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Author: Sleddering, Maria A.
Title: Insulin resistance : pathophysiology in South Asians & therapeutic strategies
Issue Date: 2014-02-04
CHAPTER 3

Higher insulin and glucagon-like peptide-1 (GLP-1) levels in healthy, young South Asians as compared to Caucasians during an oral glucose tolerance test

Maria A. Sleddering
Leontine E.H. Bakker
Laura G.M. Janssen
A. Edo Meinders
Ingrid M. Jazet

Metabolism 2013, in press
ABSTRACT

Objective
Higher insulin levels during an oral glucose test (OGTT) have been shown in South Asians. We aimed to investigate if this increased insulin response causes reactive hypoglycemia later on, and if an increased glucagon-like-peptide-1 (GLP-1) response, which could contribute to the hyperinsulinemia, is present in this ethnic group.

Methods
A prolonged, 6-hour, 75-g OGTT was performed in healthy, young Caucasian (n = 10) and South Asian (n = 8) men. The glucose, insulin and GLP-1 response was measured and indices of insulin sensitivity and beta-cell activity were calculated.

Results
Age (Caucasians (CAU) 21.5 ± 0.7 years vs South Asians (SA) 21.4 ± 0.7 years (mean ± SEM) and body mass index (CAU 22.7 ± 0.7 kg/m² vs SA 22.1 ± 0.8 kg/m²) were comparable between the two groups. South Asian men were more insulin resistant, as indicated by a comparable glucose but significantly higher insulin response, and a significantly lower Matsuda index (CAU 8.7(8.6) vs SA 3.2(19.2), median (IQR)). Also, South Asians showed a higher GLP-1 response, as reflected by a higher area under the curve for GLP-1 (CAU 851 ± 99.8 mmol/l vs SA 1235 ± 155.0 mmol/L). During the whole 6-hour period, no reactive hypoglycemia was observed.

Conclusion
Healthy, young South Asian men have higher insulin levels during an OGTT as compared to Caucasians. This does not, however, lead to reactive hypoglycemia. The hyperinsulinemia is accompanied by increased levels of GLP-1. Whether this is an adaptive response to facilitate hyperinsulinemia to overcome insulin resistance or reflects a GLP-1 resistant state has yet to be elucidated.
INTRODUCTION

Among both native and migrant South Asians the risk of developing type 2 diabetes (T2DM) is exceptionally high. Furthermore, T2DM occurs at a younger age and lower body mass index (BMI) as compared to Caucasians. Also, long-term complications start earlier and run a more serious course. The predominant mechanism involved in the pathogenesis of T2DM in South Asians seems to be a decrease in insulin sensitivity.

It has repeatedly been shown that South Asians, as compared to Caucasians, exhibit higher 2-hour insulin levels or a higher area under the curve (AUC) for insulin, with a normal glucose response, during an oral glucose or meal tolerance test (OGTT). These higher insulin levels are considered a compensatory mechanism to overcome insulin resistance and maintain glucose tolerance. The hyperinsulinemia might be caused by a decreased insulin clearance, but an increased β-cell response has been reported as well.

Glucagon-like peptide-1 (GLP-1), an incretin secreted from the enteroendocrine L-cells in the gut in response to eating, is known to stimulate insulin secretion from pancreatic β-cells. An increased GLP-1 response could therefore contribute to the glucose-stimulated hyperinsulinemia consequently seen in South Asians. However, whether GLP-1 levels are indeed higher in this ethnic group is currently unknown. Furthermore, not only the underlying mechanism, but also the consequences of the hyperinsulinemia in people of South Asian descent are not yet fully elucidated. It is, for instance, unknown whether the increased insulin response in people of South Asian descent causes reactive hypoglycemia, a condition characterized by a drop in glucose levels 4-6 hours after a glucose load, which is considered a sign of early latent diabetes. In the present study we therefore studied the glucose and insulin response during a prolonged 6-hour OGTT in healthy, young South Asian and Caucasian men. Furthermore, GLP-1 levels were assessed to investigate whether an increased GLP-1 response is present in South Asians.

SUBJECTS AND METHODS

Subjects

Eighteen healthy, young men were included in the study (10 Caucasians, 8 South Asians). Male subjects aged 18-25 years, with a body mass index (BMI) between 18.5 and 25 kg/m², and a positive family history of T2DM were eligible for enrollment. The South Asian subjects were all Hindustani Surinamese. In the Netherlands, almost all South Asians are Hindustani Surinamese, an ethnic group that has migrated from Surinam, a former Dutch colony in South America, and whose ancestors came from the Indian subcontinent. Seven of the South Asians subjects were born in the Netherlands. One was
born in Surinam and migrated to the Netherlands at the age of eight. Exclusion criteria were type 2 diabetes or any other chronic disease, smoking, use of medication known to influence glucose metabolism, and recent weight change. Subjects were recruited via advertisements placed online, in local media, and in public places. This study was approved by the Medical Ethical Committee of the Leiden University Medical Center and performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects before participation.

Oral glucose tolerance test

Following an initial screening visit, each subject was studied once. Subjects arrived at the research center at 8.00 AM after an overnight fast. Anthropometric measurements were obtained and fat mass was assessed by bioelectrical impedance analysis (Bodystatâ 1500, Bodystat Ltd., Douglas, Isle of Man, UK). After insertion of an intravenous catheter, two baseline blood samples were drawn (t = -15 and t = 0). Thereafter, subjects underwent a prolonged 75-g OGTT, with measurements of glucose and insulin at t = 15, 30, 60, 90, 120, 150, 180, 210, 240, 300, and 360 minutes. Samples for the measurement of GLP-1 were drawn at baseline and at t = 15, 30, 60, 90, 120, 150, and 180 minutes. Dipeptidyl peptidase IV (DPP-IV) inhibitor (10 µl/ml blood; Merck Millipore, Billerica, MA, USA) was added to these samples immediately. Blood samples were cooled on ice and centrifuged at 4 °C. Hereafter samples were distributed into aliquots and stored at −80 °C until analysis.

Assays

Serum glucose, total cholesterol, HDL-cholesterol and triglycerides were measured on a Modular P800 analyzer (Roche, Almere, The Netherlands). LDL-cholesterol was calculated according to Friedewald’s formula. Serum insulin levels were analyzed on an Immulite 2500 (Siemens, The Netherlands). Active GLP-1 was measured using a standardized ELISA kit (Meso Scale Diagnostics, Gaithersburg, MD, USA).

Statistical analysis and calculations

Results are expressed as mean ± standard error (SEM) or median and interquartile range (IQR) in case of non-normally distributed data. Baseline values for glucose, insulin and GLP-1 were calculated as the average of the two baseline measurements (t = -15 and t = 0). Reactive hypoglycemia was defined as a glucose level of 3 mmol/L or less between 3 and 6 hours after the oral glucose load. For type 2 diabetes patients on glucose lowering therapy usually a cut-off value for hypoglycemia of < 3.9 mmol is used. We chose a lower cut-off, because it was suggested by Marks et al. (Marks et al., Hypoglycemia, 1987) that 3.0 mmol/L is an appropriate cut-off point for evaluating hypoglycemia in healthy (non-diabetic) volunteers, since 95% of blood glucose levels in healthy volunteers are above this level. AUC values were determined using the trapezoidal rule. Incremental values
are calculated by deducting the area below the baseline value from total AUCs. Insulin sensitivity was estimated using the Matsuda index (glucose: mg/dl; insulin: mU/L).\textsuperscript{17} Recently it was shown that the Matsuda index correlates highly with insulin sensitivity measured with a hyperinsulinemic clamp in South Asians and Caucasians.\textsuperscript{18} The insulinogetic index (IGI; $\Delta I_{0-30}/\Delta G_{0-30}$) was used as a measurement of early insulin secretion (glucose: mmol/L; insulin: pmol/L).\textsuperscript{19} The oral disposition index (DI$O_{90}$; $(\Delta I_{0-30}/\Delta G_{0-30})$/fasting insulin) (glucose: mmol/L; insulin: mU/L) was used to provide an estimate of $\beta$-cell function relative to the prevailing level of insulin resistance.\textsuperscript{20,21}

The independent Student’s t-test was used for comparisons between the groups. A non-parametric test (Mann-Whitney U test) was applied when appropriate. A p-value of < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS for Windows (release 20.0, IBM, USA).

RESULTS

Anthropometric and laboratory measurements

Data on anthropometric and laboratory measurements are shown in Table 1. South Asians (SA) were significantly smaller and lighter compared to the Caucasian (CAU) subjects. BMI, however, was comparable between the groups (CAU: 22.7 ± 0.7 vs. SA: 22.1 ± 0.8 kg/m$^2$). There were no significant differences in (percent of) fat mass, waist circumference, or fasting levels of glucose, insulin and lipids.

Table 1. Anthropometric and laboratory parameters in young, healthy Caucasian and South Asian men.

<table>
<thead>
<tr>
<th></th>
<th>Caucasian (n = 10)</th>
<th>South Asian (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.5 ± 0.7</td>
<td>21.4 ± 0.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>1.82 ± 0.01</td>
<td>1.72 ± 0.02 *</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.0 ± 2.7</td>
<td>65.7 ± 2.8 *</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>22.7 ± 0.7</td>
<td>22.1 ± 0.8</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>81 ± 2.2</td>
<td>78 ± 2.1</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>14.9 ± 0.9</td>
<td>15.2 ± 1.5</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.9 ± 0.2</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>Fasting insulin (mu/l)</td>
<td>5.3 ± 1.5</td>
<td>9.5 ± 1.5</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.2 ± 0.4</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>3.68 ± 0.26</td>
<td>3.90 ± 0.19</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>2.10 ± 0.24</td>
<td>2.22 ± 0.12</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.03 ± 0.13</td>
<td>0.92 ± 0.11</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.10 ± 0.06</td>
<td>1.3 ± 0.06</td>
</tr>
</tbody>
</table>

Mean ± SEM. * p < 0.05 BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; HOMA-IR: homeostasis model of assessment - insulin resistance.
**Prolonged oral glucose tolerance test**

Time courses for glucose and insulin during the prolonged 75-g OGTT are shown in Figure 1. Insulin levels were significantly higher in the South Asian group at several time points. During the whole 6-hour period there were no differences in glucose levels between the groups and reactive hypoglycemia did not occur. The AUCs for glucose and insulin are depicted in Figure 2. The AUC\(_{360}\) for insulin was significantly higher in the South Asian group (CAU: 6596 ± 899 vs. SA: 16725 ± 4197; \(p < 0.05\)). South Asians were less insulin sensitive as reflected by a lower Matsuda index (Table 2). A compensatory increase in insulin secretion was observed in this group as shown by an increased IGI, although this was only borderline significant (\(p = 0.051\)). \(\beta\)-cell function in relation to the level of insulin sensitivity, as assessed by the oral disposition index, did not differ between the two groups.

**Figure 1.** Time courses for plasma concentrations of glucose, insulin and glucagon-like peptide (GLP)-1 during an oral glucose tolerance test (OGTT) in healthy, young Caucasian and South Asian men. Data are mean ± SEM; * \(p < 0.05\).
GLP-1
The time course and AUC for GLP-1 during the OGTT are shown in Figure 1 and 2. The AUC$_{180}$ for GLP-1 was higher in South Asian subjects compared to Caucasian subjects (CAU: 851 ± 100 vs. SA: 1235 ± 155; $p < 0.05$). The incremental AUC$_{180}$ was higher in South Asians as well, although this did not reach statistical difference (CAU: 619 ± 94 vs. SA: 851 ± 142; $p = 0.18$). In univariate analysis, fat percentage or waist circumference did not significantly predict the GLP-1 AUC$_{180}$ ($p = 0.852$ and $p =0.102$). However when included in the model, they do alter the significance level of the between group difference in GLP-1 AUC$_{180}$ ($p = 0.055$ and $p =0.103$ instead of $p = 0.046$).

GLP-1 and insulin levels were highly correlated at several time points, especially GLP-1 at $t = 15$ minutes (with insulin at $t = 15$, 30, 90, 120, 150, 210, 240, 300 minutes; $p < 0.05$). The GLP-1 AUC$_{180}$ showed a significant correlation with the insulin AUC$_{120}$ and the

![Figure 2. Area under the curve (AUC) for glucose, insulin and glucagon-like peptide (GLP)-1 during an oral glucose tolerance test (OGTT) in healthy, young Caucasian and South Asian men. Data are mean ± SEM; * $p < 0.05$.](image-url)
insulin AUC$_{360}$ (0.607, p = 0.008 and 0.599, p = 0.009) but not with the AUCs for glucose. Due to the small sample size, correlations could not be calculated for South Asians and Caucasians separately.

Table 2. Glucose, insulin and GLP-1 indices during an oral glucose tolerance test in young, healthy Caucasian and South Asian men.

<table>
<thead>
<tr>
<th></th>
<th>Caucasian</th>
<th>South Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak glucose (mmol/l)</td>
<td>8.8 ± 0.30</td>
<td>9.1 ± 0.33</td>
</tr>
<tr>
<td>Peak glucose time (minutes)</td>
<td>30.0 (30.0)</td>
<td>30.0 (22.5)</td>
</tr>
<tr>
<td>Peak insulin (mU/L)</td>
<td>59.3 ± 8.7</td>
<td>154.9 ± 39.0</td>
</tr>
<tr>
<td>Peak insulin time (minutes)</td>
<td>60.0 (30.0)</td>
<td>60.0 (0.0)</td>
</tr>
<tr>
<td>Peak GLP-1 (mmol/l)</td>
<td>9.4 ± 0.9</td>
<td>22.1 ± 6.2</td>
</tr>
<tr>
<td>Peak GLP-1 time (minutes)</td>
<td>30.0 (30.0)</td>
<td>30.0 (15.0)</td>
</tr>
<tr>
<td>AUC$_{120}$ glucose (mmol/l)</td>
<td>818 ± 34.2</td>
<td>837 ± 31.7</td>
</tr>
<tr>
<td>AUC$_{120}$ insulin (mU/L)</td>
<td>4716 ± 581.2</td>
<td>11957 ± 3033.5</td>
</tr>
<tr>
<td>AUC$_{180}$ glucose (mmol/l)</td>
<td>1924 ± 47.4</td>
<td>1936 ± 47.8</td>
</tr>
<tr>
<td>AUC$_{180}$ insulin (mU/L)</td>
<td>6596 ± 899.3</td>
<td>16725 ± 4196.5</td>
</tr>
<tr>
<td>AUC$_{180}$ GLP-1 (mmol/l)</td>
<td>851 ± 99.8</td>
<td>1235 ± 155.0</td>
</tr>
<tr>
<td>AUC$_{180}$ incremental GLP-1 (mmol/l)</td>
<td>619 ± 94.4</td>
<td>851 ± 141.8</td>
</tr>
<tr>
<td>AUC$<em>{120}$ glucose/AUC$</em>{120}$ insulin (mmol/l*mU/L$^{-1}$)</td>
<td>0.21 ± 0.33</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>Matsuda index</td>
<td>8.7 (8.6)</td>
<td>3.2 (19.2)</td>
</tr>
<tr>
<td>IGI$_{30}$ (pmol/mmol)</td>
<td>83.4 (40.7)</td>
<td>174.7 (188.7)</td>
</tr>
<tr>
<td>DI$_{0}$</td>
<td>3.1 (3.5)</td>
<td>2.4 (1.4)</td>
</tr>
</tbody>
</table>

Mean ± SEM or median (interquartile range). * p < 0.05. GLP-1: glucagon-like peptide-1; AUC: area under the curve; IGI: insulinogenic index, DI$_{0}$: oral disposition index.

**DISCUSSION**

In this study we investigated the glucose, insulin and GLP-1 response during a prolonged OGTT in young, healthy South Asian men. We confirmed that young, healthy South Asian men are more insulin resistant, as reflected by higher insulin levels during an OGTT, than their Caucasian counterparts. A novel finding is that these higher insulin levels are accompanied by increased levels of GLP-1. Also we demonstrated that no reactive hypoglycemia occurred in the South Asians.

Whether the hyperinsulinemia in South Asians is caused by reduced clearance or increased secretion of insulin is unclear. A decreased insulin clearance has been found in South Asians during a euglycemic hyperinsulinemic clamp by our group (unpublished data) and by others. On the other hand, Petersen et al. showed an increase in β-cell response (estimated using the oral C-peptide minimal model) in South Asians as compared to Caucasians during a 2-hour, 75-g OGTT. This increased β-cell response was,
however, inadequate for their degree of insulin resistance as reflected by a lower disposition index. It is well known that to maintain glucose tolerance with declining insulin sensitivity, a proportionate increase in insulin output has to occur as a compensatory mechanism. In our study, the South Asian men were more insulin resistant as shown by a comparable glucose but significantly higher insulin response and a decreased Matsuda index, and indeed showed an increased β-cell response as reflected by a higher IGI. The oral disposition index did not differ between groups, suggesting that the increased insulin output was adequate for the level of insulin resistance.

In this study, we further explored a possible consequence of the glucose-stimulated hyperinsulinemia in South Asian men: reactive hypoglycemia. Despite the higher insulin levels, no reactive hypoglycemia was seen in this group. Reactive hypoglycemia 4-6 hours after a glucose load has been observed in obese subjects and is considered an early sign of diabetes. Reactive hypoglycemia has also been found in young, lean women with polycystic ovary syndrome (PCOS), a condition known to be associated with insulin resistance and increased risk of diabetes development. The fact that hypoglycemia did not occur in our study might be due to a high level of insulin resistance on a cellular level in the South Asian subjects, or by the induction of insulin resistance and increased hepatic glucose output by counter regulatory hormones, such as glucagon, catecholamines and cortisol, which were not measured in our study. Furthermore, because of the small sample size, it is possible that a difference was missed.

A novel finding is that the South Asians displayed a higher GLP-1 response to an oral glucose load, as reflected by an increased AUC for GLP-1. To our knowledge, this is the first study investigating the GLP-1 response in subjects of South Asian descent. GLP-1 is known to have several beneficial effects on glucose regulation. It stimulates endogenous insulin secretion in response to oral glucose or eating, suppresses glucagon secretion resulting in a decreased hepatic glucose output, and is thought to exert extra-pancreatic effects, since it improves glucose disposal and decreases endogenous glucose production independent of its release of islet hormones. Therefore, we hypothesize that an increased GLP-1 response could possibly explain -or at least contribute- to the higher insulin levels in South Asians, which may initially help overcome the insulin resistance in this ethnic group.

The precise role of GLP-1 in the pathogenesis of T2DM is currently unknown. It is well known that the incretin effect (i.e. the augmented insulin secretion in response to oral compared with intravenous isoglycemic administration of glucose) is diminished in T2DM patients. It has been debated if this impaired incretin effect is caused by impaired GLP-1 and glucose-dependent insulino tropic polypeptide (GIP) secretion or by a defective insulin secretory effect of these hormones (‘incretin resistance’). Several studies showed a lower postprandial GLP-1 release in subjects with T2DM and insulin resistance, but a recent meta-analysis found that patients with T2DM on the whole do
not exhibit reduced GLP-1 secretion in response to oral glucose or meal tests, and that T2DM patients may even have higher GLP-1 peak levels. Our data suggest that a state of insulin resistance is associated with a higher GLP-1 response. Possibly, GLP-1 secretion changes during the progression from normal glucose tolerance to T2DM, which was also suggested by the authors of the aforementioned meta-analysis. Early stages of T2DM may lead to compensatory increased GLP-1 secretion from intestinal L-cells, which is then followed by the exhaustion of these cells when the disease progresses. Indeed, a study by Theodorakis et al. in newly diagnosed T2DM patients showed an increase in late-phase (20-80 min) GLP-1 secretion after a 75-g OGTT, in parallel with rising plasma insulin levels. Furthermore, they found increased numbers of L-cells in the duodenum in this group. However, in a study of Knop et al. insulin resistant -but normal glucose tolerant- obese subjects did not show an increased GLP-1 response. In this study, however, an oral glucose load of 50-g was used, instead of the 75-g OGTT in our study. Furthermore, these subjects already displayed signs of β-cell dysfunction as shown by a decreased disposition index, indicating a more progressed state of insulin resistance. In another study, insulin resistance induced in healthy, young men (using a 12 day intervention with prednisolone treatment, high-energy diet, and relative physical inactivity) led to higher fasting GLP-1 levels, but no difference in the GLP-1 response to an oral glucose load was found when comparing the baseline and insulin resistant state. This might be due to the fact that the subjects in this study not only were insulin resistant, but also less glucose tolerant. Four of the 10 subjects even displayed impaired glucose tolerance or diabetes after the intervention, whereas all our subjects were normal glucose tolerant. Hence, glucotoxicity might have attenuated the GLP-1 response.

The increased GLP-1 response found in the South Asian subjects in our study on the other hand might also indicate a state of GLP-1 resistance. Although debated, evidence suggests that GLP-1 resistance is present in T2DM patients and their healthy offspring. Furthermore, in the aforementioned study in which insulin resistance was induced in healthy subjects, although no alterations in the GLP-1 response were seen, increased fasting GLP-1 levels and a reduction in the incretin effect were shown. In addition, the insulinotropic effect of GLP-1 was impaired, suggesting that incretin resistance was present and is a consequence of insulin resistance. We also showed higher fasting GLP-1 levels in the South Asian group. However, since the incretin effect and the direct insulinotropic action of GLP-1 were not assessed in our study, it remains to be elucidated whether the higher fasting GLP-1 levels and higher GLP-1 response in South Asians are due to a compensatory increased secretion or reflecting a GLP-1 resistant state. However, the peak GLP-1 levels preceded the peak insulin response and paralleled the increased β-cell activity (IGI), suggesting a direct relation between the increased GLP-1 response and the insulin secretion by the β-cell.
A limitation of our study is the small sample size. However, even with only 18 subjects, a difference in GLP-1 response was found. Further research is required to see whether these findings can be reproduced in larger samples. In univariate analysis, fat percentage and waist circumference did influence the significance level of the between group difference in GLP-1 AUC\textsubscript{180}. It can therefore not be excluded that differences in body composition, although not significantly different between the two groups, has influenced our findings on GLP-1 levels. In addition, we do not have data on nutritional intake and exercise. It is possible that differences in intake and physical activity influenced insulin resistance and GLP-1 secretion. However, it is unlikely that differences in behavior solely explain the increased insulin sensitivity found in diverse groups of South Asians.\textsuperscript{6} Furthermore, in a previous study of our group in a similar study population no differences were found in diet and exercise.\textsuperscript{33} Hence, it seems unlikely that these factors have influenced our findings.

In conclusion, we confirmed that young, healthy South Asian men are more insulin resistant and have higher insulin levels during an OGTT than their Caucasian counterparts. The higher insulin levels were accompanied by increased levels of GLP-1. No reactive hypoglycemia was observed in the South Asians despite the hyperinsulinemia. Whether this is an adaptive response to facilitate hyperinsulinemia to overcome insulin resistance or reflects a GLP-1 resistant state, has yet to be elucidated.
REFERENCES

5. Liew CF, Seah ES, Yeo KP, Lee KO, Wise SD. Lean, nondiabetic Asian Indians have decreased insulin sensitivity and insulin clearance, and raised leptin compared to Caucasians and Chinese subjects. Int J Obes Relat Metab Disord 2003; 27(7):784-789
17. Matsuda M, Defronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999; 22(9):1462-1470
27. Holst JJ, Knop FK, Vilsboll T, Krarup T, Madsbad S. Loss of Incretin Effect Is a Specific, Important, and Early Characteristic of Type 2 Diabetes. Diabetes Care 2011; 34(Supplement 2):S251-S257
31. Hansen KB, Vilsboll T, Bagger JI, Holst JJ, Knop FK. Reduced Glucose Tolerance and Insulin Resistance Induced by Steroid Treatment, Relative Physical Inactivity, and High-Calorie Diet Impairs the Incretin Effect in Healthy Subjects. Journal of Clinical Endocrinology & Metabolism 2010; 95(7):3309-3317