Impairment of the insulinotropic effect of gastric inhibitory polypeptide (GIP) in obese and diabetic rats is related to the down-regulation of its pancreatic receptors

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Abstract. The association of obesity with type 2 diabetes mellitus has been recognized for years. In type 2 diabetes, there is a possibility that an important part of the impaired insulin secretion is due to the gastric inhibitory polypeptide (GIP) hormone. This study investigated changes that occur in the pancreatic GIP receptors' (GIP-Rs) expression and in GIP secretion in obese and type 2 diabetic rats and its relation to plasma glucose and insulin levels during oral glucose tolerance test (OGTT) compared to control rats. During the first 20 min of the OGTT, both the obese and the diabetic rats had a significant increase in the glucose excursion and a significant decrease in early-insulin secretion compared to the control group, with more prominent changes in the diabetic group. The obese rats had a significant increase in fasting GIP level and in the incremental change of GIP from 0 to 20 min (GIP Δ 0–20: 60.1 ± 6.66 pmol/l) compared to that of the control (33.96 ± 4.69 pmol/l) and the diabetic (29.34 ± 2.62 pmol/l) group, which were not significantly different from each other. However, there was a significant decrease in GIP-Rs expression in both the obese (88.07 ± 10.36 μg/ml) and diabetic (87.51 ± 4.72 μg/ml) groups compared to the control group (120.35 ± 8.06 μg/ml). During the second hour of the OGTT, plasma GIP was decreasing in all groups, however, the obese group had a significant hyperinsulinemia compared to the other two groups. Moreover, the diabetic group had a significantly lower plasma insulin level until the 90 min interval and thereafter it showed a non-significant difference compared to the control group.

In conclusion, both obese and diabetic rats had an impaired early-phase insulinotropic effect of GIP due to impaired gene expression of GIP-Rs which could be a potential target to prevent transition of obesity to diabetes and to improve insulin secretion in the latter.

Key words: Incretin — GIP receptors — Obese rats — Type 2 diabetes

Introduction

Obesity and diabetes are two of the most prevalent health conditions in industrial nations. The association of obesity with type 2 diabetes mellitus has been recognized for years. Obesity acts, at least in part, by inducing resistance to insulin-mediated peripheral glucose uptake, which is an important component of type 2 diabetes. Recent studies suggest that a gut-derived peptide, gastric inhibitory polypeptide (GIP), may be involved in the pathogenesis of type 2 diabetes and obesity induced by overnutrition (Meier et al. 2002; Miyawaki et al. 2002).

GIP is one of the major gut-derived incretin hormones, which are peptide hormones released in response to oral meal (mainly glucose) and act *via* the endocrine system to potentiate about two third of glucose-induced insulin secretion. Incretins also include the proglucagon gene-derived glucagon-like peptide (GLP)-1 (D'Alessio 1997).

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GIP is produced by K cells, a specific subtype of small intestinal entero-endocrine cells. Its secretion is triggered by ingested nutrients containing glucose or fat not by their circulation in the blood, and it is cleaved by the enzyme dipeptidylpeptidase (Fehmann et al. 1995; Drucker 2003a). GIP release is stimulated by molecules produced by enteric neurons, as bombesin-like peptides (Ramshur et al. 2002) and is inhibited by somatostatin (Walsh 1994) and possibly by enterocytes (Wang et al. 2003).

GIP was initially discovered and named for its gastric inhibitory properties, later it was found, however, that under physiological conditions in humans, GIP has a negligible effect on gastric acid secretion, while it has an important insulinotropic role. Thus, it was renamed into glucose-dependent insulinotropic polypeptide (Creutzfeldt 1979). In the pancreas, GIP stimulates glucose-dependent insulin secretion through interaction with specific heterotrimeric G-protein-coupled GIP receptors (GIP-Rs) on pancreatic β-cells. Evidences suggest that GIP also stimulates proinsulin gene transcription and translation (Wang et al. 1996), acts as a β-cell growth factor (Trumper et al. 2001) and as an antiapoptotic agent (Ehses et al. 2003a).

GIP-Rs have been demonstrated at a number of extra-pancreatic sites, including stomach, brain and adipose tissue (Gault et al. 2003), suggesting a range of additional effects of GIP on nutrient metabolism. Key among these is the ability of GIP to attenuate glucagon-stimulated hepatic glucose production, to stimulate glucose uptake in muscles and to increase both fatty acid synthesis and lipoprotein lipase activity in adipocytes (Knapper et al. 1995). The important role of GIP as an incretin hormone is demonstrated in GIP-Rs knockout mice, which show normal glucose tolerance after intraperitoneal administration of glucose and glucose intolerance accompanied by impaired insulin secretion after oral administration of glucose (Miyawaki et al. 1999).

Many studies have suggested that GIP directly links overnutrition to obesity and may be a potential target for antiobesity drugs and that weight gain and triglyceride synthesis induced by fat diet were prevented in mice lacking GIP-Rs (Miyawaki et al. 2002). Moreover, much recent attention has been devoted to enhancement of incretin action using dipeptidyl peptidase IV inhibitors or stable analogs of GIP for the treatment of type 2 diabetes (Drucker 2003b). Such an approach is reliant on the possibility that GIP action is defective in diabetes and that the underlying defects responsible for metabolic disarray might be overridden by exogenous GIP administration (Gault et al. 2003). Many studies suggested that there is mounting evidence for a beneficial and possibly therapeutic role of GIP analogs in diabetes (Meier et al. 2002; Gault et al. 2003; Holst and Gromada 2004). Nevertheless, there is no consensus regarding the circulating levels of GIP in obese and in type 2 diabetic subjects; studies have demonstrated increased, decreased and unchanged GIP levels (Fukase et al. 1993; Ahren et al. 1997). Also another human study has shown that there is a decreased GIP effect in type 2 diabetes, and this has been attributed to a change in GIP-Rs expression or a change in circulating GIP levels, although altered signal transduction pathways could also play a role (Holst et al. 1997).

The aim of the present study was to investigate the possibility of an inappropriate GIP release in response to an oral glucose tolerance test (OGTT) in fat-induced obese rats and in type 2 diabetic rats compared to control rats, to link the GIP level to both glucose and insulin levels during OGTT and to assess whether there is an associated defect in GIP-Rs expression in pancreatic β-cells in these rat models.

Materials and Methods

Animals and experimental design

All procedures that involved animals were approved by the animal care unit of Cairo Medical University. Male rats, weighing 160–200 g, were housed each in a cage in a temperature- (22–24°C) and light-controlled room on an alternating 12 : 12 h light-dark cycle. Except when scheduled for an OGTT, all animals had free access to food and water (Juan et al. 2004).

Animals were divided into the following groups (n = 10/group). Control group: receiving standard chow for 10 weeks containing 6.5% Kcal fat. Obese group: receiving the standard chow with the addition of 32% Kcal as fat for 10 weeks (Dobrian et al. 2000). Diabetic group: receiving the standard chow with the addition of 66% Kcal as fructose for 10 weeks (Matz et al. 1996).

Calculation of the obesity index

Body weights and body lengths (naso-anal) were measured initially and then weekly for calculation of the obesity index, which is equivalent to the body mass index in humans. It was calculated by dividing the body weight in grams by the square of the naso-anal length in centimeters (Dobrian et al. 2000).

Blood pressure measurement

The systolic blood pressure was measured under conscious conditions at the beginning of the experiment and after 1, 3, 6 and 10 weeks of the diet, to monitor any diet-induced changes in blood pressure. Before measuring the systolic blood pressure, the body temperature of the rats had to be adjusted to 37°C using an incubator for 10 min. The body temperature was verified by colonic temperature measurement. The systolic blood pressure was assessed with the tail-cuff method using the electro-sphygmomanometer. As

normal blood pressure shows intrinsic diurnal variation and may be disturbed by environmental conditions, all measurements were carried out in a quiet room. The average of 3 pressure readings was recorded for each measurement (Dobrian et al. 2000).

Modified oral glucose tolerance test

After 10 weeks, the rats were subjected to a modified oral glucose tolerance test, which was performed on the unanesthetized animal after an overnight fasting (6 p.m. to 9 a.m.), as described by Whittington et al. (1991). Immediately after the collection of blood sample for fasting glucose, insulin, GIP and serum triglycerides measurements, a glucose solution (1 g/ml; 1 ml/kg body weight) was given orally to each rat and then more blood samples from the tail vein were collected at 5, 10, 15, 20, 60, 90 and 120 min after oral glucose administration for measurement of plasma glucose, insulin and total GIP levels (Whittington et al. 1991). The modified OGTT was chosen to assess early-phase insulin secretion using the insulinogenic index (Bruce et al. 1988), which was calculated as the ratio of the increment in the plasma insulin level (in μU/ml) to that in the plasma glucose level (in mg/dl) during the first 20 min after ingestion of glucose. The lower the index, the worse is the insulin secretion. To estimate insulin resistance, the homeostasis model assessment for insulin resistance (HOMA-IR: insulin resistance index) (Mathews et al. 1985) was used, calculated as the product of fasting insulin (in μU) and fasting glucose (in mmol/l) divided by 22.5. A lower index indicates greater insulin sensitivity. An oral glucose tolerance curve was drawn for the plasma glucose levels obtained at various intervals and interpretation of the curve was made according to Lewis et al. (2000). Briefly, normal curve criteria should be: fasting blood glucose <6.1 mmol/l and blood glucose at 2 h post-challenge <7.8 mmol/l, while impaired glucose tolerance curve criteria would be: fasting blood glucose <7 mmol/l and blood glucose at 2 h post-challenge >7.8 mmol/l and diabetic OGTT curve criteria would be: fasting blood glucose >7 mmol/l and blood glucose at 2 h post-challenge >11.1 mmol/l. Also curves were drawn showing the plasma levels of insulin and GIP at the same time intervals.

Measurement of plasma glucose

Plasma glucose in blood samples was measured using oxidase-peroxidase method (Trinder 1969).

Measurement of plasma insulin

Plasma insulin levels were analyzed using enzyme-linked immunosorbent assay ELISA (Dako, Carpinteria, CA) according to the manufacturer's instructions (Delams 1986).

Measurement of plasma GIP

Plasma GIP in the different obtained samples was measured using the radioimmunoassay kit supplied by LINCO, Missouri, USA (Deacon et al. 2000).

Detection of pancreatic GIP-Rs gene expression

Rats were anesthetized using 5 mg/kg pentobarbital sodium and a midline incision was made. The pancreatic tissue was then removed and frozen at –80°C for detection of GIP-Rs gene expression as follows: about 30 mg of each rat pancreatic tissue was homogenized and then centrifuged at 14,000 rpm for 10 min. The supernatant was examined for detection of GIP-Rs by gene expression using RT-PCR.

1. *RNA extraction*. RNA was extracted from tissue homogenate by using SV-total RNA isolation system (Promega, Madison, WI, USA) according to manufacturer's recommendation, and the extracted RNA was measured by spectrophotometer at 280 nm.

2. *RT-PCR*. About 5 μg of RNA was reverse transcripted by using 12.5 μl of oligonucleotides primer denaturated at 70°C for 2 min. The denaturated RNA was placed on ice for 5 min, then in 5 mmol KCl, 50 mmol tris HCl at pH 8.3, 0.5 mmol dNTPS and 200 U of moleny murine leukemia virus reverse transcriptase was used. The reaction conditions were adjusted at 42°C for 1 h followed by heating at 95°C for 5 min to stop the reaction. PCR reaction was performed by adding the PCR mix to about 5 μl of CDNA, the mixture contained 10 mmol/l HCl at pH 8.3, 50 mmol KCl, 100 mmol dNTPS and 2.5 unit of tag polymerase and about 10 μmol of each of sense and antisense primer of GIP-Rs with the following sequence forward primer: 5′-CCG CGC TTT TCG TCA TCC-3′ and reverse primer: 5′-CCA CCA AAT GGC TTT GAC TT-3′. The PCR cycling conditions were 94°C for 1 min for denaturation followed by 57°C for 1 min and 72°C for 45 s for 40 cycles with final extension at 72°C for 12 min (Freeman et al. 1999).

3. *Gel electrophoresis*. 10 μl of PCR product was analyzed on 2% agarose gel with ethidium bromide as staining and the product was visualized on ultraviolet transilluminator, then gel documentation was performed. PCR products were semi-quantitated by using gel documentation system (BioDoc Analyze System) supplied by Biometra.

Statistical analysis

The results were analyzed using SPSS computer software package, version 10.0 (Chicago, IL, USA) (Norusis 1997). Data were presented as mean ± S.D. Differences among the parameters of the three groups were compared by one-way ANOVA. To study the relationship between plasma insulin

Table 1. Means of the obesity index, systolic blood pressure, serum triglycerides, fasting plasma glucose, fasting plasma insulin, fasting plasma GIP and pancreatic GIP-Rs expression with the calculated HOMA-IR and insulinogenic index in the three studied groups

Parameters	Control group	Obese group	Diabetic group
Initial obesity index (g/cm2)	0.42 ± 0.015	0.44 ± 0.015	0.42 ± 0.028
Obesity index after 10 weeks (g/cm2)	0.55 ± 0.023	0.82 ± 0.026*	0.54 ± 0.041^{+}
Initial systolic blood pressure (mmHg)	124.16 ± 3.97	125.12 ± 3.75	118.1 ± 11.02
Systolic blood pressure after 10 weeks (mmHg)	119.33 ± 3.38	156.12 ± 7.91*	157.1 ± 6.7*
Serum triglycerides (mg/dl)	69.83 ± 3.77	91.08 ± 4.72*	88.08 ± 4.27*
Fasting plasma glucose (mmol/l)	3.98 ± 0.15	5.03 ± 0. 11*	9.26 ± 0. 35*$^{+}$
Fasting plasma insulin (μU/l)	11.76 ± 0.89	13.02 ± 0. 71	12.35 ± 0. 77
HOMA-IR	2.08 ± 0.16	2.91 ± 0.22*	5.09 ± 0.5*$^{+}$
Insulinogenic index	1.64 ± 0.64	0.713 ± 0.16*	0.43 ± 0.42*
Fasting plasma GIP (pmol/l)	29.55 ±2.4	49.01 ± 2.44*	33.02 ± 1.12^{+}
GIP-Rs (μg/ml)	120.35 ± 8.06	88.07 ± 10.36*	87.51 ± 4.72*

* statistically significant difference compared with control group at $p \leq 0.002$; $^{+}$ statistically significant difference compared with obese group at $p \leq 0.001$.

and secreted GIP, Pearson's correlation coefficient r was calculated at different points. The results were considered statistically significant at $p \leq 0.05$. The area under the curve (AUC) was calculated for plasma glucose, insulin and GIP incremental changes from 0 to 20 min and from 20 to 120 min, with a data spreadsheet program Excel (Microsoft, Redmond, WA, USA) using the trapezoidal method. The mean of the three baseline values was determined, and net AUC was calculated by subtracting the areas below baseline from AUC values above baseline.

Results

Table 1 reveals that at the beginning of the study both the obese and the diabetic groups had insignificant initial obesity indices and blood pressures compared to those of the control group. At the end of the study (after 10 weeks), the obesity index in the obese group increased significantly compared to that of the control and the diabetic groups with $p < 0.001$. The difference in obesity index between the diabetic and control groups was insignificant. Moreover, the systolic blood pressure and the serum triglycerides were highly significantly raised in the obese and diabetic groups compared to those of the control group ($p < 0.001$), while they showed no significant difference between the obese and diabetic groups.

At the end of the 10 weeks, there was also a highly significant increase in both the fasting plasma glucose level and the calculated HOMA-IR index in the obese and diabetic groups compared to that of the control group although there was no significant difference in the fasting plasma insulin level in the three studied groups ($p > 0.05$) as revealed by Table 1. Furthermore, the diabetic group showed high significant increase in fasting plasma glucose and HOMA-IR index compared to the obese group ($p < 0.001$).

Thus the previous results indicate the presence of insulin resistance in obese and diabetic groups, however, much more pronounced in the diabetic group.

As revealed in Table 1, fasting plasma GIP level showed a highly significant increase in the obese group (49.01 ± 2.44 pmol/l) compared to that of the control group (29.55 ± 2.4 pmol/l) and the diabetic group (33.02 ± 1.12 pmol/l) with a $p < 0.001$, however, it was insignificantly increased in the diabetic group compared to the control group ($p > 0.05$). While GIP-Rs gene expression in the pancreatic tissue of the obese (88.07 ± 10.36 μg/ml) and diabetic (87.51 ± 4.72 μg/ml) groups showed a highly significant decrease compared to that of the control group (120.35 ± 8.06 μg/ml) with $p < 0.001$ without a significant change in its expression between the obese and diabetic groups as shown in Figure 2.

These data indicate an increase in the fasting GIP level in the obese group and an unchanged fasting GIP level in the diabetic group compared to the control group. On the other hand, both the obese and the diabetic groups showed a significant decrease in the pancreatic GIP-Rs gene expression compared to the control group.

Plasma glucose, plasma insulin, insulinogenic index and GIP responses to oral glucose administration

As revealed in Figure 1A, the means of the plasma glucose levels of the obese group were significantly higher compared

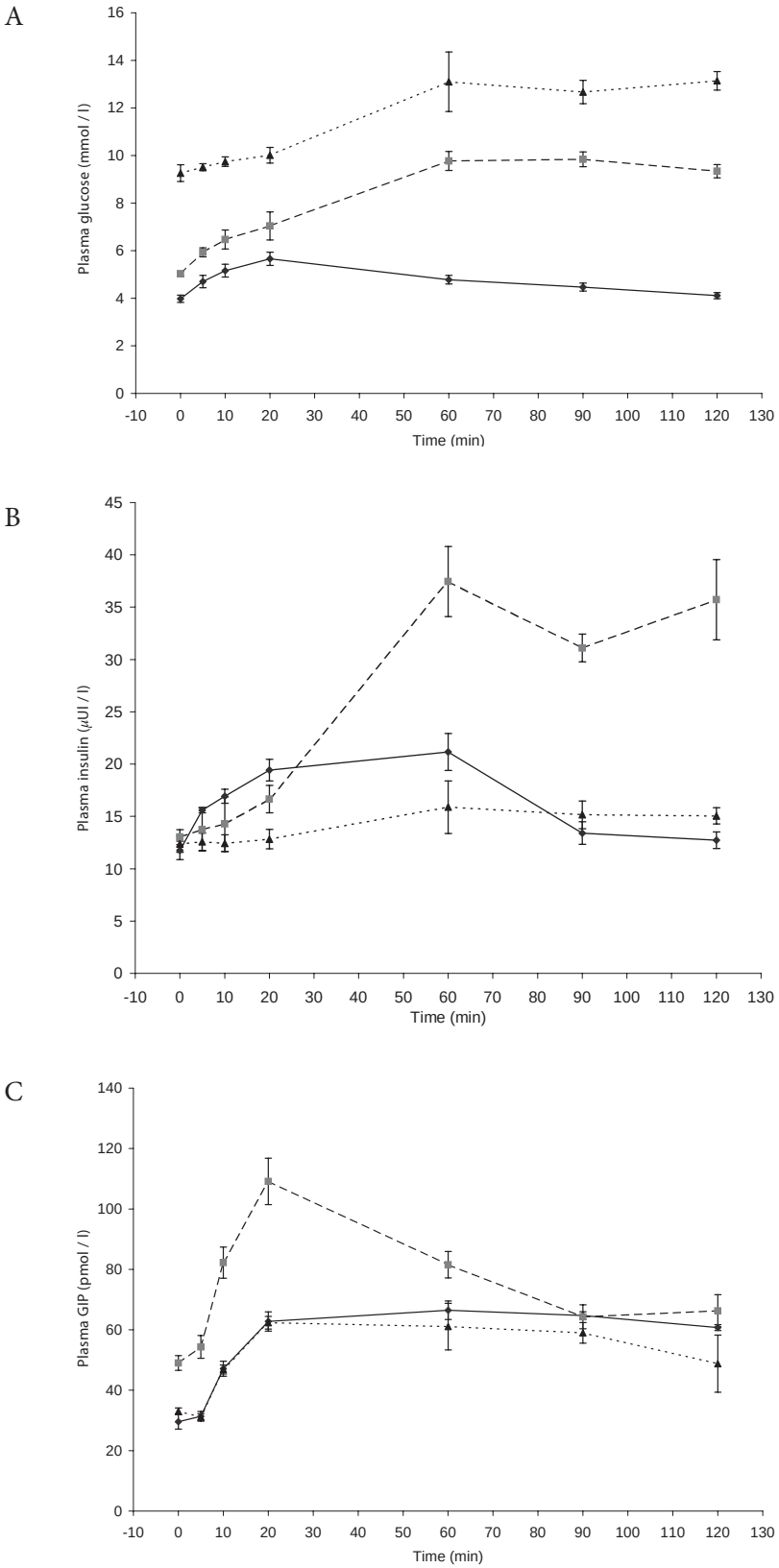

Figure 1. Plasma glucose levels (A), insulin levels (B) and GIP levels (C) in control group (——), obese group (- - - -) and diabetic group (------) during the oral glucose tolerance test. Values are expressed as means ± S.D. (n = 10/group).

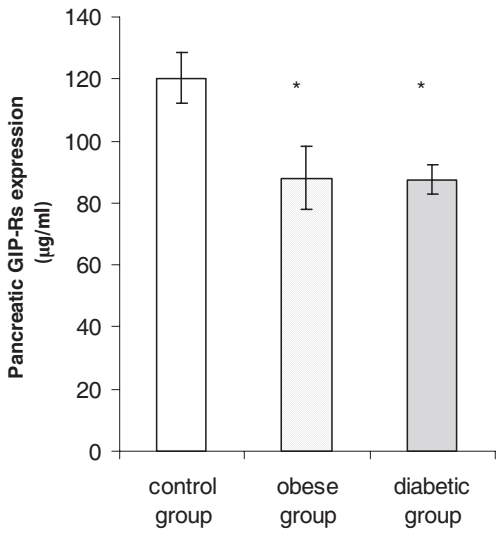

Figure 2. Pancreatic GIP receptors (GIP-Rs) expression in the three studied groups. Values are expressed as mean ± S.D. * significant ($p \leq 0.05$) compared to control group.

to those of the control group during the entire duration of the OGTT. Moreover, in the obese group, the mean of fasting plasma glucose level (5.03 ± 0.11 mmol/l) and that obtained at the 120 min post-glucose challenge (9.34 ± 0.28 mmol/l) meet the criteria of an impaired OGTT. Although the increment in plasma glucose level during the first 20 min post-glucose challenge (Glu Δ 0–20), which was calculated as plasma glucose value at 20 min minus plasma glucose value at 0 level, revealed an insignificant increase in the obese group compared to the control group (Table 2), however, the AUC of Glu 0–20 in the obese group was significantly greater than that of the control group with $p \leq 0.05$ as shown in Table 3. These data indicate a more prominent increase in the plasma glucose excursion in the obese group compared to the control group during the first 20 min post-glucose challenge.

Moreover, during the following 20 to 120 min of the OGTT, the glucose level was normally decreasing in the control group, while it was increasing in the obese group during this time period, as revealed by the significant increase in Glu Δ 20–120 and in AUC of Glu 20–120 of the obese group compared to those of the control group (Tables 2 and 3).

The oral glucose tolerance curve obtained in the diabetic group (Figure 1A) revealed that the means of plasma glucose levels were significantly increased compared to those in the other two groups during the entire duration of the OGTT. Also, the mean of its fasting plasma glucose level (9.26 ± 0.35 mmol/l) and that obtained at the end of the OGTT (120 min), which was 13.14 ± 0.39 mmol/l meet the criteria of a diabetic OGTT. The increment in plasma glucose level Glu Δ 0–20 in the diabetic group (Table 2) was significantly, lower than that of the control and obese groups due to a higher fasting plasma glucose level (0 level).

Moreover, a greater glucose excursion was observed in the diabetic group from 0–20 min and from 20 to 120 min post-glucose challenge as revealed by the significant increase in AUC of Glu 0–20, in Glu Δ 20–120 and in AUC of Glu 20–120 min compared to those of the control and the obese groups (Tables 2 and 3).

As shown in Tables 1 and 2, both the insulinogenic index used to assess the early-phase of insulin secretion (first 20 min of OGTT) and the increment in plasma insulin level from 0 to 20 min (Ins Δ 0–20) showed a significant decrease in both the obese and the diabetic groups compared to those of the control group with $p \leq 0.05$. The Ins Δ 0–20 was significantly reduced in the diabetic group compared to that of the obese group ($p \leq 0.05$) as shown in Table 2, without a significant difference between the obese and diabetic groups' insulinogenic index. Although the AUC of Ins 0–20 showed insignificant difference between the three groups with $p > 0.05$ (Table 3), however, the previous data reveal that there was an impaired early-phase of insulin secretion in both the obese and diabetic groups compared

Table 2. Incremental changes in plasma glucose (Glu), insulin (Ins) and gastric inhibitory polypeptide (GIP) levels during the oral glucose tolerance test in the studied groups

	Control group	Obese group	Diabetic group
Glu Δ 0–20 (mmol/l)	1.8 ± 0.43	2.00 ± 0.49	0.75 ± 0.2*+
Glu Δ 20–120 (mmol/l)	−1.67 ± 0.42	2.3 ± 0.69*	3.13 ± 0.42*+
Ins Δ 0–20 (μU/l)	7.65 ± 1.57	3.62 ± 0.8*	0.48 ± 0.43*+
Ins Δ 20–120 (μU/l)	−7.08 ± 1.39	19.06 ± 4.17*	2.21 ± 1.19*+
GIP Δ 0–20 (pmol/l)	33.96 ± 4.69	60.1 ± 6.66*	29.34 ± 2.62+
GIP Δ 20–120 (pmol/l)	−2.22 ± 2.17	−42.81 ± 10.71*	−13.58 ± 9.54*+

* statistically significant difference compared with control group at $p \leq 0.05$; + statistically significant difference compared with obese group at $p \leq 0.05$.

Table 3. Area under the curve (AUC) measurements of glucose (Glu), insulin (Ins) and gastric inhibitory polypeptide (GIP) incremental changes during the oral glucose tolerance test in the studied groups

	Control group	Obese group	Diabetic group
AUC of Glu 0–20 (mmol × min/l)	103.8 ± 5.1	123.8 ± 5.175*	193 ± 4.830*+
AUC of Glu 20–120 (mmol × min/l)	423.8 ± 9.161	833.8 ± 20*	1114 ± 51.25*+
AUC of Ins 0–20 (μU × min/l)	312.5 ± 35.36	287.5 ± 35.36	280 ± 42.16
AUC of Ins 20–120 (μU × min/l)	1550 ± 75	2837.5 ± 74*	1340 ± 84*+
AUC of GIP 0–20 (pmol × min/l)	875 ± 46.29	1550 ± 106.9*	920 ± 42.16+
AUC of GIP 20–120 (pmol × min/l)	5737.5 ± 168.5	6875 ± 305.9*	5270 ± 323.4*+

* statistically significant difference compared with control group at $p \leq 0.05$; + statistically significant difference compared with obese group at $p \leq 0.05$.

to the control group with a more prominent impairment in the diabetic group.

As shown in Figure 1B, peak insulin secretion in the three groups was evident at 60 min post-glucose challenge with a significant hyperinsulinemia in the obese group (37.45 ± 3.36 μU/l) compared to the insulin level of the control group (21.16 ± 1.76 μU/l) and the diabetic group (15.88 ± 2.51 μU/l). The diabetic group had a significantly decreased plasma insulin at 60 min compared to that of the control group. At 90 min post-glucose challenge, the plasma insulin level was still significantly increased in the obese group compared to the other two groups, while it was insignificantly different in the diabetic group compared to the control group. At the end of the OGTT (120 min), the plasma insulin level was still raised significantly ($p \leq 0.05$) in obese group compared to that of the control and the diabetic groups, but it was insignificantly increased in the diabetic group compared to the control group ($p > 0.05$). Moreover, it was observed in Tables 2 and 3 that the incremental change in plasma insulin level (Ins Δ 20–120) and AUC of insulin from 20 to 120 min in the obese group were significantly increased compared to those of the control and diabetic groups, while they were significantly reduced in the diabetic group compared to the control group ($p \leq 0.05$).

Figure 1C shows the changes in plasma GIP level during the OGTT in the three groups, and reveals that the fasting GIP level (0 level) which was significantly increased in the obese group compared to the control and diabetic groups, increased further more during the initial 20 min post-glucose challenge in the obese group compared to the other two groups. This was evident by the significant increase in GIP Δ 0–20 and AUC of GIP 0–20 in the obese group ($p \leq 0.05$) compared to those of the control and diabetic groups (Tables 2 and 3).

On the other hand, the diabetic group showed an insignificant difference in fasting GIP level, GIP Δ 0–20 and GIP AUC 0–20 compared to the control group. These results indicate that during the first 20 min following oral glucose administration, obesity in rats was associated with an increased GIP secretion, while the GIP secretion in the diabetic rats was similar to that of the control rats.

Peak GIP secretion in the three groups was at 20 min post-glucose challenge, and then it started to decrease gradually during the time interval of 20 to 120 min. However, the GIP level was significantly increased in the obese group compared to that of the control and diabetic groups at the 60 min interval and was insignificantly increased than that of the control and the diabetic groups at the 90 and 120 min intervals. On the other hand, the plasma GIP level in the diabetic group was only significantly decreased compared to that of the control group at the 90 and 120 min intervals ($p \leq 0.05$).

As observed in Tables 2 and 3, the incremental change in GIP from 20 to 120 min (GIP Δ 20–120) and the AUC of GIP 20–120 were significantly increased in the obese group compared to those of the control and the diabetic group with $p \leq 0.05$, indicating a rapid decrease in the GIP level in the obese group compared to the other two groups. Also both the significant increase in GIP Δ 20–120 and the significant decrease in the AUC of GIP 20–120 in the diabetic group compared to those of the control group ($p \leq 0.05$) indicate a more prominent decrease in plasma GIP level in the diabetic group compared to the control group during the time interval of 20 to 120 min of the OGTT.

The relationship between plasma insulin and total GIP values at various time points was also investigated using Pearson's correlation. There was no correlation between the fasting GIP and fasting insulin levels in all groups, while there was a positive correlation between post-glucose challenge plasma GIP level at 0–20 min with that of plasma insulin at the same time interval in the control group ($r = 0.733$, $p = 0.039$). In the obese and diabetic groups, on the other hand, there was a trend towards negative correlation between plasma insulin level from 0–20 min and that of GIP 0–20

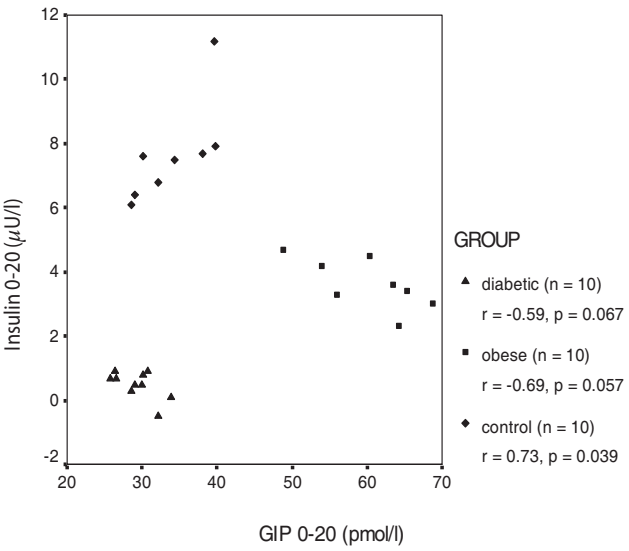

Figure 3. Correlation between plasma insulin and GIP during the first 20 min interval of the OGTT in the different studied groups.

min, although it was not statistically significant ($p = 0.057$ and 0.06, respectively) as shown in Figure 3. During the interval of 20 to 120 min following glucose administration, there was no correlation between plasma GIP and plasma insulin (at 20, 60, 90 and 120 min) in all groups.

Discussion

Insulin resistance induced by obesity is a silent condition that increases the chances of developing diabetes mellitus. The present study revealed that both the obese and diabetic rat groups had a significantly increased fasting plasma glucose level, insignificantly changed fasting plasma insulin, but a significantly increased HOMA-IR index, in addition to a higher systolic blood pressure and serum triglycerides' level compared to the control group indicating the presence of an insulin resistance state. In addition, the obese group exhibited a higher obesity index as well as a significantly raised fasting plasma GIP level compared to the control and diabetic groups, while these two parameters in the diabetic group did not differ from those of the control group.

The high fasting GIP level detected in the obese group could be attributed to excessive secretion of GIP by the K-cells, and it is unlikely that it was due to accompanying high fasting blood glucose level, since GIP release from K-cells occurs after oral intake of glucose or fat. Although K-cell function in the obese group was not studied in this work, yet, it was suggested by many other reports that in obese individuals a dysregulation of GIP secretion occurs (Elahi et al. 1994). Studies performed by Bailey et al. (1986), on genetically obese mice revealed K-cell hyperplasia and markedly elevated concentrations of intestinal and circulating GIP. Furthermore, these authors stated that fatty acids although they are weaker stimulus for GIP secretion compared to glucose, yet they represent a particularly powerful stimulus to K-cell hyperplasia and subsequent GIP secretion in ob/ob which may denote another plausible explanation for the high fasting GIP level observed in the obese group of the present study.

Normally GIP has a direct effect on adipocytes and has been shown in many studies to dose-dependently stimulate lipoprotein lipase activity, fatty acid synthesis and fatty acid incorporation into adipose tissue. Moreover, GIP through its adipocytes receptors increases the expression of acyl CoA diacylglycerol transferase-1, which catalyses the final step of triglyceride synthesis. Thus, GIP helps to clear triglycerides from plasma (Miyawaki et al. 2002). Such action could be defective in both the obese and diabetic groups included in the present study contributing to the detected hypertriglyceridemia despite the presence of a high fasting plasma GIP level in the obese group and a normal GIP level in the diabetic group. A possibility of a decreased expression and/or hyporesponsiveness of adipocytes GIP-Rs cannot be excluded.

Carlson et al. (2004) found that GIP may play an integrative role in peripheral tissues by helping to coordinate targeted nutrient absorption and distribution. They described it as a homeostatic control mechanism in which disruption could be associated with hallmarks of glucose intolerance and characteristic features of the metabolic syndrome.

GIP was claimed to have a negligible insulinotropic effect under fasting conditions. Fasting blood glucose and plasma insulin concentrations were the same in GIP-Rs knockout mice compared to wild-type mice (Miyawaki et al. 1999). However, the significantly increased fasting plasma GIP level concomitant with a normal fasting plasma insulin level observed in the obese group of this study compared to the control group indicates a defect in GIP response in the obese group. Furthermore, the nutrient-elicited chemosensory and signaling mechanisms from the appearance of breakdown products of food in the duodenum (where the bulk of K-cells reside), till the trigger of incretin release from K enteroendocrine cells are unknown (Livak and Egan 2002; Ballinger 2003). However, the data obtained in this study in the subsequently performed OGTT revealed that during the first 20 min following oral glucose load, the obese group when compared with the control group had an exaggerated GIP release which was unable to stimulate early phase insulin secretion concomitant with a significantly increased plasma glucose level. In addition, the control group showed a positive correlation between plasma insulin and plasma GIP levels from 0 to 20 min; while the obese group showed

a trend towards negative correlation between them in the same time period.

Thus the ineffectiveness of the secreted GIP is probably due to pancreatic β-cell resistance to GIP. This was confirmed in the present work, which revealed a significant decrease in the pancreatic GIP-Rs gene expression in the obese group compared to that of the control group. Most probably increased GIP secretion in the obese rats leads to down regulation of the pancreatic GIP-Rs.

Similar results to those revealed by this work, were obtained in obese individuals and were attributed to desensitization of the pancreatic GIP-Rs following prolonged exposure to increased GIP plasma levels (Vilsboll et al. 2002).

On the other hand, the diabetic group had the criteria of a diabetic oral glucose curve with insignificant change in both fasting plasma insulin and GIP compared to the control group. Also during the first 20 min of the OGTT, the diabetic group had a significantly increased plasma glucose excursion, a more prominent defect in the early phase of insulin secretion compared to similar parameters in control and obese groups and a similar GIP secretory response to the oral glucose load compared to the control group. In addition, there was an associated trend towards a negative correlation between plasma GIP and plasma insulin levels during the first 20 min of the OGTT in this group. Moreover, there was a significant decrease in the pancreatic GIP-Rs gene expression in the diabetic group compared to the control group, without a significant difference compared to the obese group. Part of the defect in the early phase insulin secretion in the diabetic group could be due to a decreased expression of pancreatic GIP-Rs despite a normal level of plasma GIP compared to control, diminishing the early β-cell response to GIP as an insulinotropic factor.

Such an insensitivity of pancreatic β-cells to GIP may reflect a generalized secretory dysfunction rather than a specific cellular defect as reported by Vilsboll et al. (2002).

The results of the present work are partly different from those suggested by Carlson et al. (2004), who found a significantly decreased secretion of GIP in diabetic subjects and a reduction of their β-cells insulin secretion in response to glucose. Therefore they suggested that diabetes is associated by both a K- and a pancreatic β-cells defect. These investigators, however, did not examine pancreatic GIP-Rs gene expression in their study. In this study feeding rats with fructose was expected to increase GIP secretion through exhaustion of pancreatic insulin secretion. Insulin normally exhibit negative feed back on GIP-Rs expression and secretion by K-cells (Kok et al. 1998). However, the GIP level in the diabetic group was insignificantly increased compared to that in the control group which can not exclude the presence of a defect in the K-cells in addition to the pancreatic GIP-Rs defect.

The presence of such defect in pancreatic GIP-Rs expression in the diabetic group raises the question whether the present hyperglycemia could be the cause of such defect as expression of other G-protein-coupled receptors in the superfamily (such as the glucagons receptor) is regulated by glucose (Abrahamsen et al. 1995). Thus, it is likely that GIP-Rs downregulation could result from inappropriate stimulation of the β-cell by abnormal levels of metabolites in this animal model. However study performed by Hinke et al. (2000) on βTC-3 cells showed that potentiation of insulin release by GIP is also attenuated after its continuous application in less than 20 h under hyperglycemic conditions (11.0 mmol/l glucose). They concluded that this was not due to loss of islet viability or reduction in the releasable pool of insulin granules. In addition, GIP-stimulated cAMP production was not greatly affected by the prevailing glucose conditions, suggesting that the glucose dependence of GIP-stimulated insulin release occurs distally to the receptor activation and involves desensitization of distal steps in the exocytosis cascade.

Similarly to the present study, Lynn et al. (2001) found that diabetic rats had defective expression of GIP-Rs and an impaired GIP insulinotropic effect although they did not measure their plasma GIP level. Moreover, studies in glucose-tolerant first degree relatives of diabetic patients showed a reduced insulinotropic effectiveness of GIP in 50% of the subjects compared with controls without a family history of diabetes, indicating that the GIP-Rs defect could be genetically determined and is possibly the primary defect (Meier et al. 2001).

In fact, the GIP role in insulin secretion is extremely important. Binding of GIP to its receptors on β-cells increases intracellular cAMP levels, acts synergistically with glucose to close K_{ATP} (ATP-sensitive K^+ channels), antagonizes the delayed rectifying K^+ channels, increases intracellular calcium concentration thus stimulating mitochondrial ATP synthesis, and enhancing the exocytosis of insulin (Wheeler et al. 1995; Ding and Gromada 1997). A number of other signaling pathways may also be activated, including MAP kinase and phosphatidylinositol 3 kinase/protein kinase B pathways (Ehses et al. 2003b,c).

The glucose-stimulated insulin secretion follows a biphasic time course; shortly after elevation of the glucose concentration, a transient first-phase insulin secretion occurs (early phase) lasting for 20 min. It consists of the release of the ready release granules which is ATP-independent. Then, it is followed by a gradually developing secondary stimulation (second-phase secretion) with slower release of insulin, which results from ATP- and time-dependent mobilization of the insulin granules from the reserve pool at a much lower rate (Rorsman et al. 2000). Thus, GIP is important for both early and late phases of insulin secretion and acts as an anticipatory signal to the β-cells to ensure insulin is rapidly

released as glucose is absorbed from the gut (Miyawaki et al. 1999).

Loss of the early-phase of insulin secretion has severe consequences for glucose homeostasis in that insulin-sensitive tissues are not adequately primed to transport glucose, and glucagon secretion, free fatty acid secretion, and hepatic glucose output are not suppressed (Mitrakou et al. 1992). Such continued delivery of glucose to the circulation, aggravates post-prandial hyperglycemia, and it can be considered as an early feature of type 2 diabetes mellitus (Rorsman et al. 2000).

In the present study, during the next 20 to 120 min following oral glucose load, plasma glucose and insulin levels were decreasing in the control group, while they were significantly increasing in the obese group compared to the control group (Glu Δ 20–120 and Ins Δ 20–120).

Peak GIP secretion in the three groups was at 20 min post-glucose challenge. At 60 min, the plasma GIP level in the obese group was still significantly increased and was accompanied by a significant hyperinsulinemia compared to the control and diabetic groups. Then insulin secretion in the obese group remained significantly elevated in the time interval of 90 and 120 min, albeit its GIP level at the same time interval was insignificantly increased compared to the control group.

These data reveal the presence of an exaggerated β-cells secretion of insulin in the absence of a correlation between GIP and insulin levels and in the presence of a significant reduction of pancreatic GIP-Rs gene expression in the obese group, denoting a possible up-regulation of a compensatory mechanism as a result of the impaired GIP insulinotropic effect.

Pamir et al. (2003), demonstrated that disruption of the GIP-Rs component of the entero-insular axis in mice resulted in increased islet sensitivity to glucagons-like polypeptide-1 (GLP-1) and changes in islet structure. In fact, GLP-1 is the other incretin secreted by intestinal L-cells in response mainly to ingested fat and weakly to ingested glucose and its gene is also expressed in pancreatic α-cells and is cleaved by the enzyme dipeptidylpeptidase. Thus fat-induced increased GLP-1 secretion in obese rats of this study can not be excluded although it was not measured. Binding of GLP-1 to its pancreatic β-receptors which belong to the same family as the GIP-Rs, causes the formation of cAMP and subsequent plethora of events similar to those activated by GIP leading to insulin secretion (Holz 2004). Most of the effects of GIP and GLP-1 are shared and important differences between their actions on β-cells have not been discovered so far except that GLP-1 is more effective as an insulinotropic substance at post-prandial glucose levels of 6 and 7 mmol/l as cited by Vilsboll et al. (2003). However, GLP-1 strongly inhibits glucagon secretion from non β-islet cells (Holst and Gromada 2004), while GIP is a weak stimulant of glucagon secretion (Meier et al. 2003).

Thus it can be suggested that a compensation for the absence of a functional GIP-Rs occurs, in part, by up-regulation of the GLP-1 component of the entero-insular axis. The physiological changes that take place in the GIP-Rs deficient (GIP-Rs$^{-/-}$) strains of knockout mice suggest that the incretins act in concert to maintain glucose homeostasis and that a balance between the two is required for proper function of the entero-insular axis. It might be predicted that an increase in β-cell GLP-1 sensitivity in the absence of GIP action could protect against a decrease in islet insulin mRNA and protein levels. Therefore, inhibition of GIP signaling to prevent triglycerides incorporation in adipose tissues as trials to treat obesity may carry the price of impaired glucose tolerance or possibly frank diabetes (Ballinger 2003).

Carlson et al. (2004) found similar results to those obtained in this study when they performed the OGTT in obese individuals. They found an impaired early phase of insulin secretion associated with elevated GIP secretion and hyperinsulinemia occurring at 60 to 120 min post-glucose challenge. These authors associated such hyperinsulinemia to GIP and stated that the elaboration of GIP could be a contributing factor in progressive exhaustion of pancreatic β-cells in obesity and is a link between obesity and diabetes. However, they did not measure the GIP-Rs expression in these individuals, which may highlight another cause for such hyperinsulinemia.

The diabetic group, on the other hand, had similar GIP levels as those observed in the control group at the time interval from 20 to 90 min. Yet, the insulin level in this group remained significantly lower than that of the control group during the same time period, indicating a defect in the insulinotropic effect of GIP during the second-phase of insulin secretion in the diabetic group which is also attributed to down-regulation of its pancreatic islet GIP-Rs. With regard to the time period from 90 to 120 min, however, the GIP level in the diabetic group dropped faster toward fasting levels than in the control group, resulting in greater incremental changes in GIP Δ 20–120 and significantly reduced AUC of GIP Δ 20–120 with a significantly lower GIP level in the diabetic than in the control group at 120 min post-glucose challenge. The insulin levels in both the control and diabetic groups were insignificantly different during this time period, because the insulin level, which had previously been high in the control group (in the 20–90 min time period), now diminished also in this group and approached fasting levels, which are similar to those of the diabetic group.

The incremental changes of plasma insulin and GIP levels were always significantly decreased in the diabetic group compared to those of the obese group from 20 to 120 min post-glucose challenge.

Thompson et al. (2003) documented that the effect of GIP is greatly reduced in diabetic individuals, mainly because of a complete loss of the normal GIP-induced potentiation of

the second-phase insulin secretion and that this might result from an enhanced desensitization and/or internalization of a normal or a reduced number of GIP-Rs.

In conclusion, this study revealed that both obese and diabetic rats had an impaired early phase insulinotropic effect of the gut-derived peptide, gastric inhibitory peptide, in response to oral glucose administration during OGTT due to impaired gene expression of its pancreatic receptors. Also there was an associated hyperinsulinemia in the obese rats during the second hour of the OGTT, which may lead to exhaustion of β-cells on the long run, increasing the incidence of diabetes mellitus. Such an observation raises the interesting question of whether an early intervention in obese individuals aiming to reduce raised GIP level and to improve GIP-Rs function could contribute to counteract the associated hyperinsulinemia and subsequently to decrease the incidence of diabetes mellitus and whether a treatment directed towards GIP-Rs could at least partially restore the GIP insulinotropic response in obesity and type 2 diabetes mellitus.

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