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

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ABSTRACT

Objective: Higher insulin levels during an oral glucose test (OGTT) have consequently 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 white 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 21.5±0.7 years *vs.* South Asians 21.4±0.7 years (mean±SEM) and body mass index (Caucasians 22.7±0.7 kg/m^2 *vs.* South Asians 22.1±0.8 kg/m^2) 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 (Caucasians 8.7(8.6) *vs.* South Asians 3.2(19.2), median(IQR)). South Asians showed a higher GLP-1 response, as reflected by a higher area under the curve for GLP-1 (Caucasians 851 ± 100 mmol/L*h *vs.* South Asians 1235 ± 155 mmol/L*h). 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 white 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 is exceptionally high. Furthermore, type 2 diabetes occurs at a younger age and lower BMI as compared to white Caucasians.[1-3] Also, long-term complications start earlier and run a more serious course. The predominant mechanism involved in the pathogenesis of type 2 diabetes in South Asians seems to be a decrease in insulin sensitivity.[4-6]

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).[7-9] 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,[5] but an increased β-cell response has been reported as well.[10]

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.[11] 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 if 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.[12-14] In the present study we therefore studied the glucose and insulin response during a prolonged 6-hour OGTT in healthy, young South Asian and white 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 white Caucasians, 8 South Asians). Male subjects aged 18-25 years, with a BMI between 18.5 and 25 kg/m^2, and a positive family history of type 2 diabetes were eligible for enrolment. 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 Asian 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 Centre 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 centre at 8.00 AM after an overnight fast. Anthropometric measurements were obtained and fat mass was assessed by bioelectrical impedance analysis (Bodystat® 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 analyser (Roche, Almere, The Netherlands). LDL-cholesterol was calculated according to Friedewald's formula.[15] Serum insulin levels were analysed 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/L is used. We chose a lower cut-off value for hypoglycemia suggested by 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.[16] 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).[17] Recently it was shown that the Matsuda index correlates highly with insulin sensitivity measured with a hyperinsulinemic clamp in South Asians and Caucasians.[18] The insulinogenic 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).[19] The oral disposition index (DI_o; ($\Delta I_{0-30}/\Delta G_{0-30}$)/fasting insulin) (glucose: mmol/L; insulin: mU/L) was used to provide an estimate of β-cell function relative to the prevailing level of insulin resistance.[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 were significantly smaller and lighter compared to the Caucasian subjects. BMI, however, was comparable between the groups (Caucasians: 22.7 ± 0.7 *vs.* South Asians: 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 white Caucasian and South Asian men.

	white Caucasians (n = 10)	South Asians (n = 8)
age (years)	21.5 ± 0.7	21.4 ± 0.7
height (m)	1.82 ± 0.01	1.72 ± 0.02
weight (kg)	76.0 ± 2.7	65.7 ± 2.8*
BMI (kg/m^2)	22.7 ± 0.7	22.1 ± 0.8
waist (cm)	81 ± 2.2	78 ± 2.1
fat mass (%)	14.9 ± 0.9	15.2 ± 1.5
fasting glucose (mmol/L)	4.9 ± 0.2	5.2 ± 0.1
fasting insulin (mU/L)	5.3 ± 1.5	9.5 ± 1.5
HOMA-IR	1.2 ± 0.4	2.2 ± 0.4
total cholesterol (mmol/L)	3.68 ± 0.26	3.90 ± 0.19
LDL-cholesterol (mmol/L)	2.10 ± 0.24	2.22 ± 0.12
triglycerides (mmol/L)	1.03 ± 0.13	0.92 ± 0.11
HDL-cholesterol (mmol/L)	1.10 ± 0.06	1.3 ± 0.06

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 (Caucasians: 6.6 ± 0.9*10^3 *vs.* South Asians: 16.7 ± 4.2*10^3 mU/L*h;

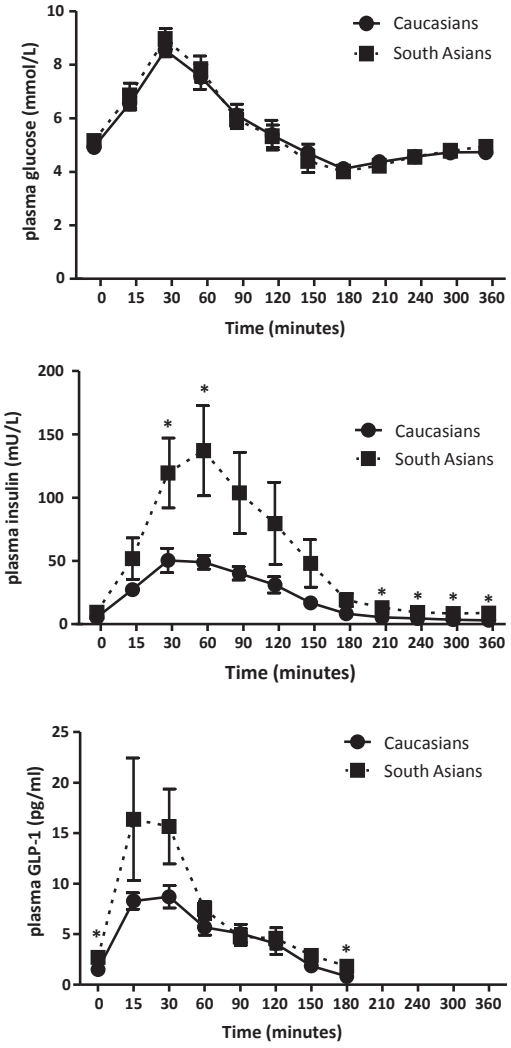

Figure 1. Time courses for plasma concentrations of glucose, insulin and glucagon-like peptide (GLP)-1 during an oral glucose tolerance test (OGTT) in healthy young white Caucasian and South Asian men. Data are mean ± SEM; * p<0.05.

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). β-cell function in relation to the level of insulin sensitivity, as assessed by the oral disposition index, did not differ between the two groups.

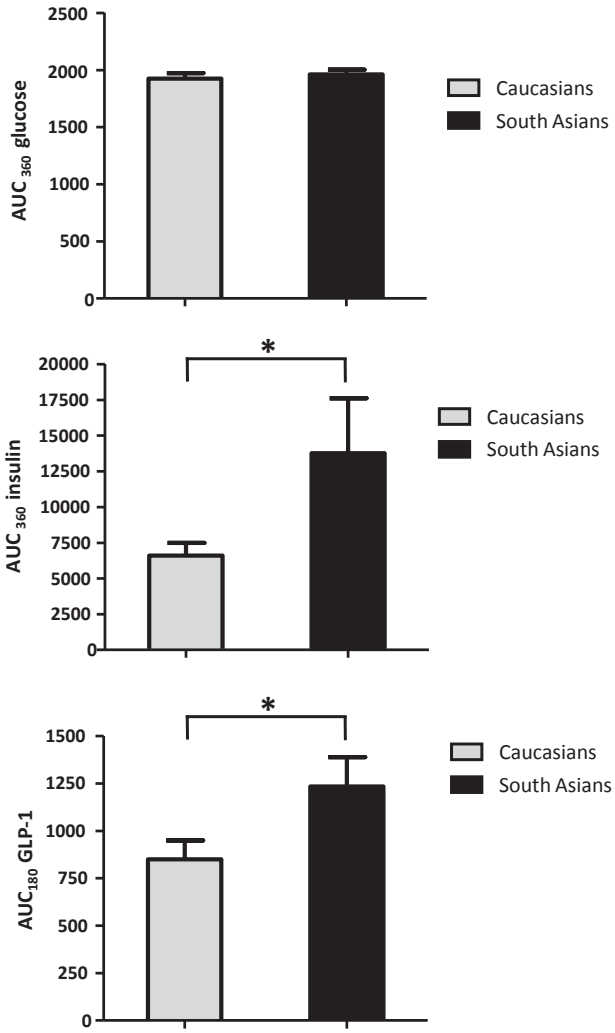

Figure 2. Area under the curve (AUC) for glucose, insulin and glucagon-like peptide (GLP)-1 during an oral glucose tolerance test (OGTT) in healthy, young white Caucasian and South Asian men. Data are mean ± SEM; * p<0.05.

Table 2. Glucose, insulin and GLP-1 indices during a 6-hour 75-g oral glucose tolerance test in young, healthy white Caucasian and South Asian men.

	white Caucasians (n = 10)	South Asians (n = 8)
peak glucose (mmol/L)	8.8 ± 0.30	9.1 ± 0.33
peak glucose time (min) (median, IQR)	30 (30)	30 (23)
peak insulin (mU/L)	59 ± 8.7	155 ± 39*
peak insulin time (min) (median, IQR)	60 (30)	60 (0)
peak GLP-1 (mmol/L)	9.4 ± 0.9	22.1 ± 6.2
peak GLP-1 time (min) (median, IQR)	30 (30)	30 (15)
AUC_{120} glucose (mmol/L * h)	818 ± 34	837 ± 32
AUC_{120} insulin (mU/L * h)	$4.7 \pm 0.6*10^3$	$12.0 \pm 3.0*10^3$*
AUC_{360} glucose (mmol/L * h)	1924 ± 47	1936 ± 48
AUC_{360} insulin (mU/L * h)	$6.6 \pm 0.9*10^3$	$16.7 \pm 4.2*10^3$*
AUC_{180} GLP-1 (mmol/L * h)	851 ± 100	1235 ± 155*
AUC_{180} incremental GLP-1 (mmol/L * h)	619 ± 94	851 ± 142
AUC_{120} glucose/AUC_{120} insulin (mmol/mU)	0.21 ± 0.33	0.13 ± 0.04
Matsuda index (median, IQR)	8.7 (8.6)	3.2 (19.2)*
IGI_{30} (pmol/mmol) (median, IQR)	83 (41)	175 (189)$^{p=0.051}$
DI_o (median, IQR)	3.1 (3.5)	2.4 (1.4)

Mean ± SEM, unless otherwise specified. * $p < 0.05$. GLP-1: glucagon-like peptide-1; AUC: area under the curve; IGI: insulinogenic index, DI_o: oral disposition index

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 (Caucasians: 851 ± 100 *vs.* South Asians: 1235 ± 155 mmol/L*h; $p<0.05$). The incremental AUC_{180} was higher in South Asians as well, although this did not reach statistical difference (Caucasians: 619 ± 94 *vs.* South Asians: 851 ± 142 mmol/L*h; 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 min (with insulin at t = 15, 30, 90, 120, 150, 180, 210, 240, 300 min; $p<0.05$). The GLP-1 AUC_{180} showed a significant correlation with the insulin AUC_{120} and the 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.

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 white 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.[5] 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.[9] 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.[22] 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.[12-14] 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.[23] 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 extrapancreatic effects, since it improves glucose disposal and decreases endogenous glucose production independent of its release of islet hormones.[24-26] Therefore, we hypothesized 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 type 2 diabetes 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 type 2 diabetes patients.[27] It has been debated if this impaired incretin effect is caused by impaired GLP-1 and glucose-dependent insulinotropic 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 type 2 diabetes and insulin resistance, but a recent meta-analysis found that patients with type 2 diabetes on the whole do not exhibit reduced GLP-1 secretion in response to oral glucose or meal tests and that type 2 diabetes patients may even have higher GLP-1 peak levels.[28] Our data suggest that a state of insulin resistance leads to a higher GLP-1 response. Possibly, GLP-1 secretion changes during the progression from normal glucose tolerance to type 2 diabetes, which was also suggested by the authors of the aforementioned meta-analysis.[28] Early stages of type 2 diabetes 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 type 2 diabetes 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.[29] 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.[30] 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.[31] 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 type 2 diabetes patients and their healthy offspring.[32] Furthermore, in the aforementioned study in which insulin resistance was induced in healthy subjects, although no alterations in the GLP-1 response were seen, a reduction in the incretin effect was shown.[31] 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 AUC180. 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 behaviour solely explain the increased insulin sensitivity found in diverse groups of South Asians.[6] Furthermore, in a previous study of our group in a similar study population no differences were found in diet and exercise.[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 white 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.

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