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Original Article

Fasting serum glucagon-like peptide-1 concentrations in obese patients with acanthosis nigricans: a pilot study

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Abstract: Objective: The aim of this study was to evaluate the fasting serum GLP-1 concentrations in Chinese obesity associated with Acanthosis nigricans (AN). Methods: This cross-sectional study included 61 subjects who were divided into two groups: 27 obese patients with AN (AN group) and 34 obese patients without AN (OB group). Data regarding weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), visceral fat fraction, lipid profile, and uric acid (UA) were determined and collected for each patient. 75 g glucose tolerance tests (OGTT) were performed and insulin levels during OGTT were measured in all subjects. Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated to estimate insulin resistance. Body composition was measured by dual energy X-ray absorptiometry (DEXA). Fasting serum total GLP-1 concentrations were measured. Results: 1) Weight, WC, HC, UA, and HOMA-IR were significantly higher in the AN group than the OB group ($P < 0.05$). However, the level of high density lipoprotein-cholesterol (HDL-C) was significantly lower in the AN group than the OB group ($P < 0.05$). 2) The level of serum GLP-1 in the AN group was the significantly higher than the OB group ($P < 0.05$). 3) The level of GLP-1 was significantly positively associated with LDL in AN group ($P < 0.05$). The level of GLP-1 was significantly positively associated with TC in AN group ($P < 0.05$). The level of GLP-1 was positively associated with plasma insulin measured at 30 min after OGTT, total cholesterol, and LDL-C in all patients with obesity ($P < 0.05$). Conclusions: Patients with obesity associated AN had severe insulin resistance accompanied with increased total fasting GLP-1 concentrations in comparison with simply obesity.

Keywords: Acanthosis nigricans, glucagon-like peptide-1, obesity, metabolism, insulin resistance

Introduction

Acanthosis nigricans (AN) is characterized by dark, coarse and thickened skin with a velvety texture and histopathologically characterized by papillomatosis and hyperkeratosis of the skin and can be distributed on the neck, axillae, antecubital and popliteal fossae, and groin folds. The first case of AN was described in 1891 [1]. AN is a cutaneous manifestation and may serve as a marker of many underlying states such as endocrine abnormalities, obesity, certain drugs, and malignancy. AN is one of the consequences in severe obesity and obesity is the most common cause of AN. A high prevalence of AN has been observed recently because of the rising prevalence of obesity. AN may appear at any age and over half of adults who weigh over 200% of their ideal body weight may develop AN. AN is closely related to insulin resistance. Hyperinsulinemia can exert more potent growth-promoting effects through binding to insulin-like growth factor 1 receptors (IGF-1Rs) and the binding stimulates proliferation of keratinocytes and fibroblasts which result in AN [2]. Improving hyperinsulinemia may reduce keratinocytic lesions. Therefore, the therapeutic approach involving weight loss and exercise improves obesity associated AN through increasing insulin sensitivity and reducing insulin levels [3].

Glucagon-like peptide-1 (GLP-1) is a 30-amino acid peptide hormone secreted from the L-cells of gastrointestinal tract in response to meal ingestion and enhances postprandial insulin secretion [4]. The level of bioactive GLP-1 in fasting serum usually ranges from 5 to 10

pmmol/L and increases approximately twofold to threefold after each meal [5]. Additionally, the postprandial peak of the serum GLP-1 level appears 20-30 minutes after a meal according to the size and nutritional composition of the meal [5]. The levels of GLP-1 are thought to be involved in the development of obesity and type 2 diabetes mellitus (T2DM) [5]. Glucose-induced GLP-1 secretion was remarkably decreased in patients with nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) compared with healthy controls [6]. These findings provided the rationale for the development of GLP-1 receptor agonist for the treatment of obesity, T2DM, and NAFLD.

Though AN has been linked to obesity and insulin resistance. And, disturbance in the secretion of GLP-1 has been observed in the states with impaired glucose regulation and obesity. However, the mechanism underlying of AN is unknown. No studies have reported the total serum GLP-1 concentrations in Chinese patients with obesity associated AN. We are wondering whether exist a difference of GLP-1 concentrations between the simply obesity and the obesity associated AN. Therefore, we compared the fasting total serum GLP-1 concentrations in patients with obesity associated AN with simple obese patients, and aimed to explore the mechanism of the obesity manifest AN. Furthermore, it may provide evidence for use of potential methods to change sensibility of GLP-1 for treatment of obesity associated with AN.

Methods

Subjects

This cross-sectional study enrolled 61 patients with obesity (age 18-53) from the outpatient Endocrinology and Metabolism clinic at Shanghai Tenth People's Hospital. The obese patients were divided into the following groups: simple obesity (OB, n = 34, BMI > 28 kg/m^2) and obesity associated AN (AN, n = 27, BMI > 28 kg/m^2). Obesity was defined as BMI > 28 kg/m^2. The diagnosis of AN mainly clinically with histopathology needed only for confirmation. The following quantitative scale of AN was applied in this study. Neck severity: 0-Absent: not detectable on close inspection; 1-Present: clearly present on close visual inspection, not visible to the casual observer, extent not measurable; 2-Mild: limited to the base of the skull, does not extend to the lateral margins of the neck (usually < 3 inches in breadth); 3-Moderate: extending to the lateral margins of the neck (posterior border of the sternocleidomastoid, usually 3-6 inches), should not be visible when the participant is viewed from the front; 4-Severe: extending anteriorly (> 6 inches), visible when the participant is viewed from the front. Axilla: 0-Absent: not detectable on close inspection; 1-Present: clearly present on close visual inspection, not visible to the casual observer, extent not measurable; 2-Mild: localized to the central portion of the axilla, may have gone unnoticed by the participant; 3-Moderate: involving entire axillary fossa, but not visible when the arm is against the participant's side; 4-Severe: visible from front or back in the unclothed participant when the arm is against the participant's side [1]. Each subject with AN enrolled in this study had a score greater than 2. Exclusion criteria: 1) malignant tumor; 2) previous use of glucocorticoids or nicotinic acid; 3) serious co-morbid medical conditions such as liver and kidney disease; 4) serious endocrine or hereditary diseases. This study was approved by the ethics committee of Shanghai Tenth People's Hospital and all participants signed an informed consent prior to participation in this study. The Clinical Trials registration Number is ChiCTR-OCS-12002381.

Anthropometric measurements

Anthropometric measurements of participants including weight, BMI, and fraction of visceral fat measured with light clothes and without shoes using a simple anthropometric measuring instrument (Omron HBF-358, Japan). The dates on which WC (measured at the level midway between the lower rib margin and the iliac crest) and HC (measured at the level of anterior superior iliac spine) were measured and recorded. All these parameters were tested twice and the averages were used for analysis.

Oral glucose tolerance test and biochemical analyses

Fasting venous blood samples were collected after fasting for at least 8 hours after which biochemical parameters including TC, TG, LDL-C, HDL-C), free fatty acids (FFA, UA, fasting plasma glucose (FPG), and insulin were determined. Additionally, a 75 g-OGTT was performed after measuring fasting glucose according to the

Table 1. Physical and biochemical characteristics of the subjects

	AN	OB	ALL
Number	N = 27	N = 34	N = 61
M/F	13/14	10/24	23/38
Age, years old	27.80±9.59[a]	34.29±8.42	31.39±9.45
Weight, kg	100.53±21.29[a]	90.68±16.05	95.11±19.08
BMI, kg/m^2	35.90±6.40	33.26±4.23	34.45±5.43
WC, cm	114.62±15.11[a]	105.45±10.85	109.54±13.60
HC, cm	115.68±12.48[a]	109.65±8.58	112.34±10.83
WHR	0.99±0.05	0.96±0.07	0.97±0.06
Visceral fat fraction	16.22±7.33	16.31±6.15	16.27±6.60
TCH, mmol/l	4.95±1.47	4.67±1.42	4.80±1.43
TG, mmol/l	1.57±0.58	1.70±0.76	1.65±0.68
HDL, mg/l	1.02±0.18[a]	1.15±0.20	1.10±0.20
LDL, mg/l	3.18±1.04	2.88±0.90	3.01±0.97
FFA, mmol/l	0.56±0.20	0.57±0.19	0.56±0.20
UA, umol/l	472.09±112.69[b]	336.60±83.02	411.53±109.29
FBG, mmol/l	5.79±1.43	5.44±1.14	5.60±1.28
FINS, mmol/l	40.59±20.54[a]	24.47±9.93	31.38±17.23
HOMA-IR	10.07±6.46[a]	6.19±2.82	7.87±5.07
Trunk fat, g	17536.31±4132.37	18475.96±3872.90	18076.61±3960.84
Trunk fat, %	40.04±4.32	43.22±5.97	42.03±5.57
Head fat, g	1814.51±274.72	1863.76±390.05	1845.30±348.28
Total fat, g	351680.28±7160.14	36390.48±6767.335	35900.64±6868.49
Total lean, g	54483.39±11482.42	52309.38±9072.09	53157.78±9998.83
Total fat, %	37.99±3.40	39.33±4.03	38.79±3.81

AN, obese group with acanthosis nigricans; OB, simple obese group; M/F, male/female; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; FFA, free fatty acids; UA, uric Acid; HOMA-IR, homeostasis model assessment-insulin resistance; vs. OB, [a]$P < 0.05$, [b]$P < 0.01$. Continuous data were described as means ± standard deviation. *p*-values < 0.05 were considered significant.

methods described by World Health Organization (WHO) [7]. The HOMA-IR was calculated to estimate insulin resistance [8]. The following formula was used to calculate the HOMA-IR: HOMA-IR = fasting plasma glucose (mmol/L) × fasting plasma insulin (uU/mL)/22.5. Additionally, the fasting serum total GLP-1 (7-36 and 9-36) concentrations were measured with an enzyme-linked immunosorbent assay (ELISA) kit (Millipore Corporation, Billerica, MA, USA).

Body composition measurements

Body fat mass and fat distribution of the subjects with obesity were measured by dual-energy X-ray absorptiometry (DEXA) (Hologic QDR4500, USA) which is able to determine fat content and distribution with high accuracy. Data from DEXA included total fat%, total fat mass, total lean mass, upper limb fat, lower limb fat, trunk fat, trunk fat%, and head fat.

Statistical analysis

Statistical analysis was performed using SPSS software (version 17.0). All continuous values were presented as means ± standard deviation ($\bar{X}$ ±s) and the count data were expressed as the number of columns (n). Comparison of quantitative data was performed using a t-test. The correlation among different variables was analyzed using correlation analysis. Statistical differences were considered significant at when $P < 0.05$.

Results

Baseline characteristics of patients

When compared with the OB group, patients in the AN group had higher weight, WC, HC, UA, HOMA-IR, and lower HDL-C levels. All baseline characteristics and metabolic parameters were

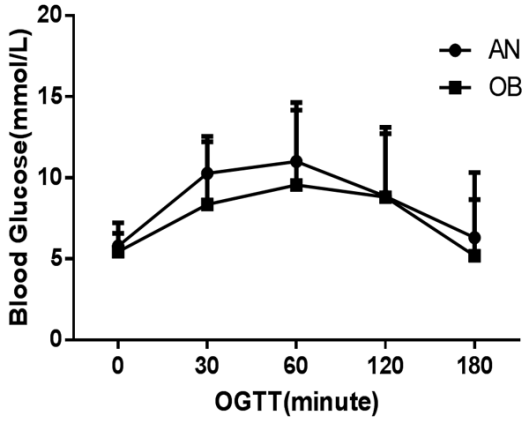

Figure 1. Comparison of blood glucose level. AN: Obese group with acanthosis nigricans. OB: Simple obese group.

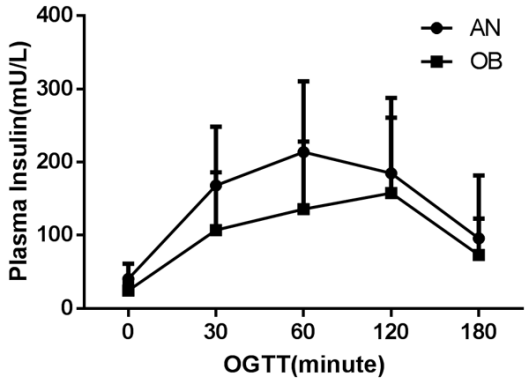

Figure 2. Comparison of insulin levels. AN: Obese group with acanthosis nigricans. OB: Simple obese group.

shown in **Table 1**. OGTT results were shown in **Figures 1** and **2**. Compared with the OB group, the AN group showed higher insulin levels at three points (0 min, 30 min, and 60 min) ($P < 0.05$) and with no significant difference at 120 min and 180 min ($P > 0.05$). Insulin resistance was the more serious in the AN group as demonstrated by HOMA-IR being higher in the AN group ($P < 0.05$) as shown in **Figure 3**.

Fasting GLP-1 concentrations

AN group had higher GLP-1 concentrations than the OB group ($P < 0.05$) as shown in **Figure 4**.

Correlation of GLP-1 levels with metabolic variables

We assessed the correlation of GLP-1 levels with metabolic variables to further analyze the

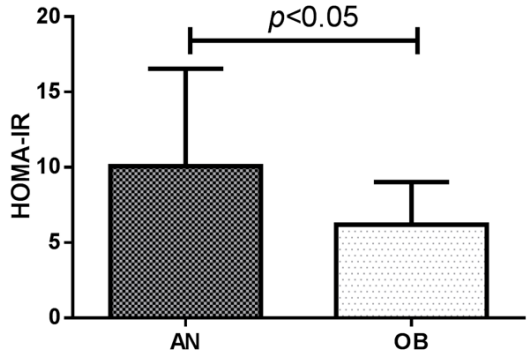

Figure 3. Comparison of insulin resistance. AN: obese group with acanthosis nigricans. OB: simple obese group.

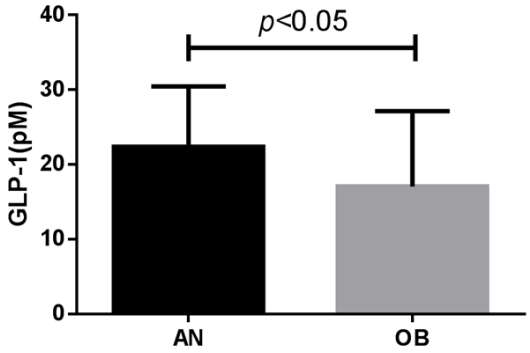

Figure 4. Comparison of GLP-1 concentrations. AN: obese group with acanthosis nigricans. OB: simple obese group.

relationship between serum GLP-1 levels with metabolic disorders as presented in **Table 2**. The results showed that the levels of GLP-1 were positively correlated with plasma insulin measured at 30 min after OGTT, as well as TC and LDL in all patients with obesity ($P < 0.05$). The level of GLP-1 was significantly positively associated with LDL in AN group and was significantly positively associated with TC in AN group ($P < 0.05$).

Discussion

AN affects localized areas of the skin in patients with obesity and/or hyperinsulinemia. The effects of topical and cosmetic treatments are limited [9]. The improvement in AN by oral retinoids (isotretinoin and acitretin) requires large doses and extended courses and recurrences have been described [10]. Treatment with metformin for six months reduces hyperinsulinemia, body weight, and fat mass and improves

Table 2. Correlation analysis of GLP-1 concentrations

Variables	AN		OB		All subjects	
	Correlation index	*P-value*	Correlation index	*P-value*	Correlation index	*P-value*
Weight	0.010	NS	0.341	NS	0.124	NS
BMI	-0.138	NS	0.173	NS	0.062	NS
WC	-0.023	NS	0.002	NS	0.019	NS
HC	-0.083	NS	0.172	NS	0.059	NS
Visceral fat fraction	0.333	NS	0.149	NS	0.101	NS
Blood glucose						
(OGTT: 0 min)	-0.180	NS	-0.051	NS	-0.087	NS
(OGTT: 30 min)	0.005	NS	-0.016	NS	-0.022	NS
(OGTT: 60 min)	-0.218	NS	-0.048	NS	-0.054	NS
(OGTT: 120 min)	-0.275	NS	-0.220	NS	-0.105	NS
(OGTT: 180 min)	-0.259	NS	-0.171	NS	-0.113	NS
Plasma insulin						
(OGTT: 0 min)	0.141	NS	0.275	NS	0.280	NS
(OGTT: 30 min)	0.261	NS	0.004	NS	0.375	0.020
(OGTT: 60 min)	0.205	NS	0.204	NS	0.309	NS
(OGTT: 120 min)	0.028	NS	0.102	NS	0.153	NS
(OGTT: 180 min)	-0.037	NS	0.106	NS	0.286	NS
HOMA-IR	0.126	NS	0.180	NS	0.212	NS
TC	0.251	NS	0.385	0.048	0.300	0.043
TG	0.221	NS	0.053	NS	0.032	NS
HDL	0.272	NS	0.376	NS	0.267	NS
LDL	0.487	0.022	0.174	NS	0.304	0.043
FFA	-0.270	NS	0.028	NS	-0.269	NS
UA	0.001	NS	0.205	NS	0.131	NS
Trunk fat	0.267	NS	0.146	NS	0.243	NS
Trunk fat%	-0.177	NS	0.065	NS	0.172	NS
Head fat	-0.285	NS	0.072	NS	-0.120	NS
Total fat	0.163	NS	0.114	NS	0.120	NS
Total lean	0.188	NS	0.200	NS	0.060	NS
Total fat%	-0.191	NS	0.031	NS	0.113	NS

AN, obese group with acanthosis nigricans; OB, simple obese group; BMI, body mass index; WC, waist circumference; HC, hip circumference; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment-insulin resistance; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; FFA, free fatty acid; UA, uric acid; NA, not assessed; NS, non-significant. *p*-values < 0.05 were considered significant.

insulin sensitivity in very obese subjects with insulin resistance and AN [11]. However, a previous study compared the efficacy of 12-weeks of treatment with metformin versus rosiglitazone for AN lesions of the neck with results showing no effect on the severity of AN; only modest improvements of skin texture occurred in both groups [3]. The underlying mechanism of AN is not sufficiently understood.

GLP-1 is an incretin hormone secreted from L cells in the gastrointestinal tract and has beneficial effects on glucose-dependent insulin responses, lowering glucagon secretion and reducing appetite [12-15]. GLP-1 has a role in the development of obesity partly due to its effects on appetite and food intake, and there is a reduced secretion of GLP-1 in obesity [16]. Impaired GLP-1 responses after food intake is associated with insulin resistance in non-diabetic men, and the area under the curve (AUC) of GLP-1 during the first hour was correlated significantly with insulin sensitivity ($r = 0.47$, $P < 0.01$) [17]. Additionally, insulin resistance but not obesity is an independent predictor of the decreased GLP-1 response [17]. The postpran-

dial GLP-1 response was lower in nine obese subjects (BMI 42±4 kg/m^2) compared to normal-weight controls [18]. Previous studies have also suggested that obese individuals have lower GLP-1 secretion in response to food intake compared with lean individuals [19, 20]. However, the secretion of GLP-1 did not increase after weight loss [19]. Another study showed that GLP-1 responses were the same in obese and lean healthy subjects [21]. Another study found no significant difference in fasting GLP-1 concentrations between overweight/obese and normal weight individuals which suggested that the decreased GLP-1 secretion in obesity is not a consistent finding [22]. Obesity is caused by a chronic energy surplus which leads to the excessive accumulation of adipose tissue. A 3-day overfeeding study enrolled 21 subjects and showed GLP-1 was unchanged [23]. A study in 26 healthy Danish young men who were overfed a high fat diet for five days also found no significant change in fasting circulating GLP-1 [24]. A small study also reported no significant difference in GLP-1 concentrations after overfeeding which increased body weight by 5% [25]. However, one study observed a significant increase in serum GLP-1 after 7 days of an overfeeding diet in seventy-two young healthy men which suggested GLP-1 secretion could increase in response to a positive energy challenge and serve a protective role which counteracts energy surplus [22]. Thus, GLP-1 is involved in energy homeostasis in addition to facilitating the glucose-dependent insulin response. In the current study, we compared the fasting serum GLP-1 concentrations in Chinese obesity associated AN patients with simply obesity. The most important finding was that GLP-1 concentrations were significantly higher in the AN group than the OB groups. As the patients in AN group had higher weight, WC, and HOMA-IR when compared to OB group, we assumed higher GLP-1 concentrations in the AN group were a homeostatic protective mechanism to improve the energy surplus in obesity-associated AN patients with higher body weights and insulin resistance. That means higher GLP-1 levels may be a compensate outcome of insulin resistance which was closely related to AN.

Obesity associated AN may result in more severe lipid metabolic dysfunction, inflammation, and insulin resistance. This study also found that when compared with the OB group, the patients in the AN group had higher weight, WC, HC, UA, and HOMA-IR but lower HDL-C levels. To explore the blood glucose and insulin levels in AN patients, we performed 75-g oral glucose tolerance tests. Individuals with AN had higher fasting insulin levels and HOMA-IR which indicated more insulin resistance when compared to the OB groups. Increased GLP-1 concentrations in the AN group could explain the higher insulin concentrations and the increased prevalence of hyperinsulinemia-associated disorders in obesity with AN. Thus, taken together, we concluded that obesity-associated AN subjects with higher circulating serum GLP-1 had a less favorable lipid profile (including lower HDL levels), inflammation state (higher UA), and more insulin resistance (increased HOMA-IR). We also suggest that the increase in GLP-1 was a compensatory response for the increase in serum lipids and insulin. Therefore, administration of GLP-1 receptor agonists can lead to a beneficial change in lipid metabolism and insulin resistance [26, 27].

Previous studies have found that GLP-1 concentrations are correlated with the percent of gynoid fat in overweight/obese individuals [22]. In this study, GLP-1 concentrations had no significant correlation with total fat mass, total fat percent, fat distribution such as trunk fat, trunk fat percent, or head fat in the OB plus AN group. Luis et al [28] found that fasting GLP-1 was negatively associated with HDL-C. Additionally, a study found that individuals with metabolic syndrome with high GLP-1 levels are at greater risk for cardiovascular disease as there is a significant interaction between circulating GLP-1 and HDL-C, TG, and serum UA [29]. A previous study has shown that fasting serum GLP-1 concentrations are negatively correlated with HDL-C and positively correlated with TG and markers of insulin resistance in the overweight/obese group [22]. Circulating fatty acids could be responsible for the GLP-1 response [30]. However, free fatty acids (FFA) levels did not influence meal-induced GLP-1 secretion in both diabetic and non-diabetic subjects [31]. In this study, we confirmed the relationship between GLP-1 and markers of glucose-lipid metabolism and insulin resistance. The level of GLP-1 was significantly positively associated with LDL in AN group and was significantly positively associated with TC in AN group. GLP-1 was positively correlated with TC and LDL-C in AN plus the OB

group. More studies are warranted to elucidate the role of GLP-1 in glucose-lipid metabolism.

There are some limitations in this study. Firstly, we only investigated the GLP-1 concentrations in Chinese subjects who were 18-53 years old. Therefore, more studies are needed in different age ranges and ethnic groups. Secondly, the number of patients in this study was relatively small. Thus, further large-scale studies assessing the GLP-1 concentrations in different types of obesity are warranted. Additionally, only total fasting serum GLP-1 was measured in this study. Active GLP-1 has a very short half-life and is quickly degraded by DPP-4 and therefore vary from total GLP-1 levels. Thus, dipeptidil peptidase activity and levels should have been tested. Also GLP-1 concentrations are varied in response to different kinds of macronutrient composition and food intake. Thus, active GLP-1 levels and postprandial GLP-1 concentrations will need to be measured in future studies. At last, as the sample of AN patients without obesity is relatively limited at present. We did not determine the GLP-1 levels on them. Thus, the GLP-1 levels on patients who have AN without obesity should be tested in the further studies.

Conclusion

Our study investigated the fasting total serum GLP-1 concentrations in Chinese patients with simply obesity and the obesity associated AN, and the results showed that the GLP-1 concentrations were higher in the AN group. We concluded that GLP-1 can potentially act as a protective mechanism to counteract dysfunctional lipid metabolism and insulin resistance in obesity associated AN.

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Disclosure of conflict of interest

None.

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References

[1] Burke JP, Hale DE, Hazuda HP, Stern MP. A quantitative scale of acanthosis nigricans. Diabetes care 1999; 22: 1655-9.

[2] Jeong KH, Oh SJ, Chon S, Lee MH. Generalized acanthosis nigricans related to type B insulin resistance syndrome: a case report. Cutis 2010; 86: 299-302.

[3] Bellot-Rojas P, Posadas-Sanchez R, Caracas-Portilla N, Zamora-Gonzalez J, Cardoso-Saldana G, Jurado-Santacruz F, Posadas-Romero C. Comparison of metformin versus rosiglitazone in patients with Acanthosis nigricans: a pilot study. J Drugs Dermatol 2006; 5: 884-9.

[4] Holst JJ. The physiology of glucagon-like peptide 1. Physiological reviews 2007; 87: 1409-39.

[5] Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007; 132: 2131-57.

[6] Bernsmeier C, Meyer-Gerspach AC, Blaser LS, Jeker L, Steinert RE, Heim MH, Beglinger C. Glucose-induced glucagon-like Peptide 1 secretion is deficient in patients with non-alcoholic fatty liver disease. PLoS One 2014; 9: e87488.

[7] Abdul-Ghani MA, Tripathy D, DeFronzo RA. Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes care 2006; 29: 1130-9.

[8] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes care 2004; 27: 1487-95.

[9] Phiske MM. An approach to acanthosis nigricans. Indian Dermatol Online J 2014; 5: 239-49.

[10] Hermanns-Le T, Scheen A, Pierard GE. Acanthosis nigricans associated with insulin resistance: pathophysiology and management. Am J Clin Dermatol 2004; 5: 199-203.

[11] Tankova T, Koev D, Dakovska L, Kirilov G. Therapeutic approach in insulin resistance with acanthosis nigricans. Int J Clin Pract 2002; 56: 578-81.

[12] Meier JJ, Deacon CF, Schmidt WE, Holst JJ, Nauck MA. Suppression of glucagon secretion is lower after oral glucose administration than during intravenous glucose administration in human subjects. Diabetologia 2007; 50: 806-13.

[13] Kazakos KA, Sarafidis PA, Yovos JG. The impact of diabetic autonomic neuropathy on the incretin effect. Med Sci Monit 2008; 14: Cr213-20.

[14] de Heer J, Rasmussen C, Coy DH, Holst JJ. Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas. Diabetologia 2008; 51: 2263-70.
[15] Larsen PJ, Vrang N, Tang-Christensen M. Central pre-proglucagon derived peptides: opportunities for treatment of obesity. Curr Pharm Des 2003; 9: 1373-82.
[16] Verdich C, Flint A, Gutzwiller JP, Naslund E, Beglinger C, Hellstrom PM, Long SJ, Morgan LM, Holst JJ, Astrup A. A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. J Clin Endocrinol Metab 2001; 86: 4382-9.
[17] Rask E, Olsson T, Soderberg S, Johnson O, Seckl J, Holst JJ, Ahren B. Impaired incretin response after a mixed meal is associated with insulin resistance in nondiabetic men. Diabetes care 2001; 24: 1640-5.
[18] Naslund E, Gryback P, Backman L, Jacobsson H, Holst JJ, Theodorsson E, Hellstrom PM. Distal small bowel hormones: correlation with fasting antroduodenal motility and gastric emptying. Dig Dis Sci 1998; 43: 945-52.
[19] Verdich C, Toubro S, Buemann B, Lysgard Madsen J, Juul Holst J, Astrup A. The role of postprandial releases of insulin and incretin hormones in meal-induced satiety--effect of obesity and weight reduction. Int J Obes Relat Metab Disord 2001; 25: 1206-14.
[20] Mannucci E, Ognibene A, Cremasco F, Bardini G, Mencucci A, Pierazzuoli E, Ciani S, Fanelli A, Messeri G, Rotella CM. Glucagon-like peptide (GLP)-1 and leptin concentrations in obese patients with Type 2 diabetes mellitus. Diabet Med 2000; 17: 713-9.
[21] Vilsboll T, Krarup T, Sonne J, Madsbad S, Volund A, Juul AG, Holst JJ. Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. J Clin Endocrinol Metab 2003; 88: 2706-13.
[22] Wadden D, Cahill F, Amini P, Randell E, Vasdev S, Yi Y, Church J, Sun G. Circulating glucagon-like peptide-1 increases in response to short-term overfeeding in men. Nutr Metab (Lond) 2013; 10: 33.
[23] Shea J, French CR, Bishop J, Martin G, Roebothan B, Pace D, Fitzpatrick D, Sun G. Changes in the transcriptome of abdominal subcutaneous adipose tissue in response to short-term overfeeding in lean and obese men. Am J Clin Nutr 2009; 89: 407-15.
[24] Brons C, Jensen CB, Storgaard H, Hiscock NJ, White A, Appel JS, Jacobsen S, Nilsson E, Larsen CM, Astrup A, Quistorff B, Vaag A. Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. J Physiol 2009; 587: 2387-97.
[25] Brands M, Swat M, Lammers NM, Sauerwein HP, Endert E, Ackermans MT, Verhoeven AJ, Serlie MJ. Effects of a hypercaloric diet on beta-cell responsivity in lean healthy men. Clin Endocrinol (Oxf) 2013; 78: 217-25.
[26] Bergenstal RM, Li Y, Porter TK, Weaver C, Han J. Exenatide once weekly improved glycaemic control, cardiometabolic risk factors and a composite index of an HbA1c < 7%, without weight gain or hypoglycaemia, over 52 weeks. Diabetes Obes Metab 2013; 15: 264-71.
[27] Giorgino F, Leonardini A, Natalicchio A, Laviola L. Multifactorial intervention in Type 2 diabetes: the promise of incretin-based therapies. J Endocrinol Invest 2011; 34: 69-77.
[28] de Luis D, Pacheco D, Conde R, Primo D, Aller R, Izaola O. Basal GLP-1 levels in morbidly obese patients following biliopancreatic diversion surgery. Ann Nutr Metab 2012; 61: 70-3.
[29] Yamaoka-Tojo M, Tojo T, Takahira N, Matsunaga A, Aoyama N, Masuda T, Izumi T. Elevated circulating levels of an incretin hormone, glucagon-like peptide-1, are associated with metabolic components in high-risk patients with cardiovascular disease. Cardiovasc Diabetol 2010; 9: 17.
[30] Ranganath L, Norris F, Morgan L, Wright J, Marks V. Inhibition of carbohydrate-mediated glucagon-like peptide-1 (7-36) amide secretion by circulating non-esterified fatty acids. Clin Sci (Lond) 1999; 96: 335-42.
[31] Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. J Clin Endocrinol Metab 2001; 86: 3717-23.