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Increased acyl ghrelin but decreased total ghrelin and unacyl ghrelin in Chinese Han people with impaired fasting glucose combined with impaired glucose tolerance

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ABSTRACT

We assessed the plasma acyl ghrelin (AG), unacyl ghrelin (UAG), and total ghrelin (TGhr) levels in Chinese adults with pre-diabetes and newly diagnosed diabetes mellitus (NDDM) after an oral glucose tolerance test (OGTT), and abdominal subcutaneous fat area and visceral fat area (VFA) were measured. Fasting AG level was increased in the impaired fasting glucose (IFG) combined with impaired glucose tolerance (IFG + IGT) and NDDM groups. AG, UAG, and TGhr levels were significantly decreased post-OGTT, and the decrements of 30-min AG, UAG, and TGhr post-OGTT were not significantly different among groups. UAG and TGhr levels did not differ significantly among the normal glucose tolerance (NGT), IFG and NDDM groups, but they decreased obviously in the IFG + IGT and impaired glucose tolerance (IGT) groups. The NDDM group had larger VFA than the NGT, IGT, and IFG + IGT groups, even after adjustment for height, it was still larger than the NGT group. The factors such as dyslipidemia and obesity which are prone to develop insulin resistance (IR) and decrease insulin sensitivity (IS) were negatively correlated with UAG and TGhr, positively with AG/UAG, while no correlations with AG. In terms of evaluating IS and IR, AG/UAG ratio may be superior in AG concentration. Our findings suggest that relative sufficiency of AG, the deficiency of TGhr and UAG are already present in IFG + IGT patients. We speculate that there is UAG resistance in severe hyperglycemia (diabetic state), which could produce elevated TGhr and UAG compared to IFG + IGT group. In the development of T2D, increase of VFA could be the initiating factor, leading elevated AG, reduced UAG, IR, decreased IS, and finally hyperglycemia.

Abbreviations: 120, 120-min post-OGTT; 30, 30-min post-OGTT; AG/UAG, the ratio of AG and UAG; AG, acyl ghrelin; BMI, body mass index; CT, computed tomography; DBP, diastolic blood pressure; F, fasting; Gutt, Gutt index; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment for insulin resistance index; HOMA-β, the homeostasis model assessment of β cell function; IFG, impaired fasting glucose; IFG + IGT, IFG combined IGT; IGT, impaired glucose tolerance; INS, insulin; IR, insulin resistance; IS, insulin sensitivity; LAP, Lipid accumulation product; LDL-C, low-density lipoprotein cholesterol; Matsuda, Matsuda index; MRI, magnetic resonance imaging; NDDM, newly diagnosed diabetes mellitus; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PG, plasma glucose; SBP, systolic blood pressure; SFA, abdominal subcutaneous fat area; T2D, type 2 diabetes; TAFA, total abdominal fat area(SFA + VFA); TC, total cholesterol; TG, triglyceride; TGhr, total ghrelin; UAG, unacyl ghrelin; VFA, visceral fat area; WC, waist circumference; WHR, waist-to-height ratio; $\Delta I_{30}/\Delta G_{30}$, early insulin secretion of β cells.

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Introduction

Ghrelin is a 28-amino-acid gastrointestinal-derived peptide predominantly produced in the stomach[25,52] and is the only known peripherally produced and centrally acting peptide hormone that stimulates food intake and digestive functions [44]. It is widely expressed in many other central and peripheral tissues, including the hypothalamus, pituitary, pancreatic islets, and adipose tissue [25,51,54]. Ghrelin is circulated in two major forms in the blood, acyl ghrelin (AG), and unacyl ghrelin (UAG). AG is the bioactive form, with an *n*-octanoyl group at the serine 3 residue, while UAG is the form lacking *n*-octanoylation [21]. The UAG represents by far the most abundant form of circulating ghrelin as indicated by an AG/total ghrelin (TGhr) ratio that varies from 1:55 to 1:5 depending upon the method of blood processing to prevent the rapid degradation of the acylated form in tissues and the circulation by endogenous esterase activity [44]. In addition to its ability to stimulate growth hormone secretion mediated by the growth hormone secretagogue receptor 1a, AG can also increase gastric motility and acid secretion, stimulate prolactin and adrenocorticotropic hormone secretion, increase body weight and adiposity, and influence glucose and lipid metabolism [13,20,30,48,49,54,58]. It has been reported that both AG and UAG can promote proliferation [3] and inhibit apoptosis of pancreatic β cells [18]. AG modulates pancreatic β-cell secretion in humans and rats, and increases plasma glucose levels by reducing insulin secretion[3,7,16,40,49] . Endogenous AG in islets acts on β cells to decrease glucose-induced insulin release at least partly via attenuation of Ca^{2+} signaling, and that the insulinostatic action may be implicated in the upward control of blood glucose [14].

TGhr level is negatively related with insulin resistance (IR) in both lean and obese individuals [4]. The potentially differential roles of the circulating acyl and unacyl forms of ghrelin in the modulation of glucose metabolism were hourly reported recently. Evidence suggest that AG and UAG may induce different physiological and metabolic effects. UAG suppresses hepatic glucose release and antagonizes the AG-induced increase in hepatic glucose output [17], as well as decreases circulating insulin levels [9]. In humans, the circulating level of AG is increased under anorexia, cachexia, obesity, IR, and type 2 diabetes (T2D), while UAG and TGhr levels among subjects with obesity, IR, or obesity-associated T2D are lower compared with parallel lean subjects [33,42]. A reciprocal relationship exists between TGhr and insulin, and TGhr is negatively correlated with the prevalence of T2D[8,38] . It has also been reported that the homeostasis model of assessment for IR index (HOMA-IR) and body mass index (BMI) are negatively associated with TGhr and UAG, but positively with the AG and AG/UAG ratio[34,35] .

For TGhr, it has been reported that there is no significant difference between T2D and impaired glucose tolerance (IGT) groups [38], or T2D and normal glucose tolerance (NGT) groups [26]. Furthermore, AG levels are higher in obese individuals with IGT and T2D compared with obese normoglycemic patients [42]. In a prospective follow-up study, there was no significant difference in AG level between NGT and those who developed impaired fasting glucose (IFG), IGT, or T2D [53]. The divergence may have resulted from the sample size, the participants with different duration of T2D and the pre-diabetes categories (i.e., whether or not all the pre-diabetes individuals with IFG, IGT, or IFG + IGT were included). Otherwise, diverse hyperglycemic conditions and different durations of pre-diabetes and diabetes may lead to divergence in the β-cell function and insulin sensitivity (IS), which may result in disparity of ghrelin levels.

The aim of the present study was therefore to investigate TGhr, AG, and UAG (0, 30 and 120 min) levels in the serum during a 75-g glucose oral glucose tolerance test (OGTT) among subjects with NGT, IFG, IGT, IFG + IGT, or newly diagnosed diabetes mellitus (NDDM). The relationships between levels of different forms of ghrelin and β-cell function, IR, IS, and visceral fat area (VFA) were also evaluated.

Materials and methods

Subjects and protocol

A total of 1200 and 530 individuals were recruited from either Yincao Community or the Hangtian Community of Chengdu (Sichuan Province, China), respectively, from September to November 2011 ($n = 1730$). All participants were aged 40–70 years, of Chinese nationality and from the Han ethnic group. OGTT was carried out for all participants. The classification of different hyperglycemic types based on OGTT results was in accordance with the diagnostic criteria given by the American Diabetes Association in 2011 [1]. The participants with IFG ($n = 87$), IGT ($n = 118$), IFG + IGT ($n = 98$), and NDDM ($n = 118$) were selected. Subsequently, the age- and sex-matched control group ($n = 135$) was randomly selected from the remaining 1309 participants with NGT. Subjects with any of the following conditions were excluded: history of cardio- or cerebro-vascular events (according to medical documents of secondary or tertiary hospitals); receiving oral or intravenous corticosteroid hormone treatment; hepatic cirrhosis and ascites; hyperthyroidism or hypothyroidism; malignant tumor; severe disability or mental disorder; or pregnant or breastfeeding women. Informed written consent was obtained from each participant and the entire study procedure was approved by the Biological Sciences Ethical Committee of West China Hospital of Sichuan University, China.

Anthropometric measurements and OGTT

Body weight and height were measured with subjects wearing light clothes without shoes. BMI was estimated by body weight (kg) dividing square of height (m^2). Waist circumference (WC, cm) was measured between the lowest rib and the iliac crest at the end of expiration with the subject in a standing position. Waist-to-height ratio (WHR) was calculated by dividing waist circumference (cm) by height (cm). Each participant underwent a standardized 75-g OGTT. After an overnight fast, blood samples were obtained from an antecubital vein without compression before 09:00 h. Each participant ingested a cup of water containing 75 g glucose before 09:00 h. Plasma samples were obtained before oral glucose loading and at 30- and 120-min post-OGTT. Lipid accumulation product (LAP) was computed by using waist circumference (WC, cm) and triglyceride (TG) level (mmol/L): $(WC - 65) \times TG(men)$ and $(WC - 58) \times TG(women)$[22] .

Measurement procedures

Blood samples were allowed to clot at room temperature for 20 min and were centrifuged at 3900 *g* for 10 min at 4–8 °C and then frozen at −80 °C in multiple aliquots until further analysis. All blood samples were assayed for plasma AG, TGhr, glucose, and insulin. Fasting serum samples were additionally assayed for triglyceride, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and glycated hemoglobin (HbA_{1c}).

Plasma glucose levels were determined in duplicate by a glucose oxidase method adapted to an automated analyzer (Hitachi 704; Boehringer Mannheim, Germany). TG, HDL-C, and LDL-C levels were determined by enzymatic methods with commercial reagent sets (Boehringer Mannheim). Serum insulin levels were measured by electrochemiluminescence (Cobase411; Roche, Switzerland)