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Early phase glucagon and insulin secretory abnormalities, but not incretin secretion, are similarly responsible for hyperglycemia after ingestion of nutrients

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ABSTRACT

Aims: Hypersecretion of glucagon and reduced insulin secretion both contribute to hyperglycemia in type 2 diabetes (T2DM). However, the relative contributions of impaired glucagon and insulin secretions in glucose excursions at the various stages of T2DM development remain to be determined.
Methods: The responses of glucagon and insulin as well as those of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) were examined before and after ingestion of glucose or mixed meal in Japanese subjects with normal or impaired glucose tolerance (NGT and IGT) and in non-obese, untreated T2DM of short duration.
Results: In OGTT, T2DM showed a rise in glucagon at 0–30 min, unlike NGT and IGT, along with reduced insulin. In MTT, all three groups showed a rise in glucagon at 0–30 min, with that in T2DM being highest, while T2DM showed a significant reduction in insulin. Linear regression analyses revealed that glucose area under the curve $(AUC)_{0–120\ min}$ was associated with glucagon-$AUC_{0–30\ min}$ and insulin-$AUC_{0–30\ min}$ in both OGTT and MTT. Total and biologically intact GIP and GLP-1 levels were similar among the three groups.
Conclusions: Disordered early phase insulin and glucagon secretions but not incretin secretion are involved in hyperglycemia after ingestion of nutrients in T2DM of even a short duration.

1. Introduction

Postprandial glucose homeostasis is controlled by the two counteracting hormones glucagon and insulin. Unger first proposed a two hormone abnormality theory of diabetes pathophysiology in which reduced secretion of glucose-lowering insulin and enhanced secretion of glucose-elevating glucagon both play key roles in postprandial hyperglycemia in diabetes (Unger & Cherrington, 2012). Many investigators have noted that glucagon secretion is enhanced in type 2 diabetes (T2DM), and might thereby aggravate postprandial glucose levels already raised by reduced insulin secretion (Reaven, Chen, Golay, et al., 1987; Seino, Goto, Kurahachi, et al., 1977). The contributions of each of these defects to glucose excursion after ingestion of glucose or mixed meal during the progression of the disease remain to be determined. In addition, studies of insulin secretion have found impairment of insulin secretion to be an early manifestation (Haffner, Miettinen, Gaskill, et al., 1996; Weyer, Bogardus, Mott, et al., 1999), but it remains unknown when impaired glucagon secretory defects appear during T2DM development.

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The mechanisms involved in nutritional regulation of glucagon secretion remain unclear, even though the mechanisms involved in regulating insulin secretion are rapidly being elucidated (Prentki, Matschinsky, & Madiraju, 2013; Seino, Shibasaki, & Minami, 2011). It has been postulated that glucagon secretion by glucose relies on several different factors: 1) neural sensing of glucose and subsequent regulation of α cells, 2) indirectly by sensing glucose from neighboring β cells, which secrete insulin as well as γ-aminobutyric acid, zinc and glutamate, and δ cells, which secrete somatostatin, and 3) intrinsically by glucose sensing of α cells by mechanisms still under investigation (Kawamori, Welters, & Kulkarni, 2010; Marroqui, Alonso-Magdalena, Merino, et al., 2014). Some amino acids (e.g., arginine, alanine, and glutamine) are potent stimulators of glucagon secretion, accounting for most glucagon release after protein intake. Chronic elevation of fatty acids, often seen in T2DM, may also enhance glucagon secretion, but by unknown mechanisms.

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are incretins secreted from the gut in response to ingestion of various nutrients, and stimulate insulin secretion from pancreatic β cells glucose-dependently (Drucker, 2013; Holst, 2007; Seino & Yabe, 2013). Interestingly, the incretins exert opposing effects on glucagon secretion: GLP-1 suppresses glucagon and GIP enhances glucagon secretion (Christensen, Calanna, Sparre-Ulrich, et al., 2014; Christensen, Vedtofte, Holst, et al., 2011; Drucker, 2013; Holst, 2007; Mentis, Vardarli, Kothe, et al., 2011; Seino & Yabe, 2013; Taminato, Seino, Goto, et al., 1977). Accordingly, impaired secretion and/or action of GLP-1 or GIP could contribute to the development of T2DM.

In the current study, we measured the levels of insulin and glucagon and those of the incretins in Japanese subjects with normal glucose tolerance (NGT), relatively mild IGT, and untreated T2DM to clarify changes in glucagon secretion during the course of T2DM development.

2. Materials and methods

The protocol (UMIN registration number: UMIN000014575) was approved by the ethics committee of each participating institute, and written informed consent was obtained from all participants. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Subjects of NGT, IGT and T2DM, with body mass index (BMI) less than 27.0 kg/m^2 and age between 20 and 65 years, were purposely recruited in order to evaluate changes in secretions of glucagon and insulin during the development of non-obese T2DM, which is typically seen among East Asians (Moller, Pedersen, Tanaka, et al., 2014; Ntuk, Gill, Mackay, et al., 2014). At screening, subjects with type 1 diabetes, gastrointestinal tract disease, cardiac disease, pancreatic disease, liver disease, renal disease, alcohol or drug abuse, anti-diabetic medication, diabetogenic medication or malignancy, or pregnancy were excluded. T2DM with HbA1c 7.9% or above were excluded from the study. Non-diabetic subjects with FPG 110 mg/dL or above were excluded from the study. Of 145 total subjects screened, 18, 17 and 4 subjects were excluded due to the inclusion criteria for BMI, age or both. One T2DM was excluded for HbA1c above 7.9%, and four non-diabetic subjects were excluded for FPG above 110 mg/dL. Diagnosis of NGT, IGT and T2DM was according to the criteria of the Japanese Diabetes Society (Seino, Nanjo, Tajima, et al., 2010).

Participants were subjected to both oral glucose and meal tolerance tests (OGTT and MTT) in the morning after an overnight fast on two separate days, as described previously (Yabe, Kuroe, Lee, et al., 2010). Briefly, 75 grams of glucose or a Japanese standard meal (480 kcal, carbohydrate: protein: fat = 2.8:1:1) was ingested within 5 and 10 min, respectively. Blood samples were withdrawn from a cubital vein of the subjects directly into evacuated sample tubes containing relevant preservatives [e.g., ethylenediaminetetraacetate-2Na and aprotinin-containing tubes (catalogue no. NP-EA0305; Terumo, Tokyo, Japan) for glucagon; and BD P700 tubes (catalogue no. 366473; BD, Franklin Lakes, NJ, USA) for GLP-1 and GIP)] using Venoject II Multisample Luer Adaptor S and tube holder D (catalogue nos. XX-MN2000S and XX-VP010HD; Terumo, Tokyo, Japan). These tubes were kept on ice until centrifugation. Separated plasma samples were frozen and kept at −70 °C until further analysis.

2.1. Laboratory determinations

Glucagon was measured using Glucagon kit "Daiichi-II" (TFB Co. Ltd., Tokyo, Japan; detection limit <30 ng/mL; intra- and interassay CV 5.0–10.0%). This glucagon assay utilizes antiserum OAL123, previously reported specific for pancreatic glucagon and showing limited cross-reactivity with gut glucagon-like immunoreactivity (Nishino, Kodaira, Shin, et al., 1981). Insulin was measured using lumipulse presto insulin (Fujirebio Inc., Tokyo, Japan; detection limit <0.1 ng/mL; intra- and interassay CV <5.0%; human proinsulin cross-reaction 0% with 2500 pg/mL of proinsulin). Because GLP-1 and GIP undergo rapid degradation by dipeptidyl peptidase-4 (DPP-4) and soon lose their biological effects, both intact and total GLP-1 and GIP were measured as described previously (Yabe et al., 2010). Briefly, aliquots of plasma were extracted with ethanol at the final concentration of 70% (vol/vol), and dried extracts were reconstituted in the original volume prior to measuring incretins. Total GLP-1 was measured using antiserum 89390 specific for the amidated COOH-terminus of GLP-1 and detection of GLP-1 (7–36) amide and GLP-1 (9–36) amide (detection limit: <1 pM). Intact GLP-1 was measured using a two-site sandwich enzyme-linked immunesorbent assay, which detects GLP-1 (7–36) amide and GLP-1 (7–37) but not the NH2-terminally truncated metabolites, using two monoclonal antibodies, the near C-terminally directed GLP1F5 as a catching antibody and the strictly N-terminally directed Mab26.1 as a detecting antibody (detection limit: <0.5 pM). This assay has 100% cross-reactivity with GLP-1 (7–36) amide and 88% with GLP-1 (7–37), but <0.1% with either GLP-1 (9–36) amide or GLP-1 (9–37). Total GIP was measured using the COOH-terminally directed antiserum R65, reacting with intact GIP and GIP (3–42) but not with 8-kDa GIP, the chemical nature and relation to GIP secretion of which is uncertain (detection limit, 2 pM). Intact, biologically active GIP was measured using antiserum 98171, specific for the intact GIP, and cross-reacting <0.1% with GIP (3–42) (detection limit: 5 pM). The intra- and inter-assay variations for intact GIP, total GIP and total GLP-1 were <15% and for intact GLP-1 was <5%. Other laboratory measurements including HbA1c, plasma glucose (PG) and serum lipids were measured by standard assays.

2.2. Calculations and statistical analyses

Results are reported as mean ± standard error of the mean unless otherwise stated. Area under the curve (AUC) of each measurement was calculated according to the trapezoidal rule. All statistical calculations, including linear regression analyses, were performed using IBM SPSS for Windows ver. 22 (SAS Institute Inc., Berkeley CA). Repeated measures were analyzed by mixed effects model. Values at single time points were compared by Wilcoxon rank sum test. A p value <0.05 was taken to indicate significant differences. Insulin resistance and β cell function was calculated according to the homeostasis model assessment (HOMA) model (Matthews, Hosker, Rudenski, et al., 1985). II and glucagonostatic index (GI) were calculated as follows: II, $\Delta\text{insulin}_{30\text{–}0\ \text{min}}/\Delta\text{PG}_{30\text{–}0\ \text{min}}$; GI, $\Sigma(\Delta\text{glucagon}_{10\text{–}0\ \text{min}}/\Delta\text{PG}_{10\text{–}0\ \text{min}} + \Delta\text{glucagon}_{20\text{–}0\ \text{min}}/\Delta\text{PG}_{20\text{–}0\ \text{min}} + \Delta\text{glucagon}_{30\text{–}0\ \text{min}}/\Delta\text{PG}_{30\text{–}0\ \text{min}} + \Delta\text{glucagon}_{60\text{–}0\ \text{min}}/\Delta\text{PG}_{60\text{–}0\ \text{min}} + \Delta\text{glucagon}_{120\text{–}0\ \text{min}}/\Delta\text{PG}_{120\text{–}0\ \text{min}})$ (Seino, Ikeda, Kurahachi, et al., 1978). Secretory units of islets in transplantation (SUIT) index and C-peptide index (CPI) were calculated as follows: SUIT index, 250 × [fasting C-peptide (nmol/L)]/([fasting plasma glucose (mmol/L)]−3.43) (Yamada, Fukuda, Fujimoto, et al., 2006); CPI, [fasting C-peptide (ng/mL)]/[fasting plasma glucose (mg/dL)] × 100 (Iwata, Maeda, Kamura, et al., 2012).

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