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Plasma Level of Glucagon-like Peptide 1 in Obese Egyptians with Normal and Impaired Glucose Tolerance

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Background and Aims. Low GLP-1 has been implicated in obesity and type 2 diabetes. Some studies reported reduced post-prandial GLP-1 levels in type 2 diabetics, whereas others reported GLP-1 levels not reduced in patients with impaired glucose tolerance (IGT) or type 2 diabetes. We undertook this study to evaluate the effect of obesity and pre-diabetes on GLP-1 levels in response to 75 g oral glucose.

Methods. Eighty subjects comprised four groups: 20 control subjects (normal weight and normal glucose tolerance (NGT)), 20 obese with NGT, 20 obese with impaired fasting glucose (IFG) and 20 obese with both IFG and impaired glucose tolerance (IGT). Laboratory tests included fasting blood glucose (FBG), 75 g glucose OGTT, fasting insulin and glucose-stimulated GLP-1 (30 min after 75 g glucose). Insulin resistance was quantified using HOMA-IR.

Results. GLP-1 levels were significantly decreased in obese subjects compared to controls (571.17 ± 170.37 vs. 908.50 ± 169.90 pg/mL, $p < 0.001$) and it was negatively correlated with body mass index (BMI) and waist circumference in all studied groups. Levels of GLP-1 were negatively correlated with HOMA-IR in all obese groups ($r = -0.75$, $p < 0.001$). No significant difference was found in GLP-1 levels between all obese subjects (611.50 ± 187.96, 577.50 ± 150.85, 524.50 ± 167.35 pg/mL respectively, $p > 0.05$). Morbidly obese cases ($n = 15$) had a significantly higher fasting insulin (25.20 ± 2.49 vs. 14 ± 3.81 μIU/ml), higher HOMA-IR (6.69 ± 1.2 vs. 3.48 ± 1.20), and lower GLP- 1 (212.0 ± 35.64 vs. 603.82 ± 136.35 pg/mL) ($p < 0.001$) compared to non-morbid obese cases ($n = 45$).

Conclusions. Obesity reduces the GLP-1 levels. In insulin resistance, GLP-1 levels were reduced and it was related to the degree of insulin resistance.

Key Words: Obesity, Type 2 diabetes, Impaired fasting glucose, Glucose tolerance.

Introduction

Pre-diabetes is an asymptomatic condition not associated with functional impairment that mostly presents prior to the individual developing type 2 diabetes (1). Current estimates indicate that most individuals (perhaps up to 70%) with pre-diabetic states eventually develop diabetes (2). The transition from the impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) to diabetes may take many years (2). The reported increase in type 2 diabetes is related to lifestyle changes that have resulted in overweight, obesity, and decreased physical activity levels. Glucagon-like peptide 1 (GLP-1) is an incretin hormone that is secreted by the L cells of the intestine upon meal ingestion and plays a key role in glucose homeostasis. It also inhibits gastric emptying, pancreatic secretion of glucagon and food intake (3,4). The impact of obesity on the incretin effect is uncertain. The mechanism whereby obesity lowers GLP-1

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secretion is not known but may be related to the insulin resistance that accompanies weight gain (5). In some studies, the GLP-1 response of obese subjects was normal (6), whereas other studies have shown reduced response to oral carbohydrate (7). In patients with type 2 diabetes, some studies reported reduced post-prandial GLP-1 levels (8,9). On the contrary, several other investigators reported that GLP-1 levels were not reduced in patients with IGT or type 2 diabetes (10,11). Because there was no concordance among previous studies, the present study aimed to evaluate the effect of obesity and pre-diabetes on GLP-1 levels in response to 75 g oral glucose.

Materials and Methods

This study was conducted on 80 subjects who were selected from the outpatient clinic of Ain Shams University Endocrinology Unit and Ophthalmology Research Institute and who underwent pre-operative assessment. They were divided into four groups: group 1: $n = 20$ (nine males and 11 females) with normal weight and normal glucose tolerance (NGT) as a control group. Group 2 included 20 obese subjects with NGT (seven males and 13 females). Group 3 included 20 obese subjects with IFG (four males and 16 females). Group 4 included 20 obese subjects with both IFG and IGT (five males and 15 females). Gender distribution was comparable among all four groups ($\chi^2 = 3.43$, p value $= 0.330$).

Obesity was defined as body mass index (BMI) $\geq$30 kg/m^2 and morbid obesity as a BMI $\geq$40 kg/m^2 (10). Pre-diabetes was defined as having IFG or IGT or both. IFG was defined as a fasting glucose level of (100−125 mg/dL), IGT was defined by a 2 h plasma glucose (2hPG) level of 140−199 mg/dL after administration of 75 g of oral glucose, whereas NGT was defined as a FBG $<$100 mg/dL and 2hPG $<$140 mg/dL. Diabetes mellitus (DM) was defined as FBG $\geq$126 mg/dL and/or 2hPG $\geq$200 mg/dL (12).

Exclusion criteria included history of DM, hepatic and kidney diseases. Any drugs affecting GIT motility such as anticholinergics and antidepressants, antidiabetics or glucocorticoids or history of surgical operations (e.g., gastrectomy or gastric bypass operation for obesity) led to exclusion from the study. Clinical examination included measurement of blood pressure, weight, height and BMI (kg/m^2). Waist circumference was measured at the highest point of the iliac crest at minimal respiration to the nearest 0.1 cm. Laboratory tests included fasting blood glucose, 75 g glucose OGTT, fasting insulin after an overnight fasting (from 8−12). Glucose-stimulated GLP-1 (30 min after 75 g glucose). Glucose was measured by colorimetric enzymatic method (13). Immunoenzymatic assay was used for *in vitro* quantitative measurements of fasting plasma insulin (BioSource INS-EASIA Kit; BioSource Europe SA, Belgium). Insulin resistance was estimated by HOMA-IR and was defined as fasting serum insulin (μU/mL) × FPG (mmol/l)/22.5 (14). WHO considered people with HOMA values above the 75th percentile (cut-off point 4.2 U) as insulin resistant (15).

The total GLP-1 levels were measured in plasma using DRG® (Springfield, NJ) Glucagon-Like Peptide-1 (Human, Rat and Mouse) ELISA (EIA-4141) kit. The blood used for the GLP-1 estimation was collected into a Lavender Vacutainer tubes containing EDTA (5 mL blood/tube) and gently rocked several times immediately after for anticoagulation, then transferred to centrifuge tubes containing aprotinin (0.6 TIU/mL of blood) and gently rocked for several times to inhibit the activity of proteinases. The blood was centrifuged at 1,600 x g for 15 min at 4°C and the plasma was collected and kept at −70°C (16).

This study was approved by the internal review board of Ain Shams University. All subjects provided written informed consent before the study.

Statistical Analysis

SPSS statistical software package (v.13.2, 2003, Echosoft Corp, Agoura Hills, CA) was used for data analysis. The mean ± SD was used for data description as regards quantitative data, whereas number and percent (%) were used for qualitative data. One-way analysis of variance (ANOVA) was used when comparing between more than two groups. Post-hoc test (Tukey's) was used to detect the least significant difference among the studied groups. Pearson's correlation coefficient (r) test was used to assess relationship between quantitative variables. Independent-samples t test was used when comparing between groups; p value <0.05 was considered statistically significant. Area under the curve (AUC) calculations of non-parametric receiver operating characteristic (ROC) curves were used to assess the sensitivity and specificity of glucose-stimulated GLP-1 in diagnosing impaired fasting blood glucose and impaired glucose tolerance.

Results

There was a highly statistically significant difference (p <0.001) between groups regarding age, BMI, waist circumference, systolic blood pressure, 2hPG, fasting insulin, HOMA-IR and glucose-stimulated plasma GLP-1 but only significant difference (p <0.05) as regards the diastolic blood pressure and fasting plasma glucose. No significant difference was found between glucose stimulated GLP-1 levels in obese subjects with NGT, obese with IFG and obese with both IFG and IGT (611.50 ± 187.96 vs. 577.50 ± 150.85 vs.524.50 ± 167.35 pg/mL respectively, p >0.05), (Table 1).

Glucose-stimulated GLP-1 plasma levels were significantly decreased in obese subjects compared to normal controls (571.17 ± 170.37 vs. 908.50 ± 169.90 pg/mL respectively, p <0.001). Also, obese cases had higher BMI, waist circumference, FPG, 2hPG, HOMA-IR, fasting