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Original Research Article

Levels of Incretin Hormones in Subjects with Type 2 Diabetes in Different Grads of BMI

Israa Habeeb Ibrahim[1*] Haider Kamel Zaidan[1] Moshtak Abduladheem Wtwt[2]
[1]College of Science, University of Babylon, Hilla, IRAQ
[2]College of Medicine, University of Babylon, Hilla, IRAQ

[*]E-mail: nectarrose69@yahoo.com

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Abstract

Incretins are gut hormones that potentiate insulin secretion after meal ingestion in a glucose-dependent manner. There are different types of incretin hormones like GLP-1, GLP-2 and GIP. DPP-IV is the enzyme that deactivate incretin hormone in the blood. The aim of this study was to evaluate the relationship between obesity and incretin hormones in patients with type 2 diabetes.The study was included (107) patients : (62) males and (45) females from Al-Hussein Medical City in Karbala province and Marjan Medical City in Babylon province. The control for this study was (32) healthy subjects: (14) males and (18) females.BMI, Waist circumference, blood pressure , HbA1c, lipid profile, AIP, GLP-1, GLP-2 , GIP and DPP-Iv were measured to all patients and control.The resultsshow significant decrease in GLP-1 (P=0.018) and GLP-2 (P=0.028) and there was no differences in GIP (P=0.74) in patients group in comparison also there was significant increase in DPP-IV (P<0.001) in patients group in comparison with control group.According to BMI grades there was some decrease in GLP-1 and GLP-2 in obese subjects but there was no differences in GIP in comparison with lean control group. Also there was significant increase in DPP-IV in obese patients in comparison with lean control. We conclude that the decrease in incretin hormones may come mostly from the increase in DPP-IV enzyme that degrade them in the blood quickly and according to the relation with obesity, DPP-IV enzyme increase significantly in obese diabetic patients in comparison with healthy lean subjects.

Key words: Incretins, Diabetes mellitus, obesity, GLP-1, DPP-IV

الخلاصة

الانكريتينات هي هرمونات معوية تنشط افراز الانسولين بعد تناول الطعام في اسلوب يعتمد على الكلوكوز. هناك انواع متعددة من هذه الهرمونات مثل GLP−1 ، GLP−2 و GIP. يعتبر الانزيم DPP−IV هو الانزيم الذي يزل فعالية هذه الهرمونات في الدم. الهدف من الدراسة لتقييم العلاقة ما بين السمنة وهرمونات الانكرتين في مرضى السكري من النوع الثاني.تضمنت الدراسة على ١٠٧ من مرضى السكري : ٦٢ من الذكور و ٤٥ من الاناث من مدينة الحسين الطبية في محافظة كربلاء ومدينة مرجان الطبية في محافظة بابل. ضمت مجموعة السيطرة لهذه الدراسة ٣٢ من الاشخاص الاصحاء: ١٤ من الذكور و ١٨ من الاناث. تم قياس دليل كتلة الجسم، محيط الخصر، ضغط الدم، السكر التراكمي، مجموعة الدهون، دليل تصلب الشرايين البلازمي، هرمونات ال GLP−1، GLP−2 و GIP مع انزيم DPP−Iv لكل من المرضى ومجموعة السيطرة.اظهرت النتائج قلة معنوية في ال GLP−1(P=0.018) وال GLP−2(P=0.028) ولا يوجد هناك اختلافات في ال GIP(P=0.74) ايضا هناك زيادة ملحوظة في انزيم DPP−IV(P<0.001) في مجموعة المرضى بالمقارنة مع مجموعة السيطرة.وفقا لمجاميع دليل كتلة الجسم هناك بعض القلة في تراكيز هرمونات ال GLP−1 و GLP−2 في الاشخاص البدناء لكن لا توجد هناك اختلافات في ال GIPبالمقارنة مع مجموعة السيطرة النحفاء. ايضا هناك زيادة واضحة في انزيم DPP−IV في المرضى البدناء بالمقارنة مع السيطرة النحفاء. نستنتج ان القلة في هرمونات الانكرتين ربما تأتي معظمها من الزيادة في انزيم DPP−IV الذي يحطم هذه الهرمونات في الدم سريعا، ووفقا للعلاقه مع السمنة، يزداد الانزيم DPP−IV زياد ملحوظة في مرضى السكري البدناء بالمقارنة مع الاشخاص الاصحاء النحفاء.

الكلمات المفتاحية: الانكريتينات، داء السكري، السمنة، GLP−1 ، DPP−IV.

Introduction

Incretins are gut hormones that potentiate insulin secretion after meal ingestion in a glucose-dependent manner. It is quantified by comparing insulin responses to oral and intravenous glucose administration, where the intravenous infusion is adjusted so as to result in the same (isoglycaemic) peripheral (preferably arterialized) plasma glucose concentrations [1, 2]. In healthy subjects, the oral administration causes a two- to threefold larger insulin response compared to the intravenous route, thought to be due to the actions of gut hormones [3].

The same gut hormones are also released by mixed meals, and given that their postprandial concentrations in plasma are similar and that the elevations in glucose concentrations are also similar, it is generally assumed that the incretin hormones are playing a similarly important role for the meal-induced insulin secretion [3].

The two best studied incretins, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), exert their insulinotropic actions through distinct G-protein-coupled receptors highly expressed on islet β cells [4].

GIP is a 42 amino acid peptide synthesized in and secreted from enteroendocrine K cells located primarily in the duodenum and proximal jejunum, and CNS production of GIP has also been described. GIP messenger RNA (mRNA) and protein have been localized to the a cell in mouse and human islets; however, the prohormone is processed by PC2 (rather than PC1/3 as seen in the K cell) to yield a 30 amino acid protein (GIP) [5].

GIP increases insulin secretion in perfused mouse pancreata, and immuno-neutralization of GIP decreased glucose-stimulated insulin secretion in isolated mouse islets, consistent with the local release of an insulinotropic GIP peptide from a cells [5]. Ectopic expression of biologically active GIP has also been localized to β cells in mice with targeted inactivation of the GCG gene [6].

GLP-1 an active glucoincretine hormone [7] is secreted by intestinal L-cells in response to carbohydrates especially glucose and fat meals [8, 9]. It is also produced from pancreatic islets [10]. The GLP-1 peptide synthesis in this cells through posttranslational processing of proglucagon by the aid of enzyme prohormone convertase 1/3 (PC1/3) and found in two biologically active amidate forms (amino acids 7-36) and unamidate hormone (7-37) [11].

Original concepts of L cells as predominantly unihormonal or bihormonal have evolved to reflect evidence that enteroendocrine L cells exhibit a molecular profile overlapping with other gut endocrine cell types and coexpress multiple peptide hormones, with diversity of peptide hormone coexpression changing along the length of the gastrointestinal tract [12].

GLP-1 is also produced in the central nervous system (CNS), predominantly in the brainstem, from where it transported throughout the brain to elicit metabolic, cardiovascular, and neuroprotective actions [4].

The physiological effects of GLP-1 make it more attractive as therapy for type 2 diabetes [13], it cause increase in insulin secretion and decrease in glucagon release only when glucose levels are elevated [14, 15] and this manner of action avoided the likelihood of hypoglycemia [13].

The other actions includes: promotes weight loss, delays gastric emptying and decreases food intake (appetite suppression) in both human and animals [14, 16].

GLP-2 Glucagon-like peptide 2 (GLP-2) was first recognized as a growth factor in the intestine in 1996 [9]. It is a 33-amino-acid peptide, formed from the cleavage of proglucagon, a large prohormone that is mainly expressed in pancreas, intestine and brain. Alternative splicing of proglucagon through prohormone convertases leads to the tissue-specific release of GLP-2 and other

peptides with diverse biological properties [17].

The biological effect of the 33 amino acid peptide GLP-2 is mediated by activation of a G-protein coupled 7 transmembrane receptor, expressed mainly in the gut and the brain [18].

Thus GLP-2 receptors are expressed in the brainstem, lungs, stomach, small intestine and colon, but not in the heart [19].

The receptor sequence is close related to the sequence of the GLP-1 and glucagon receptors [18].The receptor has been reported to localize to subepithelialmyofibroblasts[19] as well as to enteric neurons and enteroendocrine cells [20, 21]. Studies have also shown that the receptors colocalizes with nitric oxide (NO) expressing vasoactive intestinal polypeptide-positive enteric neurons, and serotonin in enteroendocrine cells, suggesting that GLP-2 induced increased intestinal blood flow is mediated by vasoactive neurotransmitters. The localization to epithelial endocrine cells [19], has not been supported by subsequent studies.

DPP-IV: Dipeptidyl peptidase (DPP)-4 is a complex enzyme that exists as a membrane-anchored cell surface peptidase that transmits intracellular signals via a short intracellular tail and as a second smaller soluble form present in the circulation [22]. DPP-4 cleaves a large number of chemokines and peptide hormones in vitro, but comparatively fewer peptides have been identified as endogenous physiological substrates for DPP-4 in vivo. Both glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are endogenous physiological substrates for DPP-4 [23], and chemical inhibition of DPP-4 activity, or genetic inactivation of DPP-4 in rodents, results in increased levels of intact bioactive GIP and GLP-1 [24, 25].

The aim of this study to evaluate the relationship between obesity and incretin hormones in patients with type 2 diabetes.

Materials and Methods

Subjects

The samples for this study was taken from diabetic clinic of two hospital: Al-Hussein Medical City in Karbala province and Marjan Medical City in Babylon province.

The study was included (107) patients : (62) males and (45) females from mentioned hospitals. The control for this study was (32) healthy subjects: (14) males and (18) females. The ages of patients and controls were ranges between 25-75 years old.

Methods

The patients and control who have essential hypertension was excluded. The patients who have hypertension before the onset of diabetes was also excluded from study. The patient considered to have hypertension dependent on the definition of hypertension: systolic blood pressure 140 mmHg or more and /or a diastolic blood pressure 90 mmHg or more [26, 27].

BMI [28, 29]:The height was recorded to the nearest centimeters and the weight was recorded to the nearest kilograms. BMI was calculated by dividing weight (Kg) by sequre of height (m^2)

$$\text{BMI}\ (\frac{\text{Kg}}{\text{m}^2}) = \frac{\text{Weight (Kg)}}{\text{Hieght (m}^2\text{)}}$$

Waist Circumference: It was measured at the midpoint between the lower rib and iliac crest with non-stretchable plastic tape. The waist circumference was recorded to the nearest centimeters [28, 29].

The lipid profile tests were performed by using of enzymatic cholorimetric procedure by using kits from RANDOX/UK company.

atherogenic index of plasma (AIP) was calculated as log(TG/HDL-C) [30, 31].

The incretin hormones and DPP-IV were measured by ELISA method by using kits from Elabscience/ China company.

Statistical analysis

Statistical analyses were performed by using IBM SPSS statistics software of

version 20. The results represented as Mean ± S.D. The analyses of variances were made by using independent sample T-test and One Way ANOVA.

Results

Table 1 shown the subjects characteristics of control and patients groups and comparison between two groups.

The results shown revealed that there was significant increase in BMI, Waist circumference, Systolic and diastolic blood pressure, HbA1c, triglyceride, VLDL and AIP in patients group in comparison with control group.

Also in the same table shown significant decrease in GLP-1 and GLP-2 and significant increase in DPP-IV in patients group in comparison with control group.

Table 1: Subjects characteristics

parameters	Control Group n=32	Patients Group n=107	*P* value
Age (years)	31.38±11.34	50.47±10.22	<0.001
BMI(kg/m^2)	21.72±2.21	28.91±4.89	<0.001
Waist circumference (cm)	81.69±9.18	100.65±12.99	<0.001
Systolic BP(mmHg)	110.13±9.46	129.07±24.06	<0.001
Diastolic BP(mmHg)	69.88±7.46	81.37±12.65	<0.001
HbA1c (%)	5.04±0.85	8.38±2.91	<0.001
S.Cholesterol (mg/dl)	215.98±39.74	188.26±46.43	0.08
S.Triglyceride(mg/dl)	135.10±59.87	224.83±103.37	0.009
S.HDL (mg/dl)	70.10±20.29	59.94±26.94	0.26
S.LDL (mg/dl)	118.86±24.13	89.29±40.84	0.03
S.VLDL (mg/dl)	27.02±11.97	44.97±20.67	0.009
AIP	0.27±0.17	0.69±0.52	0.013
GLP-1 (ng/ml)	28.94±37.33	13.78±29.11	0.018
GLP-2 (ng/ml)	13.41±17.79	7.36±11.83	0.028
GIP (pg/ml)	4479.69±3939.19	4204.76±4114.43	0.74
DPP-IV(ng/ml)	5.79±6.70	13.12±9.36	<0.001

The results represented as Mean±S.D.
The significant value at $P \leq 0.05$

The patients were divided to five groups according to BMI grouping of WHO, 2000 and compare them with lean healthy control in incretin hormones and DPP-IV levels as shown in table 2.

This table revealed that there was some significant differences among patients groups and in comparison with control group in GLP-1 and GLP-2 but there was no differences in GIP.

The most pronounced differences were found in DPP-IV levels as shown in table 2 which demonstrated that there was significant increase in DPP-IV in first four groups in comparison with control group.

Table 2: Comparison among BMI groups in control and patients

BMI Groups	GLP-1 (ng/ml)	GLP-2 (ng/ml)	GIP (pg/ml)	DPP-IV (ng/ml)
Lean control 18.5-25 kg/m^2 (n=32)	28.94±37.33	13.41±17.79	4479.69±3939.19	5.79±6.70
Patients 18.5-25 kg/m^2 (n=20)	20.80±49.22	8.88±13.39	4153.25±4203.17	12.23±8.68[a]
Patients 25-30 kg/m^2 (n=44)	7.03±12.59[a]	4.36±8.23[a]	3187.27±2955.77	13.05±9.35[a]
Patients 30-35 kg/m^2 (n=29)	12.24±14.59[a]	8.32±13.61	5343.62±5261.90[c]	13.66±8.82[a]
Patients 35-40 kg/m^2 (n=9)	24.89±48.91	11.42±14.08	5035.00±4295.21	14.94±13.62[a]
Patients ≥40 kg/m^2 (n=2)	64.25±7.14[c, d]	26.25±6.43[c]	6855.00±2446.59	7.25±1.69

Results represented as Mean ±S.D., the mean differences is significant at level $P \leq 0.05$
[a] significant differences in comparison with lean control group
[b] significant differences in comparison with 18.5-25 kg/m^2 group
[c] significant differences in comparison with 25-30 kg/m^2 group
[d] significant differences in comparison with 30-35 kg/m^2 group

Discussion

The results show significant decrease in secretion of GLP-1 and GLP-2 in all patients and there was no change in GIP secretion as comparison with control group as seen in table 1.

In table 2 the results show decrease in GLP-1 in comparison with healthy lean subjects and also some decrease in GLP-2 but there was no differences in GIP.

The increase in secretion of incretins found in last group of patients (extremely obese subjects) may come from low sample number that not give real indication of the group.

The levels of GLP-1 peptide increase in response to nutrient intake[11] and the proportion of the secretory response depends on the amount of nutrient consumed [11, 32, 33]. However, it has been clearly shown in this study that circulating levels of GLP-1 are reduced in obese patients.

Results from a study carried out more than 25 years ago indicated that the normal enteroglucagon (glicentin+oxyntomodulin), co-secreted with GLP-1 from the L cells) responses to meals were decreased by about 75% in obese subjects [34]. By contrast, GIP responses are often increased rather than decreased [35].

One study on the role of postprandial releases of insulin and incretin hormones in meal-induced satiety and the effect of obesity and weight reduction show that there was a reduced postprandial GLP-1 response in severely obese subjects and following weight reduction, GLP-1 response in the obese subjects apparently rose to a level between that of obese and lean subjects also this study suggests that postprandial insulin and GIP responses are key players in short-term appetite regulation [36].

Other study show that glucose tolerance and obesity impair the incretin effect independently of one another [37].

It is possible that incretin impairment may contribute to the pathophysiological bridge between obesity and T2D [38].

Study on theobese youth along the span of glucose tolerance from normal to prediabetes to type 2 diabetes revealed that glucose sensitivity deteriorates progressively in obese youth across the spectrum of glucose tolerance in association with impairment in incretin effect without reduction in GLP-1 or GIP, similar to that seen in adult dysglycemia[39].

Study that contestant with recent study show that while insulin secretion is increased in glucose tolerant obese individuals, secretion of glucagon-like peptides (GLP-1 and GLP-2) is lower compared to lean controls. Therefore, diminished incretin secretion is associated not only with type 2 diabetes but also with obesity and may occur early in the development of type 2 diabetes [40] and GLP-2 may associated with insulin resistance in obesity [41].

GLP-1 responses to oral stimulation have been negatively correlated to body mass index (BMI) [11],and weight loss was associated with increasing GLP-1 responses to meal ingestion [36]. It has been suggested that the decrease may be related to the insulin resistance that accompanies weight gain and/or reduced L-cell responsiveness to carbohydrates secondary to increased levels of circulating fatty acids [42].

Interestingly, one study showed that both moderate and intense exercise increased GLP-1 levels and decreased hunger and that elevations in GLP-1 were inversely correlated with energy intake post-exercise [43].

The results also show significant increase in DPP-IV in diabetic patients (Table 1) and significant increase in obese patients as comparison with healthy lean subjects (Table 2).

This result agreement with other studies that show serum CD26/DPP4 levels and enzymatic activities were higher in patients with chronic hyperglycemia and type 2 diabetes mellitus than in the control group[44-46]

Other related studies examined the effects of chemical inhibitors of DPP-4 enzymatic activity on the structure and activity of GLP-1 in normal animals and in experimental models of diabetes. The nonselective DPP-4 inhibitor valine pyrrolidide (VP) prevented the degradation of GLP-1 and GIP in anesthetized pigs and potentiated the incretin-mediated reduction of plasma glucose and stimulation of insulin secretion in response to an intravenous glucose challenge [47, 48]. Also this inhibitor acutely improved oral glucose tolerance in high fat–fed pigs, in association with increased levels of intact GLP-1 and increased levels of plasma insulin following oral glucose loading [49].

Study on the combination treatment of type 2 diabetes with DPP-4 inhibition plus metformin show that DPP-4 inhibition in combination with metformin is an efficient, safe and tolerable combination therapy for type 2 diabetes [50].

Conclusion

The decrease in incretin hormones may come mostly from the increase in DPP-IV enzyme that degrade them in the blood quickly and according to the relation with obesity, DPP-IV enzyme increase significantly in obese diabetic patients in comparison with healthy lean subjects.

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