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Original Article

Changes of circulating ghrelin and obestatin levels in obese patients with or without type 2 diabetes mellitus

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Abstract: Objective: Most patients with Type 2 Diabetes Mellitus are successful in control their blood glucose and able to stop taking anti-diabetic medications including insulin after bariatric surgery. However, the underlying mechanism of how these operations work on patients with type 2 Diabetes Mellitus is still unclear. The aim of this study was to investigate the changes of ghrelin and obestatin in obese patients and in normal lean patients with type 2 diabetes mellitus before and after operation. Materials and methods: Total plasma ghrelin and obestatin levels were measured by radioimmunoassay in patients with Type 2 Diabetes Mellitus and control subjects, at both before and one month after Mini-Bypass or laparoscopic adjustable gastric band (LAGB) operation. Body mass index, fasting blood glucose, insulin, HbA_{1C}, peptide C and waist-hip ratio were calculated. Results: Post-operative circulating ghrelin and obestatin were elevated in obese subjects, suggesting that high ghrelin and obestatin may be involved in the etiology and pathophysiology of obesity. Conclusions: Improved Type 2 Diabetes Mellitus symptoms after operation may due to the decreased circulating ghrelin.

Keywords: Ghrelin, obestatin, type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus is an epidemic health problem, affecting more than 150 million people worldwide [1-3]. This number is expected to double in the first decades of the third millennium [4]. Recently, evidence for reduction of complications of type 2 diabetes with control of hyperglycemia has been reported, however, current available therapies, including diet, exercise, behavior modification, oral hypoglycemic agents and insulin, rarely contributes to euglycemia in patients [5-8]. Currently, bariatric surgery is the most effective weight loss treatment resulting in significant weight loss that will be sustained for many years [5, 6, 9]. Most patients with Type 2 Diabetes Mellitus are successful in control their blood glucose and able to stop taking anti-diabetic medications including insulin after bariatric surgery [10]. Enough evidence has been provided to support the notion that obesity surgeries, including Mini-Bypass, laparoscopic adjustable gastric band (LAGB) and gastrectomy surgery, are effective therapy for type 2 diabetes, at least in morbidly obese condition [11, 12]. However, the underlying mechanism of how these operations work on patients with Type 2 Diabetes Mellitus is still unclear.

Ghrelin was initially discovered by Kojima et al. in 1999 as an endogenous ligand for the growth hormone secret-agogue receptor [13]. However, subsequent evidence have shown that it plays critical roles in the short-term and long-term regulation of appetite and body weight [14]. Ghrelin stimulates appetite and promotes food intake, moreover, it affects diverse processes that involved in energy expenditure and fuel utilization, all of these promote weight gain and fat accumulation [15]. Exogenous ghrelin administration causes hyperphagia and obesity in rodents.

Recently, Zhang et al. have reported to find a ghrelin preprohormone gene encoding the 23 amino acids secreted peptide, namely "obestatin" [16]. It is also reported that obestatin can

bind orphan receptor GPR-39, the G protein-coupled receptor, which belongs to the GH secret-agogue receptor family [16]. The biological activity of obestatin depends on the amidation of its carboxyl terminus [17]. Unlike ghrelin, obestatin can suppress food intake, inhibit jejunal contraction, slow down the body-weight gaining and antagonize the actions of ghrelin when both peptides were co-administered [18]. These data suggest that the intricate balance of ghrelin and obestatin is important in the regulation of physiological functions of human body.

To the best of our knowledge, the relationship between obestatin and ghrelin, especially after surgical operation for Type 2 Diabetes Mellitus, has not been studied. Therefore, we aimed to investigate the relationship between obestatin and ghrelin in bariatric surgery and Type 2 Diabetes Mellitus in both obese and normal lean patients.

Materials and methods

Subjects

We studied 27 obese patients with or without Type 2 Diabetes Mellitus from the Tenth People's Hospital of Tongji University. Diagnosis of Type 2 Diabetes Mellitus was based on fasting plasma glucose (FPG) concentrations. Type 2 Diabetes Mellitus patients should have diabetes symptoms with the FPG level higher than 7.0 mmol/l, while patients with the FPG level lower than 6.1 mmol/l were not considered as diabetes patients. The BMI of obese patients should be higher than 28 km/m^2.

All patients were treated with either LAGB or Mini-Bypass procedures. To describe these surgical techniques briefly, a sleeve gastric tube and a single gastro-jejunal anastomosis were implanted into the patients using a linear stapler technique. This procedure was proved to be a simple procedure in the treatment of obese patients. The number of patients included in each group was labeled in **Tables 1**, **2**.

Secondary diabetes induced by medication or other diseases were excluded from the study. Patients with abnormal liver, kidney, heart and lung function, as well as mental disabilities were also excluded from the study. This study was approved by the committee of human research ethics of the Tenth People's Hospital of Tongji University. The informed consent was obtained from all participants.

Sample extraction

Blood samples were extracted before breakfast. The samples were collected in ethylenediamine tetracetate-coated polypropylene tubes (1 mg/ml) or aprotinin coated tubes (0.6 TIU/ml) and kept on ice, centrifuged immediately at 1,600 rpm for 15 min at 4°C, and the clear plasma supernatant was then stored at -80°C until assayed.

Measurement of plasma biochemical parameters

Plasma biochemical parameters, including blood lipids, liver and kidney function, blood glucose, insulin expression, glycosylated hemoglobin (HbA_{1C}), C-peptide, growth hormone, T3, T4, TSH and C-reactive protein, were measured in the Tenth People's Hospital of Tongji University. Plasma insulin was measured by enzyme chemiluminescence immunoassay using Beckman Coulter ACCESS 2 (Backman Coulter, Inc. USA). Blood glucose level was assayed using HITACHI 7600-0209 (Hitachi Limited, Japan). Insulin resistance index was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) of the following equation: HOMA-IR = insulin (μU/ml) × glucose (mmol/l)/22.5.

Measurement of obestatin and ghrelin

Plasma obestatin was examined with commercial radioimmunoassay (RIA) Kits (Beijing YUCEDA Trade CO. Ltd. China) using 125I-labelled obestatin as a tracer. Plasma samples were acidified with equal amount of 1% trifluoroacetic acid and centrifuged at 12,000 g for 20 min at 4°C. Then the acidified plasma was loaded onto C18 SEP-COLUMN (Beijing YUCEDA Trade CO. Ltd. China). The columns were washed with 60% acetonitrile in 1% trifluoroacetic acid and then with 1% trifluoroacetic acid for three times. The sample was dried and diluted in 1 ml buffer for RIA. The intra-assay coefficient variation of obestatin was less than 5%, and the inter-assay coefficient variation was less than 12%. The range of examination sensitivity of the assay was 50-6,400 pg/ml.

Plasma ghrelin was examined with commercial RIA kits according to standard protocol. The range of examination sensitivity of the assay was 10~1,280 pg/ml.

Table 1. Clinical characteristics of the obese subjects

	Obese patients without Type 2 Diabetes Mellitus (n = 11)	Obese patients with Type 2 Diabetes Mellitus (n = 16)	*p* value
Gender (male = 1 female = 0)	0.45±0.52	0.44±0.51	0.933
Age	23.91±5.03	25.13±4.96	0.540
Height (cm)	166.64±6.27	168.13±4.57	0.481
Body weight (kg)	96.73±19.34	100.25±12.61	0.571
BMI (kg/m^2)	34.6±4.6	35.39±3.45	0.613
Glucose (mmol/L)	4.78±0.46	6.36±0.94	<0.001
Insulin (mIU/L)	12.67±2.58	16.3±6.39	0.054
HbA_{1C} (mmol/L)	5.46±0.47	6.78±0.77	<0.001
Peptide C (μg/L)	2.27±0.45	3.12±1.11	0.012
HOMA-IR	2.68±0.54	4.61±1.99	0.002
Ghrelin (pg/mL)	172.38±70.96	184.34±46.43	0.630
Obestatin (pg/mL)	204.6±56.47	253.24±48.64	0.025
Ghrelin/obestatin	0.85±0.34	0.72±0.11	0.258

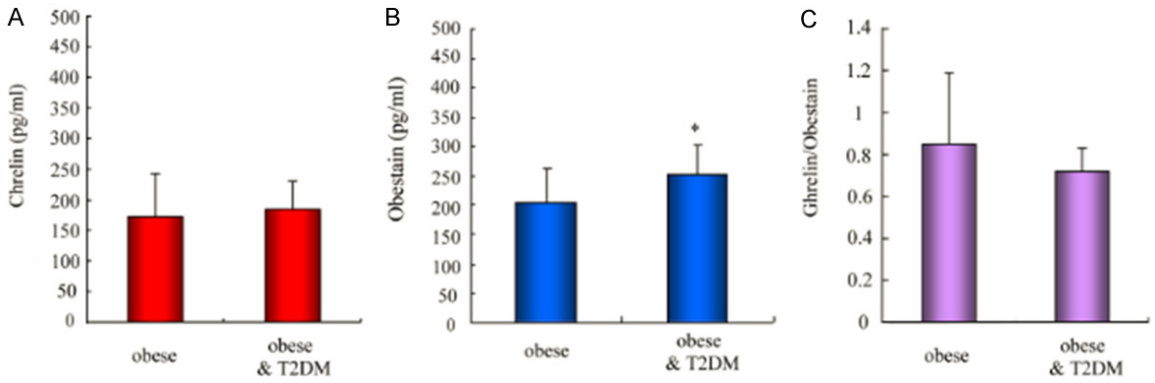

Figure 1. Expression level of ghrelin and obestatin in two groups of obese patients. A. The difference of plasma ghrelin expression between obese patients with and without Type 2 Diabetes Mellitus patients was not statistically significant. B. The difference of plasma obestatin expression between obese patients with and without Type 2 Diabetes Mellitus patients was statistically significant (*p = 0.025). C. The difference of Ghrelin/obestatin expression ratio between obese patients with and without Type 2 Diabetes Mellitus patients was not statistically significant.

Statistical analysis

All statistical analyses were performed using the statistical package SPSS 13.0 (SPSS Inc., Chicago, IL). Data are shown as mean ± standard deviation (SD). Data between two pairs were compared using independent-sample Student's t-test. The differences were considered significant at $p<0.05$ for all analyses.

Results

Changes of circulating ghrelin and obestatin levels in obese patients with type 2 diabetes mellitus

We first compared the obese patients with or without Type 2 Diabetes Mellitus. The clinical characteristics of the subjects are shown in **Table 1**. The level of blood glucose, HbA_{1C}, HOMA-IR and obestatin (**Figure 1**) were significantly higher in obese patients with Type 2 Diabetes Mellitus as compared to obese patients without Type 2 Diabetes Mellitus, however, the plasma obestatin/ghrelin ratio was not significantly different in those obese patients (**Figure 1**). After surgery, both groups of patients exhibited decreased body weight and BMI, as well as increased expression of obestatin and ghrelin (**Figure 2**). Obese patients with Type 2 Diabetes Mellitus under Mini-Bypass treatment also showed decreased plasma insulin and HOMA-IR (**Table 2**). So, the decreased body weight of obese patients after surgery is most likely due to the increased circulating ghrelin and obestatin levels.

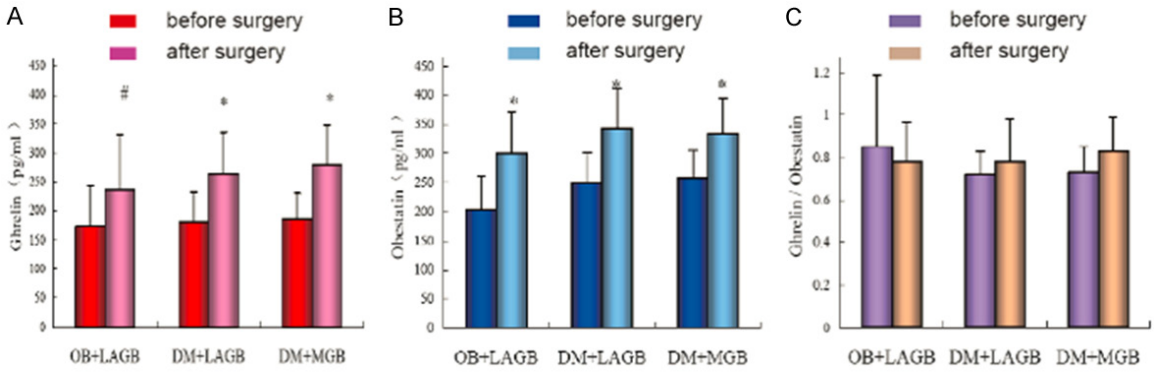

Figure 2. Expression of ghrelin and obestatin was increased after surgery in both groups of obese patients. A. The expression of ghrelin was increased after surgery in both groups of patients. (OB, obese patients, DM, obese patients with Type 2 Diabetes Mellitus, MGB, Mini-Bypass surgery) (#p = 0.014, *p<0.01). B. The expression of obestatin was increased after surgery in both groups of patients. (*p<0.01). C. The Ghrelin/obestatin expression ratio was not significantly changed after surgery in all conditions.

Table 2. Clinical characteristics of the obese subjects after procedures

	Obese patients without Type 2 Diabetes Mellitus		Obese patients with Type 2 Diabetes Mellitus			
Procedure	LAGB (n = 11)	*p*	Mini-Bypass (n = 7)	*p*	LAGB (n = 9)	*p*
Body weight (kg)	96.73±19.34	0.628	99.14±15.04	0.497	101.33±11.35	0.812
BMI (kg/m^2)	34.6±4.46	0.673	35.1±3.85	0.695	36.2±3.35	0.835
Glucose (mmol/L)	0.14±0.88	0.617	1.11±1.24	0.055	0.38±0.76	0.176
Insulin (mIU/L)	1.37±3.76	0.254	6.81±7.33	0.049	0.8±4.23	0.585
HbA_{1C} (mmol/L)	0.07±0.24	0.341	0.61±0.71	0.061	0.52±0.78	0.081
Peptide C (μg/L)	0.23±0.44	0.110	0.61±1.04	0.170	0.21±1.25	0.635
HOMA-IR	0.33±0.89	0.243	2.48±2.48	0.038	0.39±0.81	0.182
Ghrelin (pg/mL)	-64.47±72.34	0.014	-90.97±28.77	<0.001	-81.88±27.85	<0.001
Obestatin (pg/mL)	-95.67±93.03	0.007	-77.21±12.29	<0.001	-93.85±64.21	0.002

Changes of circulating ghrelin and obestatin levels in normal patients with type 2 diabetes mellitus

To further study changes of obestatin and ghrelin in patients with Type 2 Diabetes Mellitus, we next examined the level of obestatin and ghrelin in patients of normal body weight with or without Type 2 Diabetes Mellitus. Type 2 Diabetes Mellitus patients did not exhibit significant difference in biochemical parameters except that they have higher blood sucrose level. The level of plasma obestatin and ghrelin were significantly decreased in normal patients after subtotal gastrectomy surgery and in Type 2 Diabetes Mellitus patients after partial gastrectomy surgery (**Figure 3**). We also detected decreased blood sucrose in Type 2 Diabetes Mellitus patients after surgery (**Table 3**). These results suggested that decreased circulating ghrelin probably result in the improvement of type 2 diabetes.

Discussion

Both ghrelin and a ghrelin-associated peptide (obestatin) originate from the same peptide precursor (preproghrelin). Obestatin can bind to the orphan G-protein coupled receptor, which presents in the brain, making the function of obestatin in the central nervous system available [16]. In rodents, it has been reported that obestatin reduced food ingestion and body weight after peripheral administration, as opposed to ghrelin, which increased food intake and weight [19]. This raises the possibility that obestatin might be involved in the regulation of energy balance and bodyweight. So in this paper, we investigated the role of obestatin in obesity and Type 2 Diabetes Mellitus in human. We found that the level of blood glucose, HbA_{1C}, HOMA-IR and obestatin were significantly higher in obese patients with Type 2 Diabetes Mellitus as compared to obese patients without Type 2 Diabetes Mellitus (**Table 1**).

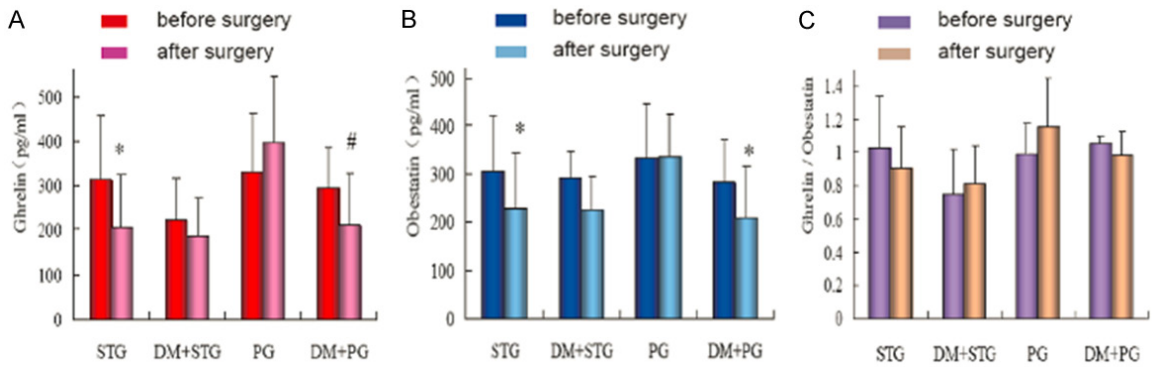

Figure 3. Expression of ghrelin and obestatin was decreased after surgery in both groups of non-obese patients. A. The expression of ghrelin was decreased in normal patients after STG surgery and in Type 2 Diabetes Mellitus patients after PG surgery. (STG, subtotal gastrectomy, DM, patients with Type 2 Diabetes Mellitus, PG, partial gastrectomy) (#p<0.05, *p<0.01). B. The expression of obestatin was decreased in normal patients after STG surgery and in Type 2 Diabetes Mellitus patients after PG surgery. (*p<0.01). C. The Ghrelin/obestatin expression ratio was not significantly changed after surgery in all conditions.

Table 3. Clinical characteristics of patients with normal body weight after procedures

	Patients without Type 2 Diabetes Mellitus (n = 20)		Patients with Type 2 Diabetes Mellitus (n = 9)	
	Difference	*p*	Difference	*p*
Glucose (mmol/L)	-0.46±0.95	0.094	2.9±2.46	0.034
Insulin (mIU/L)	-1.94±1.94	0.042	0.2±0.6	0.490
HbA_{1C} (mmol/L)	-0.18±0.59	0.277	-1.69±1.97	0.369
C peptide	-0.36±0.56	0.133	1.03±2.56	0.019
HOMA-IR	-0.59±0.68	0.406	0.16±1.12	0.736
Ghrelin (pg/mL)	109.37±109.14	0.002	33.33±24.11	0.020
Obestatin (pg/mL)	76.26±67.02	0.001	63.61±27.51	0.002
Ghrelin/obestatin	0.12±0.31	0.176	-0.06±0.11	0.217

In a previous study, Cummings et al. stated that plasma ghrelin levels were significantly decreased in obese subjects after weight loss or gastric bypass surgery [20], which is inconsistent with our finding. We also did not detect any changes in the ratio of obestatin and ghrelin, which is reported to be particularly important in keeping the balance of physiological functions [18]. Neither did we found any correlation between changes of obestatin and other physiological index.

Nevertheless, after either LAGB or Mini-Bypass surgery, both groups of patients exhibited decreased body weight as well as increased expression of obestatin and ghrelin. These results suggested that the decreased body weight of obese patients after surgery is most likely due to the increased circulating ghrelin and obestatin levels. Also, we found that patients exhibited significant decrease of glucose and insulin resistance after Mini-Bypass as compared to LAGB. The increased expression of ghrelin and obestatin may due to feedback mechanism in the regulation of physiological functions.

We also examined the circulating ghrelin and obestatin in lean patients with or without Type 2 Diabetes Mellitus. We found that obestatin and ghrelin were significantly decreased in Type 2 Diabetes Mellitus patients after partial gastrectomy surgery. Since ghrelin is a well-known hormone in stimulating appetite and increase the absorption of nutrition, changes of obestatin and ghrelin are hotly debated clinical index in evaluating patients with obesity and Type 2 Diabetes Mellitus. Consistent with previous reports, changes in ghrelin is negatively correlated with circulating insulin and HOMA-IR [21], indicating decreased ghrelin may be involved in the improvement of Type 2 Diabetes Mellitus after surgery.

To our knowledge, this is the first report to describe the plasma level of obestatin and ghrelin in control subjects and patients with obesity and Type 2 Diabetes Mellitus. The reason for the increased obestatin levels in patients with Type 2 Diabetes Mellitus needs to be clarified in further studies. Our study is mainly based on single measurements of blood

obestatin, it would be more interesting to examine serial changes of plasma obestatin levels in obese, prediabetic, diabetic subjects to further clarify the role of obestatin in the pathogenesis of Type 2 Diabetes Mellitus.

The decreased body weight of obese patients after surgery is most likely due to the increased circulating ghrelin and obestatin levels. The improvement of type 2 diabetes after surgery is probably due to the decreased circulating ghrelin expression.

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Disclosure of conflict of interest

None.

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