0.2084
Uric acid (mg/dL)	6.8 ± 1.5	5.6 ± 1.4	＜0.0001
GH (ng/mL)	0.3 ± 0.7	1.7 ± 1.6	＜0.0001
IGF-1 (ng/mL)	104.2 ± 45.6	143.2 ± 57.7	0.1017
CRP (mg/dL)	0.6 ± 0.8	0.2 ± 0.3	＜0.0001

Table 1. Comparison of clinical parameters between pre- and post-VSG.

Data are expressed as means ± SD. Abbreviations: EWL, excess weight loss; BMI, body mass index; VFA, visceral fat area; SFA, subcutaneous fat area; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate transaminase; ALT, alanine transaminase; γGTP, gamma-glutamyl transpeptidase; GH,