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Decreased gastric body mucosa obestatin expression in overweight and obese patients

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ABSTRACT

Obestatin is a recently discovered gastrointestinal hormone. It might play a role in the pathophysiology of obesity. We tried to investigate the expression of obestatin in gastric body mucosa in overweight (BMI $\geq$ 24 kg/m^2)/obese (BMI $\geq$ 28 kg/m^2) patients. Thirty overweight/obese patients and 20 healthy controls were included in the study. Biopsy specimens of gastric mucosa were obtained from the middle body of the greater curvature. Obestatin expression in gastric mucosa was evaluated by immunohistochemistry. Fasting plasma obestatin levels were measured by radioimmunoassay. The number of obestatin-positive cells in gastric body mucosa was significantly lower in overweight and obese patients than that in healthy subjects. The plasma concentrations of obestatin were also decreased in overweight and obese patients. There was a positive correlation between the numbers of obestatin-positive cells in the gastric body mucosa and circulating obestatin levels. The results indicate that overweight and obese subjects have a reduction in the number of obestatin-positive cells in gastric body mucosa.

1. Introduction

Ghrelin is a 28 amino acid peptide, which is involved in the regulation of food intake and energy homeostasis [22]. In 2005, a new peptide which named obestatin had been found. It is derived from the same gene as ghrelin [24]. The emerging evidence implies that obestatin may have some unknown but important biological functions [4]. Its potential role in the pathophysiology of obesity has been of much interest. The effects of obestatin on food intake and body weight regulation in rodents by peripheral or central administration were controversial. Zhang et al. [24] reported that obestatin decreased food intake and body weight by decelerating gastric emptying. In recent research, daily caloric intake and body weight was decreased by obestatin treatment in rats fed a standard laboratory diet [2]. But several studies could not reproduce the anorexigenic and reduced body weight gain property of it [9,5]. Peripheral obestatin had no effect on food intake and brain Fos expression in rats [14]. Lagaud et al. [15] demonstrated that obestatin decreased the body weight gain and food consumption of mice with an unusual dose–response relationship. It might explain the difficulties in reproducing the effects of obestatin with different dose by some groups. Plasma obestatin concentrations in obese patients were studied by many investigators. In most research of them, circulating obestatin levels were significantly changed in obese subjects compared with normal weight individuals [11,21]. These raised the possibility that obestatin might have the effect of decreasing body weight. Obestatin was isolated from rat stomach at first. The obestatin-producing cells in stomach were A-like cells which distributed in the basal part of the oxyntic mucosa [25]. To our best knowledge, no data has been published about obestatin expression in gastric mucosa in overweight and obese patients. It is not known whether the expression of obestatin in gastric mucosa is changed by obesity. In the present study, we try to explore the difference of the prevalence of obestatin immunoreactivity in gastric body mucosa tissues between overweight/obese patients and healthy subjects.

2. Materials and methods

2.1. Subjects and research design

The study was approved by Harbin Medical University Ethics Committee. All samples were obtained with written informed consent of the patients prior to their inclusion, in accordance with the Helsinki Declaration. According to the criteria of the guidelines for prevention and control of overweight and obesity in Chinese adults, 30 overweight/obese patients (BMI $\geq$ 24 kg/m^2 or

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BMI $\geq$ 28 kg/m^2) and 20 normal weight healthy controls (18.5 kg/m^2 < BMI < 24 kg/m^2) were enrolled in the study. Exclusion criteria were: age < 18 or >80 years, pregnancy, diabetes mellitus, hypertension, cachectic state including cancer, systemic infection, thyroid and liver diseases, serious heart diseases, lung disease, renal impairment, alcoholic abuse, drug addiction, and chronic corticosteroid or nonsteroidal antiinflammatory drug use. None had the history of gastrointestinal surgery or any other conditions known to affect endocrine and gastrointestinal function.

2.2. Specimen collection

2.2.1. Blood sample collection

After overnight fasting for 12 h, blood samples were drawn in all 50 subjects at 8:00 a.m. Blood samples for the measurement of obestatin were drawn into chilled polypropylene tubes containing EDTA-2Na (1 mg/ml) and aprotinin (Phoenix Pharmaceuticals, Belmont, CA; 100 μl containing 0.6 trypsin inhibitor units per milliliter of blood). After immediate centrifugation at 4 °C for 15 min, plasma was stored at −80 °C until assayed.

2.2.2. Biopsy sample collection

All subjects were performed upper gastrointestinal endoscopy immediately after blood sample collection. Biopsy specimen from gastric body mucosa was obtained from the middle body of the greater curvature.

2.3. Immunohistochemistry

Expression of obestatin in the gastric body mucosa was studied by immunohistochemistry. Gastric mucosa tissues were formalin-fixed and paraffin-embedded. Following deparaffinization and rehydration, sections were irradiated in 0.1 mol/L sodium citrate buffer (pH 6.0) in a microwave oven (medium low temperature) for 12 min. Sections were treated with 3% H_2O_2 to inhibit endogenous peroxidase activity, then 1% bovine serum albumin to block nonspecific protein binding sites. Obestatin expression was assessed using a rabbit anti-human obestatin antibody (Phoenix Pharmaceuticals Inc, Belmont, CA, USA) in a dilution of 1:2000. After incubating with primary antibodies overnight at 4 °C, the secondary antibody incubation was performed by applying biotinylated goat anti-rabbit IgG (Boster Biotechnology Co., Wuhan, China) for 20 min at room temperature. Subsequent immunostaining was done using the biotin–streptavidin–peroxidase method with 3,3′-diaminobenzidine (Boster Biotechnology Co., Wuhan, China). Finally, the sections were counterstained in Mayer's hematoxylin for 1–2 min, dehydrated and cover slipped. Negative immunohistochemical controls used lacked primary antibody. Biopsy slides were read under a light microscope by a single pathologist without knowledge of clinical features. Obestatin densities were expressed as the number of stained cells per 0.1662 mm^2.

Table 1
Characteristics of the two different groups. Values are mean ± SD.

	Overweight/obesity patients (n = 30)	Healthy subjects (n = 20)
Age (years)	41.9 ± 7.8	43.7 ± 7.1
Male/female	16/14	11/9
BMI (kg/m^2)	27.5 ± 1.3••	21.8 ± 0.8
Fasting blood glucose (mmol/l)	5.01 ± 0.30	4.88 ± 0.34
HOMA-IR	2.10 ± 0.30••	1.71 ± 0.25
Total cholesterol (mmol/l)	5.48 ± 0.75••	4.68 ± 0.40
Triglyceride (mmol/l)	1.97 ± 0.39••	1.47 ± 0.38
HDL cholesterol (mmol/l)	1.03 ± 0.12••	1.23 ± 0.25
Plasma obestatin concentrations (pg/ml)	52.93 ± 7.89••	69.66 ± 7.45

••P < 0.01, compared with control group.

2.4. Plasma obestatin levels and biochemical analysis

Fasting plasma obestatin levels were measured by radioimmunoassay with commercial RIA kit (Phoenix Pharmaceuticals).

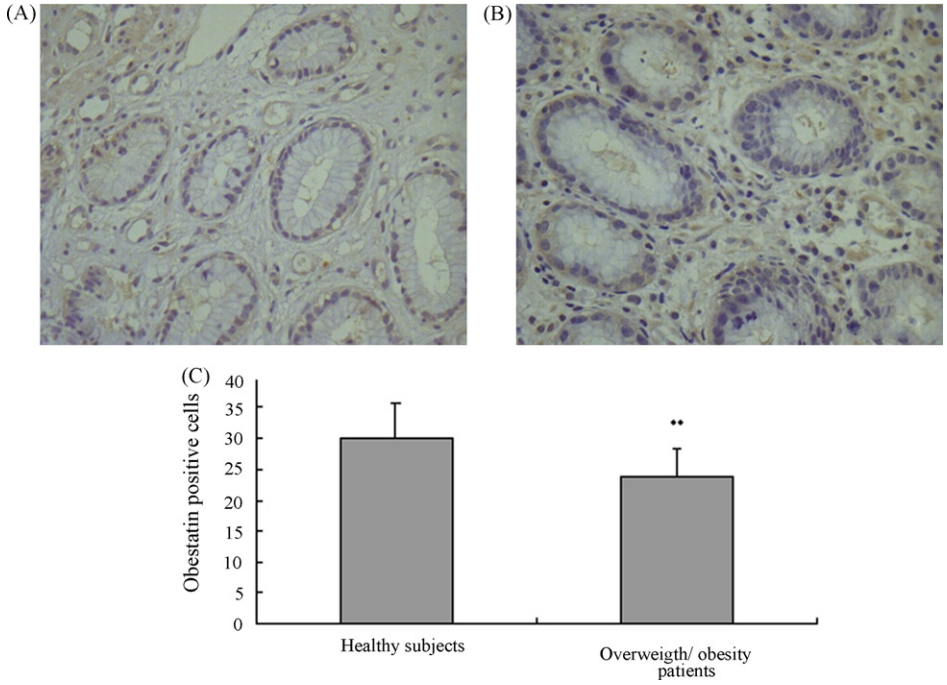

Fig. 1. (A) Immunostaining of obestatin in the gastric body mucosa from overweight, obese patients (magnification, ×200). (B) Immunostaining of obestatin in the gastric body mucosa from controls (magnification, ×200). (C) Comparison of the numbers of obestatin-positive cells in the gastric body mucosa between overweight/obese patients and healthy subjects. ••P < 0.01 compared with healthy subjects.