Acta Diabetol 32: 165–169, 1995

ACTA
DIABETOLOGICA

Response of truncated glucagon-like peptide-1 and gastric inhibitory polypeptide to glucose ingestion in non-insulin dependent diabetes mellitus

Effect of sulfonylurea therapy

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Received: 20 August 1993 / Accepted in revised form: 25 March 1995

Abstract. Gastric inhibitory polypeptide (GIP) and truncated glucagon like peptide-1 (tGLP-1) are potent gastrointestinal insulinotropic factors (incretin), mostly released after a meal or ingestion of glucose in man and animals. To investigate whether sulfonylurea (SU) affects the secretion of incretin, the modulation of plasma GIP and tGLP-1 levels following glucose ingestion in non-insulin-dependent diabetic type 2 patients with or without SU therapy was studied. A 75-g oral glucose tolerance test (OGTT) was carried out on 9 healthy subjects (controls) and 18 patients with non-obese type 2, 9 of whom were treated by diet alone (NIDDM-diet) and the other 9 with SU (glibenclamide 2.5 mg or gliclazide 40 mg) once a day (NIDDM-SU). Plasma GIP was measured by radioimmunoassay (RIA) with R65 antibody, and GLP-1 was measured by RIA with N-terminal-directed antiserum R1043 (GLP-1NT) and C-terminal-directed antiserum R2337 (GLP-1CT). Following OGTT, plasma glucose, GIP, GLP-1NT, and GLP-1CT in type 2 patients increased more markedly than in controls, despite the lower response of insulin. However, there were no significant differences in plasma levels of these peptides between the NIDDM-diet and NIDDM-SU groups. Therefore, it is unlikely that SU is involved in the high response of GIP and GLP-1s to OGTT in type 2 patients.

Key words: Gastric inhibitory polypeptide – Truncated glucagon-like peptide-1 – Incretin – Sulfonylurea

Introduction

Besides its major effect on pancreatic B cells to release insulin, sulfonylurea (SU) acts on extrapancreatic tissue to reduce blood glucose levels [1]. The effect of SU on the liver was determined, and that on the secretion of gastrointestinal insulinotropic factors (incretin) is proposed [2, 3]. Fasting and postprandial hyperinsulinemia in patients with non-insulin-dependent diabetes (type 2) [4, 5] and obesity [6] may be induced by the hypersecretion of incretin.

SU has a major effect on insulin release from the pancreatic islets. However, whether SU exerts an insulinotropic action through gastrointestinal insulinotropic factor(s) (incretin) has yet to be determined. Many reports indicate that SU has a direct effect on pancreatic B cells to release insulin, independent of the circulating glucose concentrations [7]. This effect is not beneficial, since the ideal drug for diabetes mellitus should be effective only on hyperglycemia. The agent should exert its hypoglycemic effect through the potentiation of glucose- or nutrient-induced insulin release. Since the meal- or oral glucose-induced insulin release is mediated in part by gastrointestinal insulinotropic factors, whether oral hypoglycemic agents have an effect on the release of incretin should be determined. Gastric inhibitory polypeptide (GIP) and truncated glucagon-like peptide-1 (tGLP-1) are potent incretin candidates. GIP and tGLP-1 are released upon the ingestion of glucose [8–10]. These peptides have a strong effect to potentiate glucose-induced insulin secretion [9–11]. As for the effect of SU on GIP secretion in type 2, there are at least three possibilities. Firstly, Creutzfeldt et al. proposed that SU stimulates GIP secretion, leading to insulin secretion, and the resultant hyperinsulinemia ameliorates GIP secretion. Secondly, hypersecretion of GIP may occur irrespective of the action of SU. Thirdly, SU stimulates insulin secretion by enhancing GIP secretion without the feedback control of insulin on GIP. However, the effect of SU on tGLP-1 secretion has never been reported. Therefore, we investigated the modulation of GIP and tGLP-1 response upon ingestion of glucose in type 2 patients by treatment with SU. To eliminate a variety of metabolic and hormonal changes during the initial stage of diet or SU treatment, we chose patients in the steady state of glycemic control during chronic treatment.

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Table 1. Clinical data on the subjects (mean ± SE)

	Normal subjects	NIDDM-diet	NIDDM-SU
n	9	9	9
Age (years)	25.8 ± 0.6	52.8 ± 2.3[a]	49.1 ± 2.4[a]
BMI (kg/m^2)	22.3 ± 0.4	23.1 ± 0.3	22.7 ± 0.6
HbA_{1c} (%)	–	5.7 ± 0.3	6.0 ± 0.2

[a] $P<0.01$ compared with controls

Materials and methods

Subjects

Nine subjects with normal weight and normal glucose tolerance (controls) (9 men) and 18 patients with non-obese type 2 (13 men and 5 women) were studied. Informed consent was obtained from all subjects. The clinical characteristics of each group are shown in Table 1. The type 2 patients were divided into two groups, those treated by diet only (NIDDM-diet) and those treated with SU agents (NIDDM-SU). Body mass index (BMI) of any subject did not exceed 24 kg/m^2. Age was significantly higher in the NIDDM groups than among normal subjects. Age, BMI, and hemoglobin A_{1c} were not significantly different for the NIDDM-diet and NIDDM-SU groups (Table 1). NIDDM-SU patients were treated with gliclazide (40 mg) or glibenclamide (2.5 mg) once daily for 3–6 months. No patients were taking drugs except SU agents for NIDDM-SU. Pregnant women, those with other medical problems or liver or renal impairment were excluded. Diets were adjusted so as to be weight-maintaining.

Protocols

A cannula was inserted into the antecubital vein and kept patent by the slow infusion of physiological saline. Ten-milliliter blood samples were taken from the antecubital vein 0, 15, 30, 60, 90, and 120 min after 75-g glucose ingestion and placed in glass tubes containing 500 KIU/ml aprotinin (Bayer, Germany) and 1 mmol/l EDTA$_2$Na and immediately centrifuged at 2000×g for 15 min at 4°C. Plasma samples were frozen and stored at –20°C.

Laboratory analysis

Plasma concentration of GIP was measured with the C-terminal-specific antiserum R65 (Novo, Denmark) [12]. Plasma concentration of GLP-1s was measured with the C-terminal-specific (R2337) and N-terminal-specific (R1043) antisera [13]. R2337 reacted 100% with GLP-1 (1–37), GLP-1 (1–36 amide), GLP-1 (7–37) and GLP-1 (7–36 amide), and GLP-1 (6–37), 28% with GLP-1 (7–35), and 1.5% with GLP-1 (1–20), but less than 0.3% with GLP-2, glucagon, secretin, vasoactive intestinal polypeptide (VIP), GIP, and somatostatin. R1043 reacted 100% with GLP-1 (1–37) and GLP-1 (1–36 amide) and GLP-1 (1–20), but not significantly with GLP-1 (7–37), GLP-1 (7–36 amide), GLP-1 (7–35), and the other peptides mentioned above. Therefore, the value measured with R2337 was referred to as GLP-1 CT and that with R1043 as GLP-1 NT, respectively. In plasma, GLP-1 CT mainly corresponded to GLP-1 (1–37), GLP-1 (1–36 amide), GLP-1 (7–37), and GLP-1 (7–36 amide), and GLP-1 NT to GLP-1 (1–37) and GLP-1 (1–36 amide), respectively. The sum of GLP-1 (7–37) and GLP-1 (7–36 amide), referred to as truncated GLP-1 (tGLP-1), can be estimated by the subtraction of GLP-1 NT from GLP-1 CT.

GLP-1 (1–37) was used as the standard and labeled antigen in radioimmunoassay (RIA). [^{125}I]GLP-1 was prepared by the chloramine T method and purified by Sephadex G10 column-chromatography.

Plasma concentrations of glucagon were measured using a RIA kit with the C-terminal-specific antiserum OAL-123 (Otsuka Lab., Tokushima, Japan) [14]; plasma glucagon-like immunoreactivity (GLI) was measured by the method of Harris et al. [15] using antiserum K4023 (Novo, Denmark). OAL123 reacted 100% with glucagon, but only 2% with glycentin, whereas K4023 reacted equally with glucagon and glycentin [14, 15]. Neither antisera crossreacted with any other peptide mentioned above. Plasma immunoreactive insulin (IRI) was measured with a RIA beads kit (Dainabot, Japan). Plasma glucose concentrations were determined by the glucose oxidase method using a glucose analyzer (Hitachi, Japan). Hemoglobin A_{1C} was determined by the high-pressure liquid chromatography method (Daiichikagaku, Kyoto, Japan).

Statistical analysis

All values were expressed as the mean ±SE. Changes in plasma levels of IRI and glucose among normal, NIDDM-diet, and NIDDM-SU were assessed by comparing mean changes at each time interval by analysis of variance (ANOVA). The suppression or stimulation of GIP, GLP-1 NT, and GLP-1 CT in normal subjects or type 2 patients was expressed in terms of mean increment or decrement from the value at zero time. Total integrated increment over the zero-time value was examined to determine statistical significance using ANOVA from a comparison of the means and Wilcoxon signed-rank test. Differences were considered to be significant at $P<0.05$.

Results

Plasma glucose

As shown in Fig. 1a, in the basal state plasma glucose levels in NIDDM-diet and NIDDM-SU were significantly higher than the controls (6.6±0.4 and 7.5±0.4 vs 4.3±0.07 mmol/l, $P<0.01$) and increased more markedly than the controls from 0 to 120 min ($P<0.01$ or less) following an oral glucose challenge. Plasma glucose in NIDDM-diet was the same as that in NIDDM-SU. Integrated increments of plasma glucose in NIDDM-diet and NIDDM-SU were greater than the controls ($P<0.01$). A difference between the two NIDDM groups could not be found (Fig. 4).

Plasma immunoreactive insulin

As shown in Fig. 1b, basal IRI in NIDDM-diet and NIDDM-SU was less than the controls, but the difference was not significant (48.9±5.5 and 43.3±2.7 vs 68.3±8.9 pmol/l). The IRI response of NIDDM-diet and NIDDM-SU to OGTT was significantly less than that of the controls from 15 to 60 min ($P<0.01$). There was no significant difference between the two diabetic groups (Fig. 1b). Integrated increments of plasma IRI in the NIDDM-diet and NIDDM-SU groups were significantly lower than the controls ($P<0.01$). NIDDM-diet and NIDDM-SU did not show any significant difference (Fig. 4).

Plasma immunoreactive glucagon

As shown in Fig. 2a, after OGTT, the plasma glucagon levels in the control apparently decreased, but not signifi-

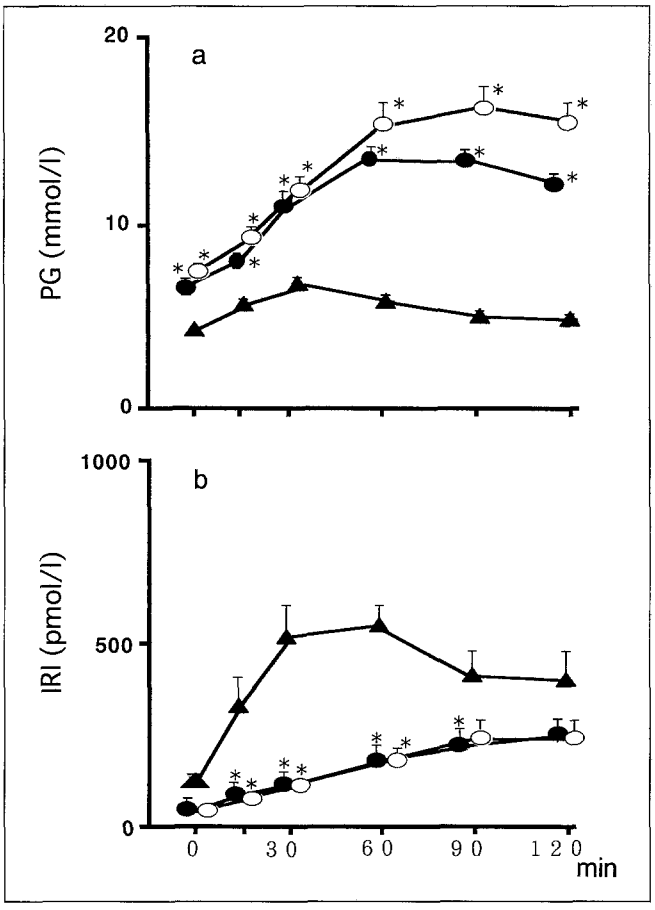

Fig. 1. Modulation of (**a**) plasma glucose (*PG*) and (**b**) immunoreactive insulin (*IRI*) in nine normal controls (*solid triangles*), nine non-insulin-dependent diabetes mellitus patients treated by diet alone (NIDDM-diet group; *solid circles*), and nine type 2 patients treated with sulfonylurea agents (NIDDM-SU group; *open circles*) following the oral administration of 75 g glucose. All values are expressed as means ± SE. **P*<0.05 or *P*<0.01 (NIDDM-diet group or NIDDM-SU group versus controls)

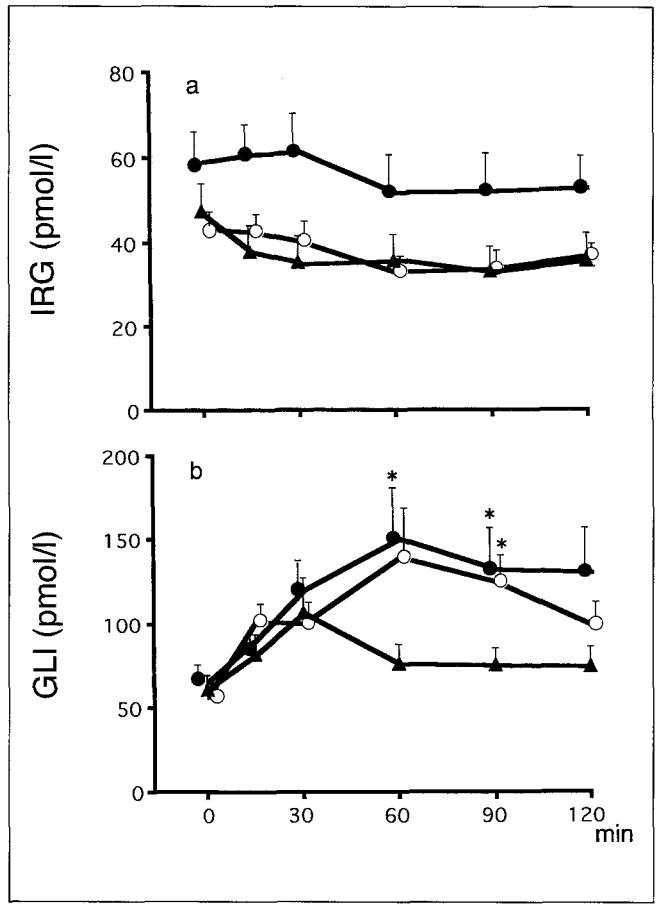

Fig. 2. Modulation of (**a**) plasma immunoreactive glucagon (*IRG*) and (**b**) glucagon-like immunoreactivity (*GLI*) in nine normal controls (*solid triangles*), nine type 2 patients treated by diet alone (NIDDM-diet group; *solid circles*) and nine type 2 patients treated with sulfonylurea agents (NIDDM-SU group; *open circles*) following the oral administration of 75 g glucose. All values are expressed as means ± SE. **P*<0.05 or *P*<0.01 (NIDDM-diet group or NIDDM-SU group versus controls)

cantly so. In contrast, in NIDDM-diet and NIDDM-SU the plasma glucagon levels did not decrease from the basal value.

Plasma glucagon-like immunoreactivity

As shown in Fig. 2b, plasma GLI levels increased to a peak at 30 min and thereafter decreased to near the basal level from 60 to 120 min in the controls. In contrast, plasma GLI levels increased markedly to a peak at 60 min and remained high up to 90 min in NIDDM-diet and NIDDM-SU.

Gastric inhibitory polypeptide

Plasma basal GIP in the diabetic groups was the same as that of the controls. The plasma GIP levels during OGTT in the two NIDDM groups increased markedly, peaked at 30 min ($P<0.05$), and remained high until 120 min ($P<0.05$). However, the responses in the NIDDM-diet and NIDDM-SU groups were not different (Fig. 3a). Integrated increments of plasma GIP in the diabetic groups were significantly greater than the controls ($P<0.05$), but the difference between NIDDM-diet and NIDDM-SU was not significant (Fig. 4).

Plasma glucagon-like peptide-1

As shown in Fig. 3b, basal GLP-1NT was not different among the three groups. On OGTT, plasma GLP-1 NT in the controls declined gradually and was significantly lower than the basal level at 90 and 120 min ($P<0.05$). In the NIDDM-diet group, plasma GLP-1 NT was slightly higher than the controls from 15 to 120 min ($P<0.05$). In the NIDDM-SU group, it exceeded that of the control at 60 and 120 min ($P<0.05$). Integrated increments of plasma GLP-1 NT in the NIDDM groups were significantly greater than the controls ($P<0.05$) (Fig. 4).

Basal GLP-1 CT was the same for all groups. On OGTT, plasma GLP-1 CT in the controls was significantly high from 30 to 120 min and increased from the basal level of

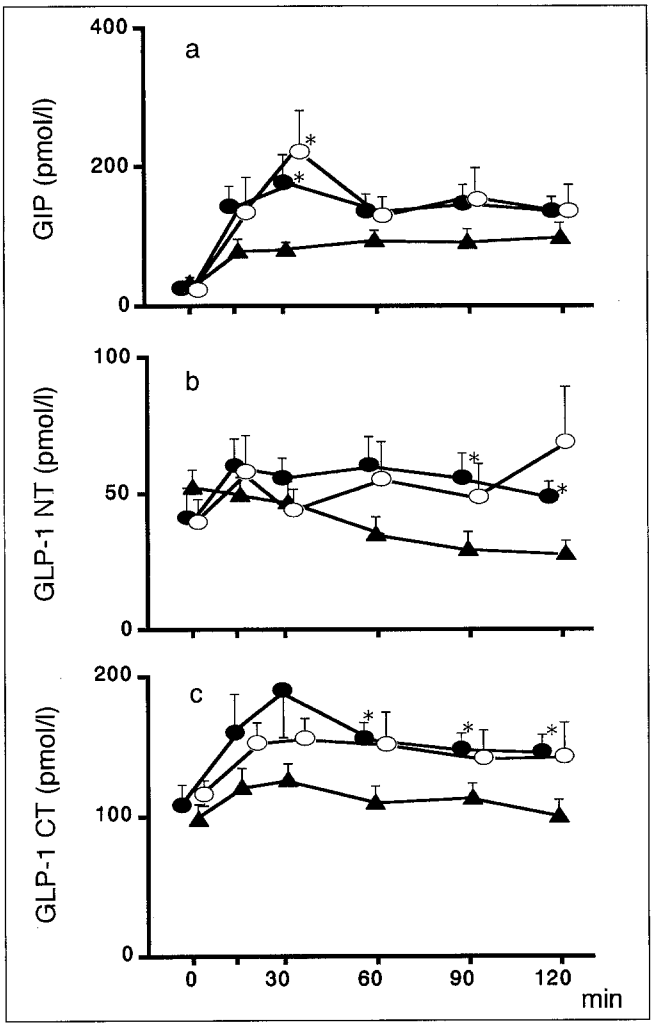

Fig. 3. Modulation of plasma (**a**) gastric inhibitory peptide (*GIP*), (**b**) plasma glucagon-like peptide-1 *(GLP-1) NT*, and (**c**) *GLP-1 CT* in nine normal controls (*solid triangles*), nine type 2 patients treated by diet alone (NIDDM-diet group; *solid circles*), and nine type 2 patients treated with sulfonylurea agents (NIDDM-SU group; *open circles*) following the oral administration of 75 g glucose. All values are expressed as means ± SE. **P*<0.05 or *P*<0.01 (NIDDM-diet group or NIDDM-SU group versus controls)

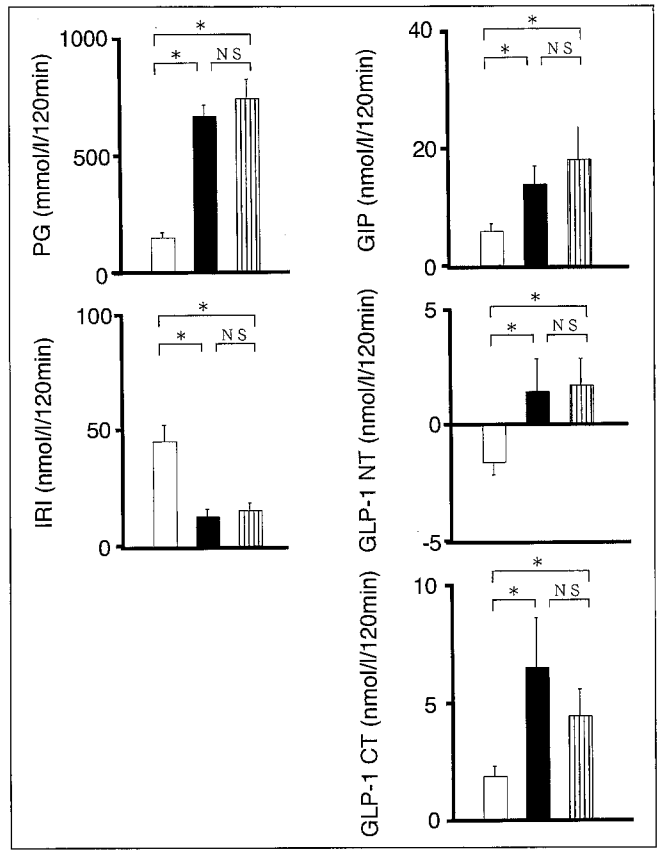

Fig. 4. Integrated increments of PG, IRI, GIP, GLP-1 NT, and GLP-1 CT in nine normal controls (*open bar*), nine type 2 patients treated by diet only (NIDDM-diet group; *solid bar*), and nine type 2 patients treated with SU agents (NIDDM-SU group; *striped bar*) following the oral administration of 75 g glucose. All values are expressed as means ± SE. **P*<0.05

97.7±9.7 pmol/l to the peak of 126.2±11.3 pmol/l at 30 min. An increase in plasma GLP-1 CT was more marked in NIDDM-diet and NIDDM-SU than the controls and higher than the controls from 60 to 120 min. A difference in the NIDDM-groups could not be seen (Fig. 3c). Integrated increments of plasma GLP-1 CT in NIDDM-diet and NIDDM-SU were significantly higher than the controls ($P<0.05$), but the difference between NIDDM-diet and NIDDM-SU was not significant (Fig. 4).

Discussion

The rise in serum GIP after ingestion of nutrients is greater in some type 2 patients or obesity [6] than normal subjects [16]. This may contribute to hyperinsulinemia in obesity and early type 2 diabetes [17, 18]. Increase in circulating GIP in these disorders may be induced by a defect in the feedback inhibition of GIP by insulin [17, 18]. In some type 2 patients, an increase in endogenous insulin secretion could result in attenuation of the GIP response to nutrients. Creutzfeldt and Ebert [19] observed a decrease in meal-stimulated GIP release after glyburide treatment and considered that the reduced response of GIP is due possibly to the effect of increased secretion of endogenouse insulin. However, Coxe et al. could not find a change in insulin and GIP after tolazamide treatment [20]. We found an exaggerated GIP response associated with a low response of insulin in both diabetic groups compared with the controls, but there was no significant difference between NIDDM-diet and NIDDM-SU. These changes in GIP and insulin would thus appear to be a characteristic of type 2 diabetes, and not the result of SU therapy.

Plasma GLP-1 NT was suppressed after ingestion of glucose in the controls, whereas it increased significantly in the diabetic groups. These findings are similar to changes observed in plasma IRG following OGTT, which was suppressed in normal subjects [21], but increased in diabetic patients. A similar elevation of plasma IRG following OGTT was found in patients with gastrectomy or pancreatectomy [22], suggesting that increased IRG is induced by a gastrointestinal stimulus, possibly due to the

glucagonotropic effect of GIP [11] released from the intestine. This glucagonotropic effect is also augmented in streptozotocin-induced diabetes, B-cell-depleted and A-cell-preserved animals [23]. The main products of preproglucagon in the human pancreas are glucagon, GLP-1, and a major proglucagon fragment [24]. It is thus of particular interest in this study that the peak of plasma GLP-1 NT in type 2 diabetes coincided with that of plasma GIP. Rise in plasma GLP-1 NT would thus appear to be induced by the glucagonotropic action of GIP. This may be more obvious in the relative failure of insulin. Since the marked increase in plasma GLP-1 CT was the same in the two diabetic groups, SU agents would not likely affect GLP-1CT secretion in type 2 diabetes.

Although integrated increments of GLP-1 NT and GLP-1 CT in the diabetic groups were significantly greater than in controls, values obtained by subtracting GLP-1 NT from GLP-1 CT, representative of tGLP-1, were not significantly different between the diabetic groups and the controls. The secretion of tGLP-1 thus does not increase in mild nonobese type 2 diabetes nor does SU influence the secretion of tGLP-1 in type 2 diabetes.

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