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Ethnic differences in insulin secretory function between Black African and White European men with early type 2 diabetes

Short title: Ethnicity and type 2 diabetes pathophysiology

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ABSTRACT

AIMS. Populations of African ancestry suffer high rates of type 2 diabetes (T2D) compared to Caucasians. Phenotypic differences in pre-diabetic populations, particularly marked hyperinsulinaemia, suggest ethnic distinctions in T2D pathophysiology. We tested the hypothesis that men of Black (West) African (BAM) ethnicity with early T2D would have greater insulin secretory deficits compared to White Europeans (WEM), following the pre-diabetic hypersecretion.

METHODS. In 19 BAM and 15 WEM, matched for age, BMI and duration diabetes, we assessed and modelled insulin secretory responses to hyperglycaemia stimulated intravenously (hyperglycaemic clamp) and orally (meal tolerance test).

RESULTS. With comparable post-challenge glucose responses, BAM exhibited lower second phase c-peptide response to intravenous (BAM 70.6 *vs* WEM 115.1nmol/l min^{-1} (ratio of geometric mean 0.55, 95%CI 0.37,0.83) p=0.006) and oral (BAM 65.4 *vs* WEM 88.5nmol/l min^{-1} (mean difference -23.2 (95%CI -40.0,-6.3) p=0.009) glucose. BAM peripheral insulin response to oral glucose was preserved (BAM 47.4 *vs* WEM 59.4nmol/l min^{-1} (ratio of geometric mean 0.89 (95%CI 0.59,1.35) p=0.566), with relative reductions in insulin clearance (BAM 506.2 *vs* WEM 630.1 mL/m^2 BSA min^{-1} (mean difference -123.9 (95%CI -270.5, 22.6) p=0.095), associated with enhanced incretin responses (GIP iAUC: BAM 46.8 *vs* WEM 33.9µg/l min^{-1} (mean difference 12.9 (95%CI 2.1,23.7) p=0.021).

CONCLUSIONS. In early T2D, BAM exhibit significantly lower insulin secretory responses to intravenous and oral stimulation compared to WEM. Lower insulin clearance, potentially driven by increased incretin responses, may act to preserve peripheral insulin concentrations. Tailoring early management strategies to reflect distinct ethnic-specific pathophysiology may improve outcomes for this high risk population.

Keywords: type 2 diabetes, ethnicity, insulin secretion, beta-cell, African

Abbreviations:

BAM: Black West African men

CRF: Clinical research facility

MRI: Magnetic resonance imaging

NEFA: Non-esterified fatty acids

VAT: Visceral adipose tissue

WC: Waist circumference

WEM: White European men

INTRODUCTION

Populations of African ancestry are disproportionately affected by type 2 diabetes (T2D) (1); it develops at younger age (2) and lower body mass (3) than amongst Caucasians.

The main pathophysiological processes of insulin secretory failure and insulin resistance that underlie T2D are well documented (4) but differences in the pathogenesis based on ethnicity are increasingly recognised. There is a growing literature examining metabolism in non-diabetic Black populations, with studies in non-diabetic African-American children and adolescents describing marked hyperinsulinaemia compared to other ethnicities (5-11) and extensive reports that Black populations, both indigenous (12) and diasporic (13-19), exhibit a hyperinsulinaemic response to glucose. Conventionally hyperinsulinaemia is understood to occur in response to heightening insulin resistance, however this does not fully explain the response in Black populations (6, 9, 11). Studies in children measuring c-peptide have described a combination of increased insulin secretion and reduced hepatic insulin clearance (7, 9). Studies in healthy and prediabetic adults have shown lower rates of insulin clearance (13, 15, 19) but heterogeneity in the populations has made independence from insulin resistance and body weight/composition differences difficult to ascertain. If intensified hyperinsulinaemia represents greater insulin secretion, it may predispose to earlier beta-cell exhaustion in the development of T2D. To date no studies have undertaken comparisons of beta-cell function in Black African and White European populations with recent-onset T2D. As this may be the time people first present to health services, this is an important phase to understand.

The measurement of insulin secretory capacity is complex. Techniques based on the measurement of circulating insulin concentrations only partially reflect insulin secretion and fail to account for hepatic insulin clearance. Measurement of c-peptide overcomes this and reflects more precisely true pancreatic insulin secretion. The intravenous glucose tolerance test is the most commonly used method but it is often restricted to assessing only first phase

secretion; the hyperglycaemic clamp is a more rigorous method that distinguishes first and second phase secretion however it does not account for the role of incretin hormones, which can be assessed by a meal tolerance test.

The purpose of this study was to assess comprehensively insulin secretory function, in response to both intravenous and oral stimulation, to explore the hypothesis that men of Black (West) African (BAM) ethnicity will have significantly greater insulin secretory deficits compared to White European men (WEM) by the time they manifest T2D.

MATERIALS & METHODS

The study was conducted at the Clinical Research Facility (CRF), King's College London, UK and approved by the London Bridge National Research Ethics Committee (12/LO/1859); all participants provided informed consent. Recruitment and data collection took place April 2013-January 2015.

Participants

Men of Black West African or White European ethnicity (self-declared, confirmed by grandparental birthplace), aged 18-65 years, BMI 25-35 kg/m^2, with a documented diagnosis of T2D within 5 years, treated with lifestyle advice ± metformin, and HbA_{1c} ≤63·9 mmol/mol (<8%) were recruited from South London General Practices taking part in an early detection T2D screening programme (20). Participants were deemed ineligible if: treated with other diabetes medications, chronic oral steroids, beta-blockers; serum creatinine >150 mmol/l; serum alanine transaminase level >2.5-fold above the upper limit of the reference range; positive auto-antibodies for anti-insulin, anti-GAD or anti-A2; sickle cell disease (trait permitted); or medications believed to affect the outcome measures. Participants completed a medical screening before study entry. BAM were matched with WEM for age (± 5 years) and BMI (± 3 kg/m^2).

Study design

Assessment visits were completed in random order and separated by a minimum of 7 days. For each assessment participants arrived having refrained from eating or drinking anything other than water from 10pm the night prior. Participants were instructed to refrain from strenuous exercise and physical activity in the preceding 48 hours and from alcohol in the preceding 24 hours , and to consume a standardised diet the day prior (~50% of calories from carbohydrate, evenly spread throughout the day, with no more than 30% of daily carbohydrate consumed in

the evening meal). Participants on metformin were instructed to cease taking it for 7 days prior to the visit.

Hyperglycemic clamp assessment of first and second phase insulin secretory function. A two-hour hyperglycaemic clamp was conducted (21). Participants were weighed in light clothing and their body surface area (BSA) calculated (22). An antecubital fossa vein was cannulated for administration of intravenous glucose; a second cannula was inserted retrogradely into the dorsum of the hand, and placed in a warming unit, to achieve arterialised venous blood samples. Three fasting samples (-20, -10 and 0 minutes) were collected before starting the glucose infusion (20% glucose) at time 0 minutes; a priming regimen, based on BSA (23), was used for the first 15 minutes to increase rapidly the plasma glucose to 6.9 mmol/l above fasting. The glucose infusion rate was then adjusted to maintain plasma glucose at 6.9 mmol/l above fasting for a further 105 minutes. Blood sampling occurred every 2 minutes for the first 10 minutes and every 5 minutes thereafter to inform adjustment of the glucose infusion rate for 'clamping' the plasma glucose. Blood samples were drawn at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105 and 120 minutes for the assessment of plasma glucose, and serum insulin and c-peptide.

Mixed meal tolerance test assessment of insulin and incretin secretion. A three-hour meal tolerance test was conducted using a liquid milkshake (Ensure Plus, Abbott Nutrition, UK), providing 6 kcals/kg body weight. An antecubital fossa vein was cannulated for blood sampling. Following the collection of fasting samples at time -10 and 0 minutes the participants consumed the drink within 5 minutes. Blood was collected at 10, 20, 30, 40, 50, 60, 75, 90, 120, 150 and 180 minutes for the assessment of glucose, non-esterified fatty acids (NEFA), insulin and c-peptide, and for GLP-1 and GIP at 30, 60 and 120 minutes.

Magnetic resonance imaging assessment of visceral fat deposition. Visceral fat and skeletal muscle mass were assessed using MRI, in a 1.5T Siemens scanner. Participants lay supine and

a single T1-weighted axial image, of 3mm thickness, was acquired at the L4-L5 region of the abdomen and the thighs (20cm below the neck of the femur), using a two-point variant Dixon imaging protocol. The area of visceral fat and volume of skeletal muscle was quantified using Osirix image processing software, version 6.0.2 (Pixemo, Switzerland).

Analyses of samples and calculations. We measured plasma glucose by automated glucose analyser (Yellow Spring Instruments, Ohio, USA); serum insulin by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare Ltd. Camberly, UK); serum c-peptide by radioimmunoassay (Millipore Ltd, Hertfordshire, UK); plasma NEFA by enzymatic colorimetric assay (Wako Diagnostics, Richmond, VA, USA) on an automated clinical chemistry analyser (ILab 650, Instrument Laboratories, Holliston, MA, USA); and GLP-1 and GIP (total) by fluorescent ELISA methods (EGLP-35K and EZHGIP-54K, Merck Millipore, UK).

The area under the curve (AUC) and incremental AUC (iAUC) were calculated, using the trapezoidal rule, for insulin, c-peptide, glucose, NEFA, GLP-1 and GIP. To calculate an index of first and second phase insulin secretion in the hyperglycaemic clamp we measured the iAUC for c-peptide over 0-10 minutes for first phase, and 10-120 minutes for second phase, in analogy to DeFronzo *et al.* (21).

Model-based measurement of beta-cell function: the glucose, insulin and c-peptide curves during the hyperglycaemic clamp and meal tolerance test were modelled using methods previously described (24-26) (SAAM-II 1.2 software; SAAM Institute, Seattle, WA). The main outputs of the hyperglycaemic clamp model are: glucose sensitivity of first-phase secretion (σ^1), expressed as the amount of insulin secreted in response to a rate of increase in glucose of 1 mmol/l between time 0 and 1 min of the study, in $(pmol \cdot m^{-2}\, BSA)/(mmol \cdot l^{-1} \cdot min^{-1})$; glucose sensitivity of second-phase secretion (σ^2), expressed as the steady state insulin

secretion rate in response to a step increase in glucose of 1 mmol/l above baseline, in $(pmol \cdot min^{-1} \cdot m^{-2}\, BSA)/(mmol \cdot l^{-1})$. Modelling of the glucose and c-peptide curves of the meal test enables an estimation of the equivalent of first phase insulin secretion (σ^1), whereas second phase insulin secretion is assessed and presented through the stimulus response curve of the insulin secretion rates at 4, 5.5, 8, 11 and 15 mmol/l of glucose. The parameter σ^2, as defined above is the slope of the rising branch of the curve relating plasma glucose concentration to insulin secretion rate.

In both the hyperglycaemic clamp and meal test, average insulin clearance was computed according to the following formula (derivation and correct interpretation are presented in *Supplementary Material*):

$$Clearance_{Ins} = \frac{AUC_{ISR}}{AUC_1 + (I_{Final} - I_{Basal}) \cdot MRT_{Ins}}$$

in which AUC_{ISR} is the area under the curve of insulin secretion rate, AUC_I is the area under the curve of insulin concentration, I_{Final} is insulin concentration at the end of the study, I_{Basal} is insulin concentration at the beginning of the study, and MRT_{Ins} is the mean residence time of insulin, which was assumed to be 27 minutes as reported in (27).

The reconstructions of beta cell function during the hyperglycaemic clamp and the meal tolerance test were combined to enable modelling of the effect of incretins on insulin secretion: the 'meal effect'. This was done by taking the beta-cell reconstructed from the hyperglycaemic clamp and challenging it, in an *in silico* experiment, with the plasma glucose curve of the meal test, thus computing the time course and the total amount of insulin secretion rate; this is the *in silico* equivalent of infusing *in vivo* intravenous glucose to mimic the glucose curve seen during the meal tolerance test. The effect of the meal on beta-cell insulin secretion can be measured

by comparing the total insulin secretion of the meal *in vivo* with that of the *in silico* simulation of intravenous glucose infusion to mimic the glucose curve elicited by the meal test, i.e.:

$$Meal\ effect = \frac{AUC\ ISR_{Meal} - AUC\ ISR_{Intravenous}}{AUC\ ISR_{Meal}}$$

Further details of the computation of the 'meal effect' are provided in *Supplementary Material*.

Statistics

All datasets were tested for normality (Shapiro-Wilks test) and non-normally distributed variables were transformed (log 10) for analysis. Normally-distributed data are expressed as mean ± standard deviation, and log-normal data were back transformed to give geometric mean and 95% CI for the ratio of the geometric mean. Differences between ethnic groups were determined by independent samples t-test using the raw data where they were normally distributed or logarithmic-transformed data where not. $p \leq 0.05$ was considered statistically significant. Note that for the data analysed on the natural scale, the null value is 0 and so where $p<0.05$, the 95% CI will exclude 0 but for the data analysed on the log scale and back-transformed to give the ratio of geometric means, the null value is 1 and so where $p<0.05$, the 95% CI will exclude 1. The relationship between average insulin clearance and average insulin concentration was analysed by linear and nonlinear regression analysis, as described in *Supplementary Material*. Analyses were performed using SPSS software, version 24 (IBM Analytics, NY).

RESULTS

Thirty-four participants, 19 BAM and 15 WEM, were studied, mean age 54.7 9 (SD 7.4) years, BMI 29.7 (SD 2.7) kg/m^2. The participants had been diagnosed with diabetes for 2.9 (SD 1.1) years, mean HbA1c was 49.3 (SD 7.6) mmol/mol; 65% of participants were treated with metformin, the remainder with lifestyle management alone. By design, there were no significant ethnic differences in age, BMI, duration of diabetes, HbA1c, or management (Table 1). Mean visceral fat was significantly lower, and skeletal muscle area significantly higher in BAM (Table 1). The BAM were first-generation West African migrants (born in Nigeria, n=11; Ghana, n=5; Sierra Leone, n=2, Ivory Coast, n=1).

Beta-cell insulin secretory function

In the hyperglycaemic clamp there were no ethnic differences in mean fasting (Table 2) or 'clamped' glucose (BAM 14.4 ± 1.28 *vs* WEM 14.8 ± 1.68 mmol/l, p=0.45). Fasting c-peptide was lower in BAM (Table 2). There were no significant ethnic differences in first phase c-peptide or insulin iAUC. Second phase c-peptide secretion (iAUC) was significantly lower in BAM, with a trend for 2nd phase insulin iAUC that did not achieve statistical significance (Figure 1, Table 2). The modelled glucose sensitivity of the beta cell (σ^1 and $\sigma 2$) showed similar trends.

During the meal tolerance test the two ethnic groups exhibited the same glucose response, however, mean c-peptide iAUC was significantly lower in BAM. The meal insulin iAUC was not significantly different between ethnic groups (Table 3, Figure 2). The modelled data from the meal tolerance test showed no significant ethnic differences in first-phase insulin secretory function (Table 3) but second-phase secretory function was lower in BAM (p=0·01). The insulin secretion rate was lower amongst BAM at 4 (p=0·019) and 5·5 mmol/l (p=0·02). This

difference was lost at higher glucose concentrations of 8 mmol/l (p=0·112), 11 (p=0·199) and 15 mmol/l (p=0·247) (Figure 3).

Insulin clearance

There were no ethnic differences in average insulin clearance during the intravenous challenge (hyperglycaemic clamp; Table 2). In response to oral glucose the average clearance appeared lower in BAM but this difference was not statistically significant (Table 3). However, when average clearance was plotted against average insulin concentration of each test a hyperbolic relationship was apparent (Supplementary Material, Figure S8), with a clear, significant difference between the groups, implying that in BAM average insulin clearance was lower at any average insulin concentration achieved during meal/clamp tests (Figure 4).

Incretin responses

Mean secretion of GIP was significantly higher in BAM in response to the meal challenge (Table 3). There were no ethnic differences in GLP-1 secretion, or in the "meal effect", the modelled effect of the mixed meal, including incretin hormones, on insulin secretion (Table 3).

DISCUSSION

This study demonstrates differences in the metabolic processes involved in glucose dysregulation in men of Black African ethnicity with early T2D compared to White Europeans. Our participants had both very short duration of diagnosed disease and good metabolic control on minimal therapy (lifestyle +/- metformin only) and thus our data extend into early diabetes existing data from healthy and pre-diabetic populations. Those studies report marked hyperinsulinaemia amongst people of African ancestry (13, 15, 16); we provide novel data to show that in early T2D there is reduced insulin secretory function in response to both intravenous and oral stimuli in BAM. Whilst the insulin iAUC in the hyperglycaemic clamp was reduced in BAM, there were no differences in insulin when the meal was used to invoke hyperglycaemia via the gut. Importantly this demonstrates that the reduced hepatic insulin clearance, which has been reported in studies of healthy and prediabetic populations of African ancestry, is maintained through to early T2D and may act to maintain peripheral insulin levels, but may occur only in response to oral stimuli. Furthermore BAM exhibited significantly greater GIP responses, which may have contributed to lower average insulin clearance rates, and may have important clinical implications.

Our study provides the most comprehensive assessment of the impact of Black ethnicity on beta-cell function to date. We used the intravenous glucose challenge of the hyperglycaemic clamp to distinguish first and second phase secretion, whilst the meal tolerance test assessed the physiological response of the beta-cells to nutrients, and incretin effects. In our study we have demonstrated significantly lower fasting c-peptide concentrations amongst BAM, compared to WEM of similar duration of diagnosed diabetes indicating significantly greater reduction in basal insulin secretion, although circulating insulin concentrations were not different. We also found reduced second phase insulin and c-peptide response to intravenously stimulated hyperglycaemia amongst BAM. Previous studies assessing insulin secretion in non-

diabetic populations, have provided inconsistent findings (7, 10, 11); reporting higher first and second phase secretion (8), or the difference occurring only in the first (6) or second phase (5). In the aetiology of T2D, impairments in both the first and second phase insulin responses have been recognised (28, 29). The second phase response, which can only be triggered and sustained by glucose and fuel secretagogues, is quantitatively very important in the maintenance of glucose homeostasis, given that it can be sustained in response to prolonged hyperglycaemia (30). Our modelling methods enabled us to investigate the impact of ethnicity on glucose dose effects on insulin secretion, which has not previously been examined amongst populations of African ancestry. Interestingly ethnic differences in second phase insulin secretion rates at lower glucose levels were lost at higher glucose concentrations (over 8 mmol/l). Since both basal and glucose tolerance are similar in the two groups, this result suggests that in the post-absorptive state insulin secretion plays a different adaptive role in the two groups.

We are not aware of other studies comparing beta-cell function between BAM and WEM with T2D using the hyperglycaemic clamp. The majority of ethnic comparisons have focused on healthy or individuals at increased risk of T2D, and have predominantly used the intravenous glucose tolerance test (IVGTT) to measure the 'acute insulin response' (AIR), which is comparable to the first phase response of the hyperglycaemic clamp, but often only insulin is measured and rarely is the second phase response assessed. These investigations have consistently demonstrated an higher AIR among non-diabetic Black groups (6, 7, 15, 31-33). To date only one ethnic comparison has been performed in people with T2D (the Insulin Resistance Atherosclerosis Study (IRAS); (34)), reporting significantly higher AIR amongst African-Americans compared to Whites, although not among the participants with newly diagnosed T2D, who are a nearer comparison to our participants. Notably IRAS did not assess c-peptide so it is not possible to determine beta-cell secretion, and the second phase response

was not assessed. There are other distinctions. It is well established that the phenotype of T2D in Black populations is gender specific (16, 17); higher insulin levels (16, 17), and obesity-driven T2D is more common in women (16), hence our study included only men, whereas IRAS consisted of both males and females. There is a need for further studies to examine gender-specific mechanisms.

Our findings of significantly lower basal c-peptide but not insulin raise concerns regarding the use of beta-cell indices based on fasting insulin, such as HOMA-B (35). These are often used to assess beta-cell function in epidemiological studies but our data suggest they may misrepresent beta-cell function in Black populations and findings of ethnic differences (16) may need to be considered with caution.

When we studied beta-cell function using an oral stimulus, we recognised a significantly lower second phase c-peptide response in BAM, consistent with the hyperglycaemic clamp. However there were no differences in insulin concentrations and model derived data brought to recognise lower insulin clearance amongst BAM (Figure 4). The implication of Figure 4 is that at the same total insulin output during an intravenous or an oral challenge BAM achieve higher insulin curves, which may compensate for reduced beta-cell secretion and contribute to peripheral insulin levels. A number of previous investigations have reported reduced insulin clearance amongst non-diabetic Black populations (5-7, 13, 15, 36) and we here demonstrate that this is maintained into early T2D. The mechanisms underlying this are largely unknown, however, recent advancements in modelling techniques, that allow for hepatic versus extrahepatic clearance to be quantified, have concluded that ethnic differences in insulin clearance are solely hepatic with no extra-hepatic contribution (37).

A reduction in insulin clearance is typically found following oral glucose or meal ingestion, and is characteristically of a much greater magnitude than that observed after intravenous

induction of hyperglycaemia (38-40). Reduction in insulin clearance upon increasing levels of insulin secretion is proposed to be due to the saturable nature of hepatocellular insulin receptors (41, 42). However there is also evidence that incretin hormones affect insulin clearance (39, 40, 43). There has been very little study of incretin hormones and how these vary according to ethnicity. In the current study, BAM exhibited significantly higher postprandial GIP concentrations, which may have contributed to the non-significant trend for lower average insulin clearance that was observed. The effect of GIP on insulin clearance is unclear; some authors have demonstrated an insulin clearance reducing effect of GIP (44, 45), whilst others have shown no effect (42, 46). Some of the conflict in these findings may have occurred because insulin clearance appears to adapt to insulin resistance and glucose intolerance, a potential mechanism by which beta-cell function is preserved in the progression to T2D (47, 48). There has been very little investigation of incretin hormones within Black populations and in those which have the focus has been on the role of incretins in the upregulation of insulin secretion; African-American children have been reported to have lower GLP-1, but similar GIP secretion compared to European-American children (49) whereas in a study of Black and White obese adolescents, Michaliszyn *et al* (2017) reported no difference in GLP-1 or GIP amongst Blacks (36), and Velasquez-Mieyer *et al.* (2003) found higher GLP-1 in obese African-American adults compared with European-Americans, with no measurement of GIP (50). We modelled the impact of the mixed meal, including, but not limited to, the incretin response on insulin secretory function ('meal effect'), but detected no ethnic differences. Michaliszyn *et al* (2017) modelled the 'potentiation factor', which describes the modulation of the relationship between glucose concentration and insulin secretion and comprises several mechanisms including the release of endogenous incretin hormones. In contrast to our data they found no differences in incretin concentrations in response to an oral glucose challenge but report a significantly higher early potentiation factor in Blacks (36). Our data suggest that by the time

diabetes develops, BAM may have no greater beta-cell response to GIP than WEM, but that their higher GIP response may concur to cause lower average insulin clearance in response to hyperglycaemia, which results in maintenance of peripheral insulin concentrations, and that these mechanisms provide some compensation for the significantly lower insulin secretory capacity of the beta-cells.

The strengths and limitations of our work warrant discussion. We have not investigated the cellular mechanisms that underlie the differences in metabolic function between BAM and WEM. Additionally we have only captured the metabolic phenotype of T2D, and of men, therefore we cannot allude to the mechanisms by which hyperglycaemia progresses and how this may be distinct among BAM, and our findings may not extrapolate to women. Our study has explored ethnic differences in insulin secretory function and in doing so has *a-priori* assessed a comprehensive portfolio of measures that attempt to thoroughly characterise insulin secretory function. Although we have conducted a large number of comparisons we have not corrected for multiple testing because our outcome variables are not independent of one another and the differences are very large and highly significant, therefore we are confident that the differences we have observed are likely to represent real differences. Finally, our model aided computation of the meal effect on beta cell function (see *Supplementary Material*) has not been validated with ad hoc experiments.

Major strengths of our work are our use of intensive, sophisticated techniques, and our well-matched participant groups; our ethnic groups had the same duration of diagnosed diabetes, HbA1c, fasting glucose and clinical management, and exhibited almost identical glucose responses to a meal challenge. We are therefore confident we have recognised novel ethnic distinctions in T2D pathophysiology which may have important clinical implications. The intensive nature of our protocol precludes a much larger study, and may have missed additional more subtle ethnic differences, but the value of our approach is perhaps best seen in the way

our data have been able to extend the conclusions of epidemiological studies such as IRAS, discussed above. Our data suggest that loss of beta-cell insulin secretory function occurs earlier in the development of T2D in BAM compared to WEM, however the mechanisms that drive beta-cell dysfunction in BAM are not clear. Potentially BAM may have lower beta-cell mass or a steeper slope of decline in beta-cell function as T2D develops.

In conclusion we have recognised in this study that deficits in beta-cell function may effect hyperglycaemia in BAM more strongly than WEM. Further studies are needed to ascertain whether the incretin hormones play a damage-limitation role in maintaining peripheral insulin concentrations by reducing insulin clearance in BAM. Meanwhile, it may be pertinent to consider therapeutic strategies that augment these physiological processes; BAM may achieve greater clinical benefit from therapeutic agents that support beta-cell function such as the incretin therapies.

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Louise Goff is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of the data and the accuracy of data analysis.

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Duality of interests: The authors declare that there is no duality associated with this manuscript.

Author contributions

LMG, SAA, JLP, AMU formulated the research question and designed the study. LMG, SAA and KGMMA supervised data collection and interpretation. CM coordinated the study and data acquisition, and performed the metabolic assessments. FS-M assisted with the metabolic assessments. LB, RB and LMG performed the minimal modelling analysis. LMG undertook data analysis, statistical analysis and drafted the manuscript. All authors contributed to the intellectual content of the submitted manuscript.

418 **Table 1. Clinical characteristics of Black African and White European participants**

	BAM (n = 19)	WEM (n = 15)	Mean difference (95% CI)	*p*-value
Age (years)	54.1 (7.7)	55.5 (7.1)	-1.3 (-6.6 to 3.9)	0.602
Weight (kg)	90.6 (9.2)	94.2 (11.6)	-3.6 (-10.8 to 3.7)	0.326
Height (cm)	175.4 (7.4)	176.8 (5.8)	-1.4 (-6.1 to 3.4)	0.561
BMI (kg/m^2)	29.5 (2.6)	30.1 (2.7)	-0.62 (-2.5 to 1.3)	0.510
Waist circumference (cm)	103.7 (8.2)	107.5 (8.8)	-3.86 (-9.8 to 2.1)	0.194
Visceral fat area (cm^2)†	130.8 (54.1)	189.0 (75.7)	-58.2 (-104.2 to -12.2)	**0.015**
Thigh skeletal muscle area (cm^2)†	434.2 (49.6)	379.2 (57.2)	55.0 (17.0 to 93.0)	**0.006**
Duration of diabetes (years)	2.8 (1.2)	2.9 (1.0)	-0.09 (-0.88 to 0.69)	0.815
Fasting glucose (mmol/l)	6.67 (0.97)	6.81 (1.37)	-0.14 (-0.95 to 0.68)	0.732
HbA1c (%)	6.7 (0.68)	6.6 (0.72)	0.11 (-0.38 to 0.60)	0.650
HbA1c (mmol/mol)	49.9 (7.7)	48.6 (7.8)	1.26 (-4.15 to 6.74)	0.631
Systolic blood pressure (mm Hg)	137.3 (14.1)	131.8 (13.9)	5.5 (-3.3 to 15.4)	0.262
Diastolic blood pressure (mm Hg)	85.6 (7.4)	82.9 (10.1)	2.7 (-3.4 to 8.8)	0.376
Total cholesterol (mmol/l)	4.12 (0.70)	4.30 (0.72)	-0.18 (-0.68 to 0.32)	0.470
LDL-cholesterol (mmol/l)	2.34 (0.53)	2.29 (0.70)	0.06 (-0.37 to 0.48)	0.794
HDL-cholesterol (mmol/l)	1.17 (0.38)	1.24 (0.24)	-0.07 (-0.29 to 0.16)	0.557
Triacylglycerol (mmol/l)	1.32 (0.75)	1.70 (0.71)	-0.38 (-0.89 to 0.14)	0.143
Metformin use (%)	74	53		0.09

419 Data are arithmetic mean (standard deviation). Differences between ethnic groups tested using independent
420 samples t-test. †data obtained for 14 WEM and 19 BAM. BMI, body mass index; HbA1c, glycated haemoglobin;
421 HDL, high density lipoprotein (-cholesterol); LDL, low density lipoprotein (-cholesterol).

422

Table 2. Hyperglycaemic clamp assessment of insulin secretory function in Black African and White European participants

	BAM (n = 19)	WEM (n = 15)	Mean difference/Ratio of geometric mean (95% CI)	*p*-value
Fasting glucose (mmol/l)	7.39 (1.59)	7.20 (1.12)	0.19 (-0.80 to 1.18)	0.699
Fasting insulin (pmol/l)[#]	66.6 (50.8 to 87.4)	84.0 (57.3 to 123.3)	0.81 (0.51 to 1.23)	0.290
Insulin iAUC 0-10 mins (pmol/l min^{-1})[#]	103.8 (28.5 to 378.1)	75.0 (13.8 to 408.5)	0.77 (0.13 to 4.48)	0.764
Insulin iAUC 10-120 mins (pmol/l min^{-1})[#]	14454 (8430 to 24786)	21999 (13636 to 35498)	0.57 (0.32 to 1.02)	0.060
Fasting c-peptide (nmol/l)	0.576 (0.193)	0.837 (0.299)	-0.261 (-0.433 to -0.089)	**0.004**
C-peptide iAUC 0-10 mins (nmol/l min^{-1})[#]	0.697 (0.131 to 1.546)	1.227 (0.273 to 2.897)	0.98 (0.20 to 4.86)	0.984
C-peptide iAUC 10-120 mins (nmol/l min^{-1})[#]	70.6 (52.5 to 94.8)	115.1 (84.8 o 156.3)	0.55 (0.37 to 0.83)	**0.006**
σ^1 [(pmol/m^2 BSA)/(mmol/l min^{-1})][#]	65.6 (27.0 to 159.2)	95.3 (42.5 to 213.8)	0.69 (0.21 to 2.20)	0.507
σ^2 [(pmol min^{-1} m^2 BSA)/mmol/l)][#]	6.8 (4.1 to 11.4)	12.4 (7.2 to 21.6)	0.55 (0.26 to 1.14)	0.105
M value (mg/m^2 BSA min^{-1})	167.2 (38.4)	185.4 (37.0)	-18.2 (-44.7 to 8.4)	0.173
Average insulin clearance (mL/m^2 BSA min^{-1})[#]	897.6 (699.0 to 1152.4)	830.8 (637.2 to 1082.9)	1.08 (0.76 to 1.55)	0.663

Data are mean (SD) or geometric mean (95% CI) for log-normal data[#]. Positively skewed data were transformed (log^{10}) prior to statistical testing. Differences between ethnic groups tested using independent samples t-test. σ^1, glucose sensitivity of β cell during first-phase insulin secretion; σ^2, glucose sensitivity of β cell during second-phase insulin secretion; BSA, body surface area; iAUC, incremental area under the curve, calculated using the trapezoidal rule; M, glucose disposal in final 60 minutes of the clamp; SI, insulin sensitivity. iAUC 0 – 10 mins represents first phase, iAUC 10 – 120 mins represents second phase.

Table 3. Meal tolerance test assessment of insulin secretory function in Black African and White European participants

	BAM (n = 18)	**WEM (n = 15)**	**Mean difference/Ratio of geometric mean (95% CI)**	***p*-value**
Fasting glucose (mmol/l)	7.34 (1.35)	7.28 (1.34)	0.10 (-0.86 to 1.06)	0.839
Glucose iAUC (mmol/l min^{-1})[#]	378.4 (250.1 to 572.3)	476.2 (377.7 to 600.5)	0.86 (0.57 to 1.29)	0.459
Fasting insulin (pmol/l)[#]	85.1 (67.6 to 107.2)	102.3 (74.1 to 141.3)	0.82 (0.57 to 1.19)	0.284
Insulin iAUC (nmol/l min^{-1})[#]	47.4 (32.6 to 68.8)	59.4 (42.3 to 84.8)	0.89 (0.59 to 1.35)	0.566
Fasting c-peptide (nmol/l)	0.603 (0.216	0.881 (0.340)	-0.278 (-0.477 to -0.080)	**0.008**
c-peptide iAUC (nmol/l min^{-1})	65.4 (17.7)	88.5 (29.4)	-23.2 (-40.0 to -6.3)	**0.009**
Fasting GLP-1 (pmol/l)	12.1 (8.6)	11.7 (6.7)	0.48 (-5.07 to 6.03)	0.861
GLP-1 iAUC (pmol/l min^{-1})[#]	810.2 (519.2 to 1264.7)	861.0 (536.2 to 1382.6)	0.95 (0.57 to 1.57)	0.832
Fasting GIP (ng/l)	44.6 (25.3)	31.8 (13.8)	12.8 (-2.10 to 27.7)	0.089
GIP iAUC (µg/l min^{-1})	46.8 (17.4)	33.9 (12.0)	12.9 (2.1 to 23.7)	**0.021**
Fasting NEFA (µmol/l)	600.0 (186.4)	631.0 (192.4)	-31.0 (-165.9 to 103.9)	0.643
NEFA iAUC (µmol/l min^{-1})	-52576 (26325)	-61337 (26442)	8760 (-10047 to 27568)	0.349
σ^1 [(pmol/m^2 BSA)/(mmol/l min^{-1})]	1420.1 (1184.2)	1134.6 (710.7)	285.5 (-447.2 to 1018.2)	0.432
Average insulin clearance (mL/m^2 BSA min^{-1})	506.2 (194.2)	630.1 (218.6)	-123.9 (-270.5 to 22.6)	0.095
Meal effect (%)	51.0 (12.9)	49.5 (6.4)	1.5 (-6.2 to 9.1)	0.700

Data are mean (SD) or geometric mean (95% CI) for log-normal data[#]. Positively skewed data transformed (log^{10}) prior to statistical testing. Differences between ethnic groups tested using independent samples t-test. σ^1, glucose sensitivity of β cell during first-phase insulin secretion; iAUC, incremental area under the curve, calculated using the trapezoidal rule; NEFA, non-esterified fatty acids.

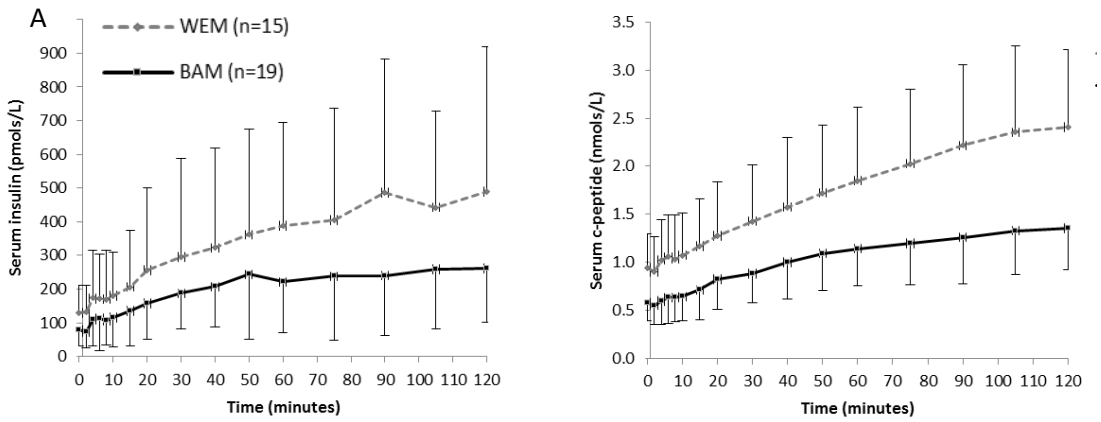

Figure 1. Serum insulin (A) and c-peptide (B) responses in the hyperglycaemic clamp in BAM and WEM

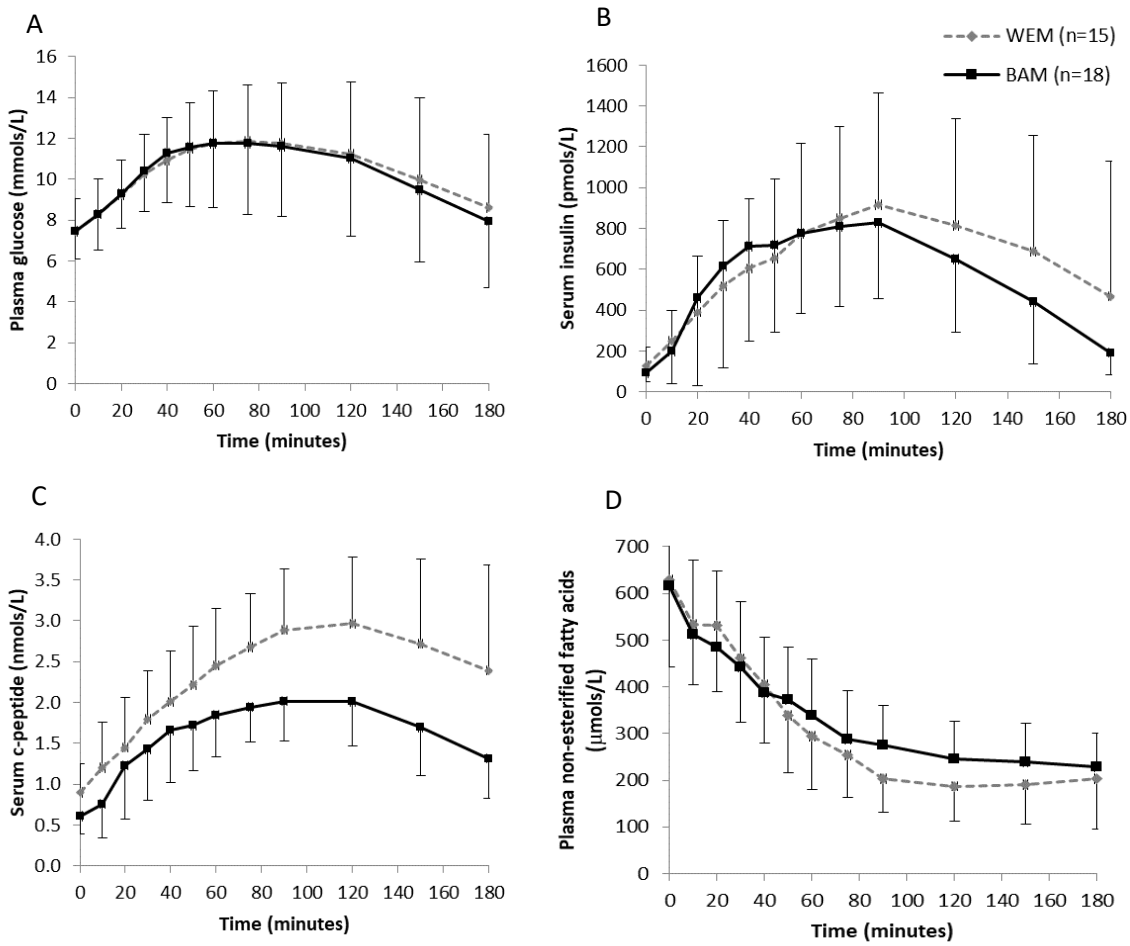

Figure 2. Plasma glucose (A), serum insulin (B), c-peptide (C), and non-esterified fatty acid (D) responses to a mixed meal tolerance test in BAM and WEM

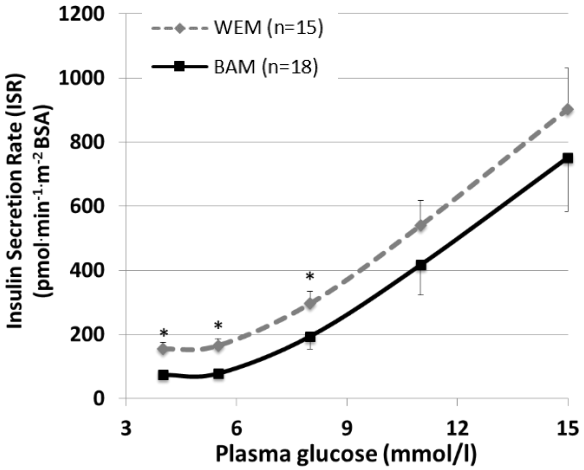

Figure 3. Insulin secretion rates at 5 increasing plasma glucose concentrations during the meal tolerance test, as reconstructed by mathematical modelling of beta cell function, in BAM and WEM.

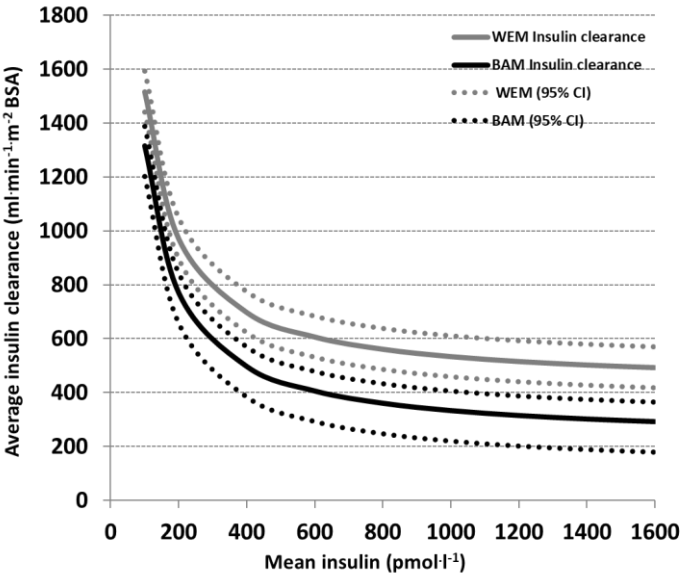

Figure 4. Relationship between average insulin clearance and average insulin concentration during the hyperglycemic clamp and the mixed meal test in BAM and WEM. Average insulin clearance (ml·min^{-1}·m^{-2} BSA): BAM = 224 + (109151/average insulin concentration), WEM = 425 + (109151/average insulin concentration).

REFERENCES

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes research and clinical practice. 2011;94(3):311-21.
2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;384(9945):766-81.
3. Ntuk UE, Gill JM, Mackay DF, Sattar N, Pell JP. Ethnic-specific obesity cutoffs for diabetes risk: cross-sectional study of 490,288 UK biobank participants. Diabetes Care. 2014;37(9):2500-7.
4. DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes. 1988;37(6):667-87.
5. Goran MI, Bergman RN, Cruz ML, Watanabe R. Insulin resistance and associated compensatory responses in african-american and Hispanic children. Diabetes Care. 2002;25(12):2184-90.
6. Hannon TS, Bacha F, Lin Y, Arslanian SA. Hyperinsulinemia in African-American adolescents compared with their American white peers despite similar insulin sensitivity: a reflection of upregulated beta-cell function? Diabetes Care. 2008;31(7):1445-7.
7. Arslanian SA, Saad R, Lewy V, Danadian K, Janosky J. Hyperinsulinemia in african-american children: decreased insulin clearance and increased insulin secretion and its relationship to insulin sensitivity. Diabetes. 2002;51(10):3014-9.
8. Uwaifo GI, Nguyen TT, Keil MF, Russell DL, Nicholson JC, Bonat SH, et al. Differences in insulin secretion and sensitivity of Caucasian and African American prepubertal children. The Journal of pediatrics. 2002;140(6):673-80.
9. Gower BA, Granger WM, Franklin F, Shewchuk RM, Goran MI. Contribution of insulin secretion and clearance to glucose-induced insulin concentration in african-american and caucasian children. The Journal of clinical endocrinology and metabolism. 2002;87(5):2218-24.
10. Arslanian S, Suprasongsin C. Differences in the in vivo insulin secretion and sensitivity of healthy black versus white adolescents. The Journal of pediatrics. 1996;129(3):440-3.
11. Arslanian S, Suprasongsin C, Janosky JE. Insulin secretion and sensitivity in black versus white prepubertal healthy children. Journal of Clinical Endocrinology and Metabolism. 1997;82:5.
12. Amoah AG, Owusu SK, Ayittey OM, Schuster DP, Osei K. Minimal model analyses of beta cell secretion, insulin sensitivity and glucose effectiveness in glucose tolerant, non-diabetic first-degree relatives of Ghanaian patients with type 2 diabetes and healthy control subjects. Ethnicity & disease. 2001;11(2):201-10.
13. Osei K, Schuster DP, Owusu SK, Amoah AG. Race and ethnicity determine serum insulin and C-peptide concentrations and hepatic insulin extraction and insulin clearance: comparative studies of three populations of West African ancestry and white Americans. Metabolism. 1997;46(1):53-8.
14. Haffner SM, Howard G, Mayer E, Bergman RN, Savage PJ, Rewers M, et al. Insulin sensitivity and acute insulin response in African-Americans, non-Hispanic whites, and Hispanics with NIDDM: the Insulin Resistance Atherosclerosis Study. Diabetes. 1997;46(1):63-9.
15. Osei K, Schuster DP. Ethnic differences in secretion, sensitivity, and hepatic extraction of insulin in black and white Americans. DiabetMed. 1994;11(8):755-62.
16. Carnethon MR, Palaniappan LP, Burchfiel CM, Brancati FL, Fortmann SP. Serum insulin, obesity, and the incidence of type 2 diabetes in black and white adults: the atherosclerosis risk in communities study: 1987-1998. Diabetes Care. 2002;25(8):1358-64.
17. Harris MI, Cowie CC, Gu K, Francis ME, Flegal K, Eberhardt MS. Higher fasting insulin but lower fasting C-peptide levels in African Americans in the US population. Diabetes/metabolism research and reviews. 2002;18(2):149-55.
18. Lee CC, Haffner SM, Wagenknecht LE, Lorenzo C, Norris JM, Bergman RN, et al. Insulin clearance and the incidence of type 2 diabetes in Hispanics and African Americans: the IRAS Family Study. Diabetes Care. 2013;36(4):901-7.

19. Osei K, Cottrell DA, Harris B. Differences in basal and poststimulation glucose homeostasis in nondiabetic first degree relatives of black and white patients with type 2 diabetes mellitus. J ClinEndocrinolMetab. 1992;75(1):82-6.
20. Winkley K, Thomas SM, Sivaprasad S, Chamley M, Stahl D, Ismail K, et al. The clinical characteristics at diagnosis of type 2 diabetes in a multi-ethnic population: the South London Diabetes cohort (SOUL-D). Diabetologia. 2013;56(6):1272-81.
21. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 1979;237(3):E214-23.
22. Mosteller RD. Simplified calculation of body-surface area. The New England journal of medicine. 1987;317(17):1098.
23. DeFronzo RA, Tobin, J. Andres, R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 1979;237:E214-E23.
24. Cobelli C, Toffolo GM, Dalla Man C, Campioni M, Denti P, Caumo A, et al. Assessment of beta-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests. American journal of physiology Endocrinology and metabolism. 2007;293(1):E1-E15.
25. Cali AM, Bonadonna RC, Trombetta M, Weiss R, Caprio S. Metabolic abnormalities underlying the different prediabetic phenotypes in obese adolescents. The Journal of clinical endocrinology and metabolism. 2008;93(5):1767-73.
26. Malandrucco I, Pasqualetti P, Giordani I, Manfellotto D, De Marco F, Alegiani F, et al. Very-low-calorie diet: a quick therapeutic tool to improve beta cell function in morbidly obese patients with type 2 diabetes. The American journal of clinical nutrition. 2012;95(3):609-13.
27. Navalesi R, Pilo A, Ferrannini E. Kinetic analysis of plasma insulin disappearance in nonketotic diabetic patients and in normal subjects. A tracer study with 125I-insulin. J Clin Invest. 1978;61(1):197-208.
28. Gerich JE. Is reduced first-phase insulin release the earliest detectable abnormality in individuals destined to develop type 2 diabetes? Diabetes. 2002;51 Suppl 1:S117-21.
29. Kahn SE. Clinical review 135: The importance of beta-cell failure in the development and progression of type 2 diabetes. The Journal of clinical endocrinology and metabolism. 2001;86(9):4047-58.
30. Wang Z, Thurmond DC. Mechanisms of biphasic insulin-granule exocytosis - roles of the cytoskeleton, small GTPases and SNARE proteins. J Cell Sci. 2009;122(Pt 7):893-903.
31. Ellis AC, Alvarez JA, Granger WM, Ovalle F, Gower BA. Ethnic differences in glucose disposal, hepatic insulin sensitivity, and endogenous glucose production among African American and European American women. Metabolism. 2012;61(5):634-40.
32. Goff LM, Griffin BA, Lovegrove JA, Sanders TA, Jebb SA, Bluck LJ, et al. Ethnic differences in beta-cell function, dietary intake and expression of the metabolic syndrome among UK adults of South Asian, black African-Caribbean and white-European origin at high risk of metabolic syndrome. Diab Vasc Dis Res. 2013;10(4):315-23.
33. Goran MI, Bergman RN, Cruz ML, Watanabe R. Insulin resistance and associated compensatory responses in african-american and Hispanic children. Diabetes Care. 2002;25(12):2184-90.
34. Haffner SM, Howard G, Mayer E, Bergman RN, Savage PJ, Rewers M, et al. Insulin sensitivity and acute insulin response in African-Americans, non-Hispanic whites, and Hispanics with NIDDM: the Insulin Resistance Atherosclerosis Study. Diabetes. 1997;46(1):63-9.
35. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
36. Michaliszyn SF, Lee S, Bacha F, Tfayli H, Farchoukh L, Mari A, et al. Differences in beta-cell function and insulin secretion in Black vs. White obese adolescents: do incretin hormones play a role? Pediatric diabetes. 2017;18:143-51.

37. Piccinini F, Polidori DC, Gower BA, Bergman RN. Hepatic but Not Extrahepatic Insulin Clearance Is Lower in African American Than in European American Women. Diabetes. 2017;66(10):2564-70.
38. Madsbad S, Kehlet H, Hilsted J, Tronier B. Discrepancy between plasma C-peptide and insulin response to oral and intravenous glucose. Diabetes. 1983;32(5):436-8.
39. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, et al. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. The Journal of clinical endocrinology and metabolism. 1986;63(2):492-8.
40. Shuster LT, Go VL, Rizza RA, O'Brien PC, Service FJ. Incretin effect due to increased secretion and decreased clearance of insulin in normal humans. Diabetes. 1988;37(2):200-3.
41. Knutson VP. Cellular trafficking and processing of the insulin receptor. FASEB J. 1991;5(8):2130-8.
42. Meier JJ, Holst JJ, Schmidt WE, Nauck MA. Reduction of hepatic insulin clearance after oral glucose ingestion is not mediated by glucagon-like peptide 1 or gastric inhibitory polypeptide in humans. American journal of physiology Endocrinology and metabolism. 2007;293(3):E849-56.
43. Groop PH, Groop L, Totterman KJ, Fyhrquist F. Relationship between changes in GIP concentrations and changes in insulin and C-peptide concentrations after guar gum therapy. Scand J Clin Lab Invest. 1986;46(6):505-10.
44. Kindmark H, Pigon J, Efendic S. Glucose-dependent insulinotropic hormone potentiates the hypoglycemic effect of glibenclamide in healthy volunteers: evidence for an effect on insulin extraction. The Journal of clinical endocrinology and metabolism. 2001;86(5):2015-9.
45. Rudovich NN, Rochlitz HJ, Pfeiffer AF. Reduced hepatic insulin extraction in response to gastric inhibitory polypeptide compensates for reduced insulin secretion in normal-weight and normal glucose tolerant first-degree relatives of type 2 diabetic patients. Diabetes. 2004;53(9):2359-65.
46. Meier JJ, Gallwitz B, Siepmann N, Holst JJ, Deacon CF, Schmidt WE, et al. The reduction in hepatic insulin clearance after oral glucose is not mediated by gastric inhibitory polypeptide (GIP). Regulatory peptides. 2003;113(1-3):95-100.
47. Ahren B, Thorsson O. Increased insulin sensitivity is associated with reduced insulin and glucagon secretion and increased insulin clearance in man. The Journal of clinical endocrinology and metabolism. 2003;88(3):1264-70.
48. Jones CN, Pei D, Staris P, Polonsky KS, Chen YD, Reaven GM. Alterations in the glucose-stimulated insulin secretory dose-response curve and in insulin clearance in nondiabetic insulin-resistant individuals. J ClinEndocrinolMetab. 1997;82(6):1834-8.
49. Higgins PB, Fernandez JR, Garvey WT, Granger WM, Gower BA. Entero-insular axis and postprandial insulin differences in African American and European American children. The American journal of clinical nutrition. 2008;88(5):1277-83.
50. Velasquez-Mieyer PA, Cowan PA, Umpierrez GE, Lustig RH, Cashion AK, Burghen GA. Racial differences in glucagon-like peptide-1 (GLP-1) concentrations and insulin dynamics during oral glucose tolerance test in obese subjects. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2003;27(11):1359-64.

Supplementary material

Derivation of the formula to compute insulin clearance

The derivation of the formula to compute insulin clearance starts from the general formula:

$$Clearance = \frac{Outflux}{Concentration}$$

Over the 180 minutes of the mixed meal tolerance test or the 120 minutes of the hyperglycemic clamp, the clearance can be computed as a ratio of the total areas under the curves of insulin outflux rate and insulin concentration:

$$Clearance_{Ins} = \frac{AUC_{Outflux}}{AUC_I}$$

However, total insulin outflux equals total insulin secretion rate minus the amount of insulin secreted and not yet irreversibly lost. Then:

$$AUC_{Outflux} = AUC_{ISR} - (I_{Final} - I_{Basal}) \cdot DV_{Ins}$$

in which $I_{Final}$is insulin concentration at the end of the study, I_{Basal} is insulin concentration at the beginning of the study and DV_{Ins} is the volume of distribution of insulin.

Since:

$$DV = Clearance \cdot MRT$$

in which *MRT* is the mean residence time of insulin, it follows:

$$AUC_I \cdot Clearance_{Ins} = AUC_{ISR} - (I_{Final} - I_{Basal}) \cdot MRT_{Ins} \cdot Clearance_{Ins}$$

The final formula becomes:

$$Clearance_{Ins} = \frac{AUC_{ISR}}{AUC_I + (I_{Final} - I_{Basal}) \cdot MRT_{Ins}}$$

As to the value used for MRT_{Ins} in the present paper, we used the values reported by Navalesi R *et al.* (1978) J Clin Invest;61(1):197-208; in that paper, the average MRT in people with type 2 diabetes was ≈27 min, whereas it was ≈18 min in healthy controls.

This formula assumes that at the final time point a new steady state is achieved, i.e. that the plasma compartment is in equilibrium with all the other compartments in which insulin distributes. In the present paper, the almost flat insulin concentration during the last 30 min of the hyperglycemic clamp shows that the above assumption was fulfilled. As to the mixed meal test, a nonsteady state, hallmarked by steadily decreasing insulin concentrations, was still present at the end of the test. However, since the final value of insulin was very close to the basal concentration, the product $(I_{Final} - I_{Basal}) \cdot MRT_{Ins}$ and its potential error were small numbers, which minimally affected the computation of average clearance.

Finally, this formula computes the average insulin clearance over a time interval during which insulin concentration achieves a measured average value (these are the numbers plotted in fig. 4 of the main text), not the insulin clearance at a determined insulin concentration.

Computation of the "meal effect" on the beta cell response to glucose

Computation of the 'meal effect' took advantage of the two reconstructions of beta cell function carried out in each subject, one after intravenous glucose administration (hyperglycemic clamp), the other after oral administration in the mixed meal tolerance test. We report an index case (SDGS003) for the sake of clarity.

The plasma glucose curve of the meal test of SDGS003is shown in Figure S1. Data modelling generated a mathematical reconstruction of the beta cell response to glucose during a mixed meal test which fitted the C-peptide experimental points as shown in Figure S2 with the corresponding insulin secretion rate shown in Figure S3.

The plasma glucose curve of the hyperglycemic clamp of SDGS003 is shown in Figure S4. Data modeling generated a mathematical reconstruction of the beta cell response to glucose during intravenous glucose administration which fitted the C-peptide experimental points as shown in Figure S5 with the corresponding insulin secretion rate shown in Figure S6.

At this point, the mathematical reconstruction of the beta cell response to glucose of SDGS003 during intravenous glucose administration was "fed" with the plasma glucose concentration of Figure S1, i.e. the glucose curve of the meal test, and generated the insulin secretory response of Figure S7. The meal effect on beta cell function was computed with the formula:

$$Meal\ effect = \frac{AUC\ ISR_{Meal} - AUC\ ISR_{Intravenous}}{AUC\ ISR_{Meal}}$$

in which $AUC\ ISR_{Meal}$ is the area under the curve of Figure S3 and $AUC\ ISR_{Intravenous}$ is the area under the curve of Figure S7. Both areas were computed by the SAAM II 1.2 modeling software.

Nonlinear regression analysis of the relationship between insulin clearance and insulin concentration

Figure S8 shows the plot of mean insulin concentration during either the hyperglycemic clamp or the meal tolerance test (x axis) and average insulin clearance (y axis). The relationship is strongly nonlinear and apparently hyperbolic, as confirmed by regression analysis (linear regression: R^2 0.338; hyperbolic relationship: R^2 0.834; $p<0.01$); in agreement with existing evidence (Cobelli C. & Pacini G. (1988) Diabetes; 37(2):223-31. Van Cauter E *et al.* (1992) Diabetes; 41(3):368-77), we did not find this to be the case when we investigated the relationship between c-peptide concentration and c-peptide clearance, no inverse relationship was evident ($p>0.05$; Figure S9). For the insulin data the best fitting hyperbola was found by identifying the unknown parameters b1 and b2 of the following equation:

$y = b1 + (b2/x)$

We then tested the hypothesis that the hyperbola describing the relationship average insulin clearance/concentration may not be the one and the same in WEM and BAM. To do so, we repeated the nonlinear regression analysis with the following equation:

$y = (b1+b3*Ethnicity)+[(b2+b4*Ethnicity)/x]$

in which Ethnicity takes the value 0 or 1 if the individual is WEM or BAM, respectively. If the same hyperbola can describe WEM and BAM together, b3 and b4 will not be statistically different from 0. The unknown parameter b3, but not b4, turned out to be statistically different from 0. Thus, two different hyperbolas (fig 4 of the main paper) are needed to best describe the relationship between average insulin clearance and average insulin concentration in WEM and BAM.

Figures

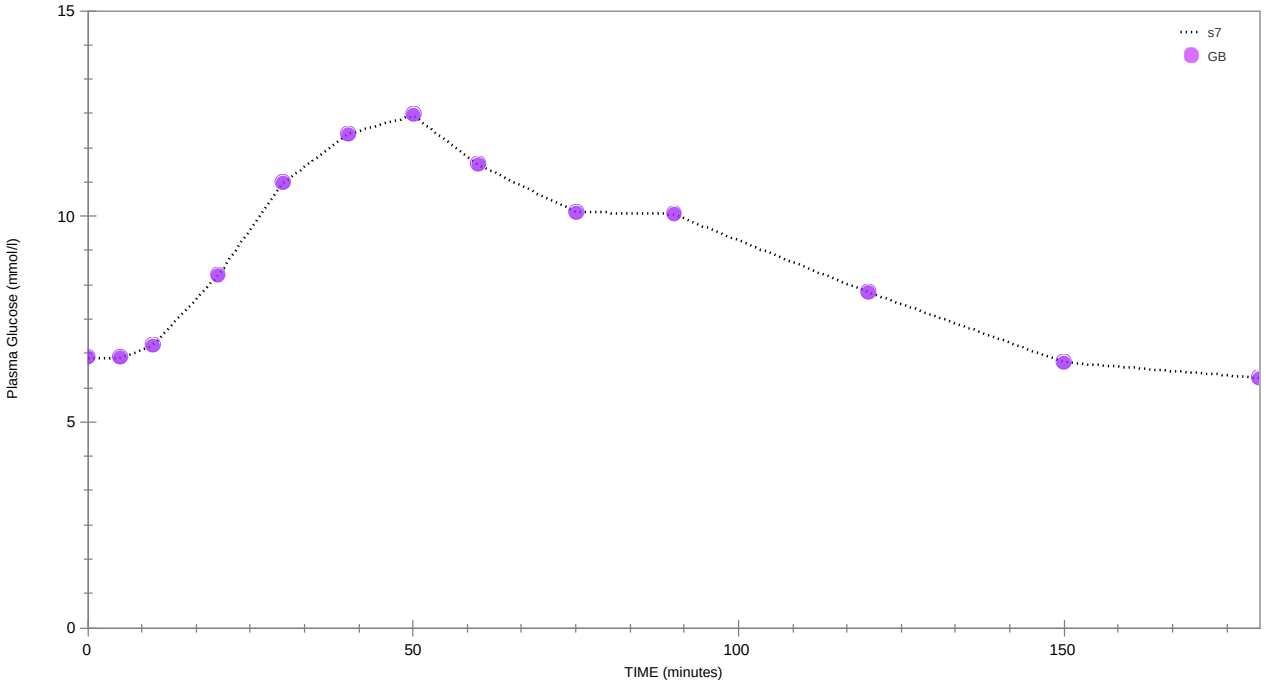

Fig. S1. Plasma glucose experimental points (filled circles) and input function (dotted line) of the mathematical model of beta cell function during the MMTT in the index case SDGS003

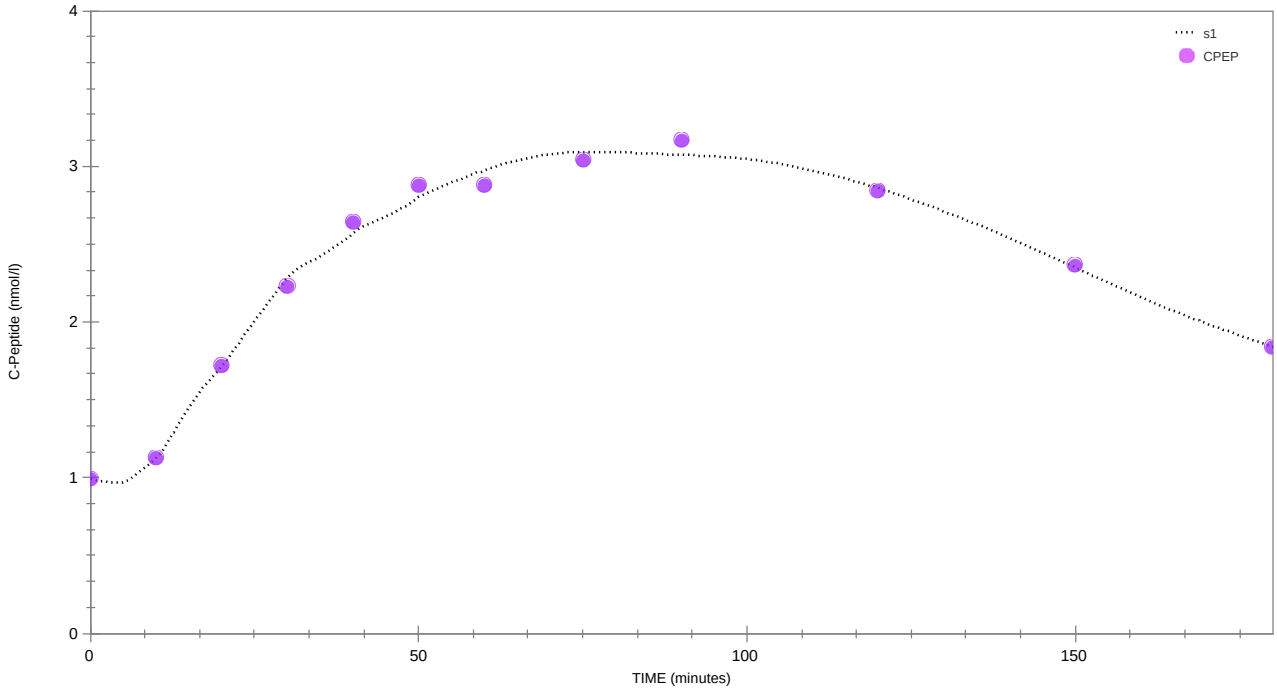

Fig. S2. Plasma C-peptide experimental points (filled circles) and model fit (dotted line) to the data of the MMTT in the index case SDGS003

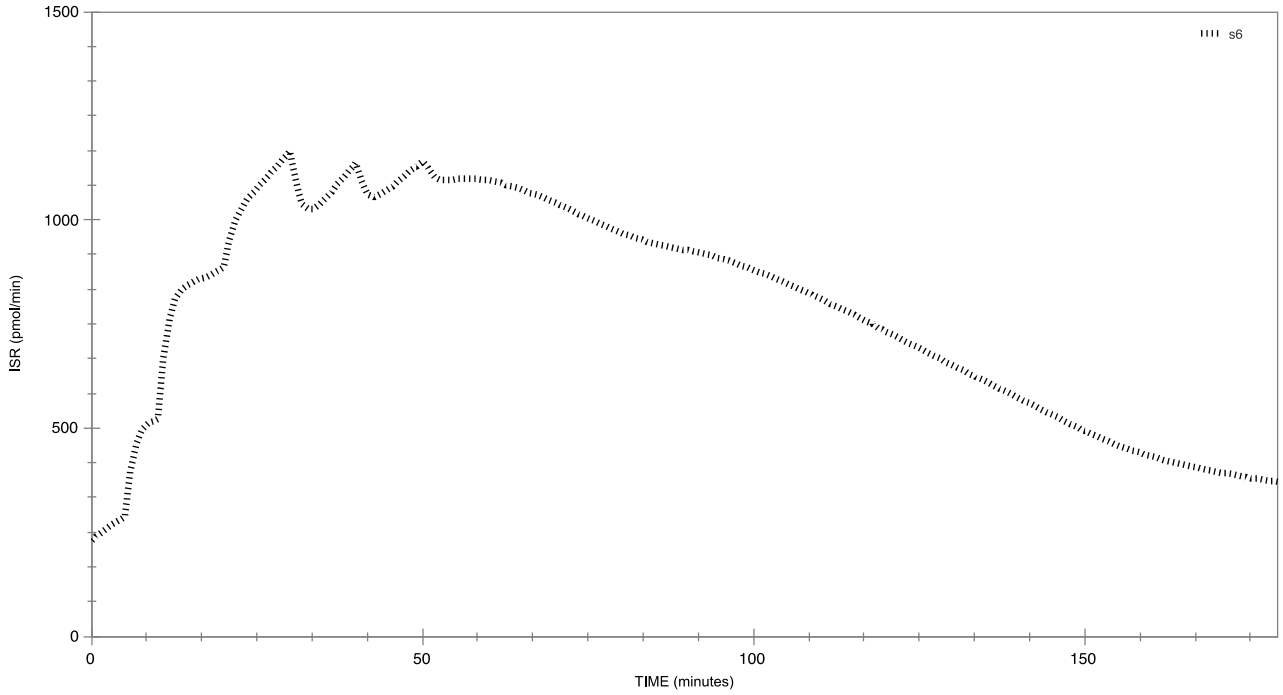

Fig. S3. Insulin secretion rate (dotted line) during the MMTT in the index case SDGS003 as computed by the mathematical model.

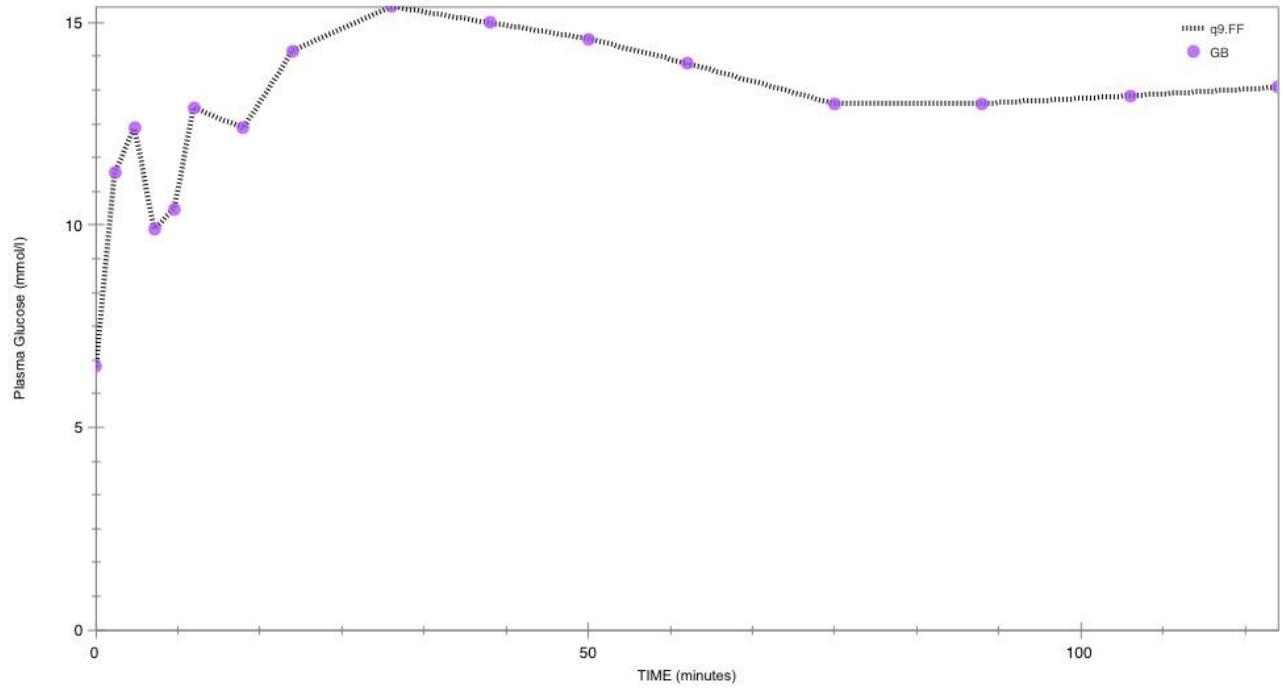

Fig. S4. Plasma glucose experimental points (filled circles) and input function (dotted line) of the mathematical model of beta cell function during the hyperglycemic clamp in the index case SDGS003

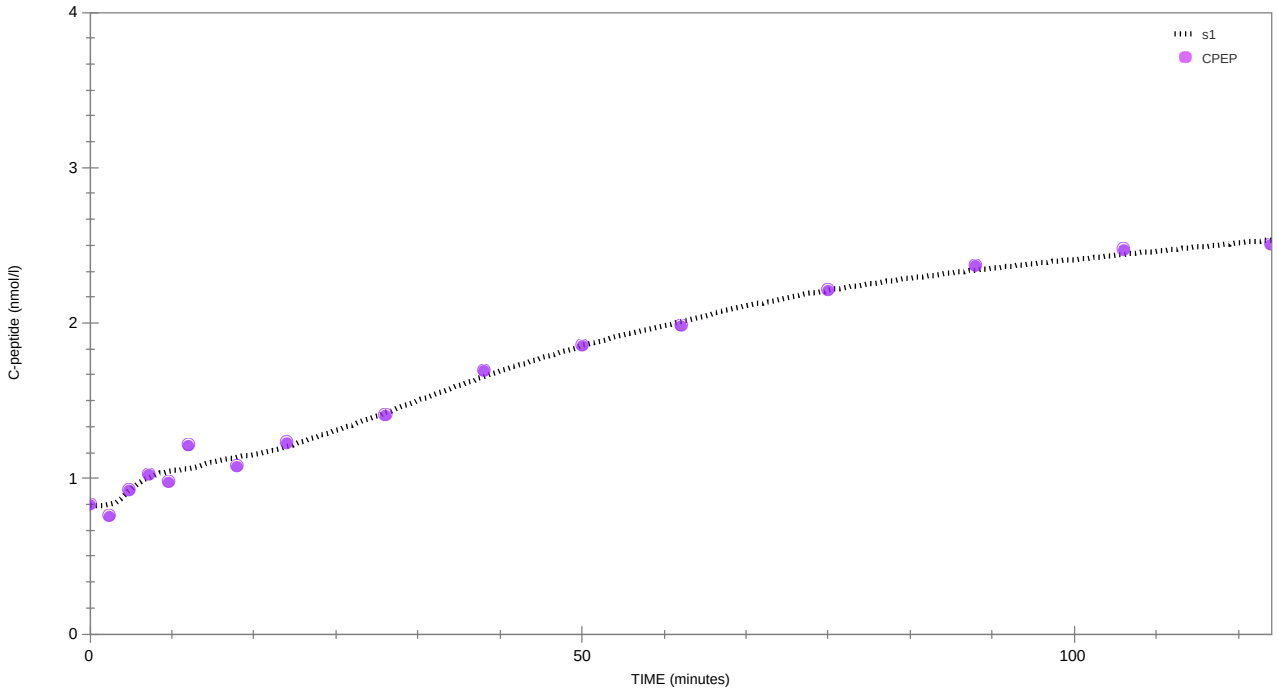

Fig. S5. Plasma C-peptide experimental points (filled circles) and model fit (dotted line) to the data of the MMTT in the index case SDGS003.

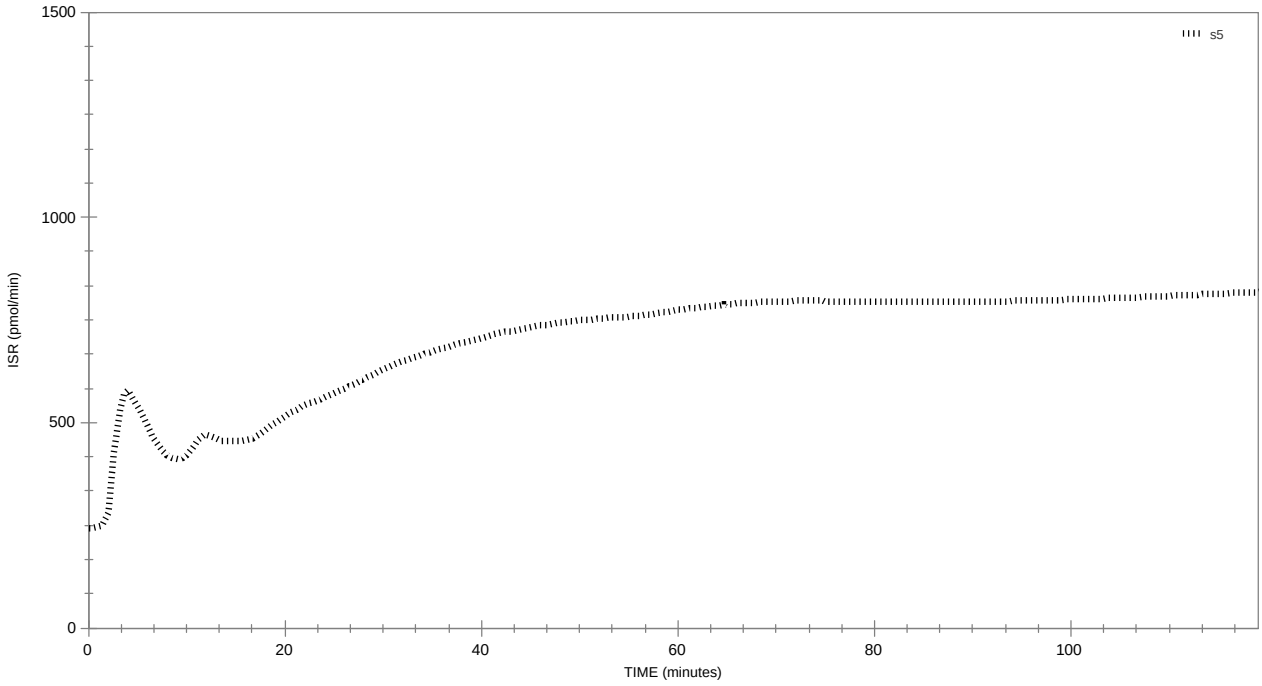

Fig. S6. Insulin secretion rate (dotted line) during the hyperglycemic clamp in the index case SDGS003 as computed by the mathematical model.

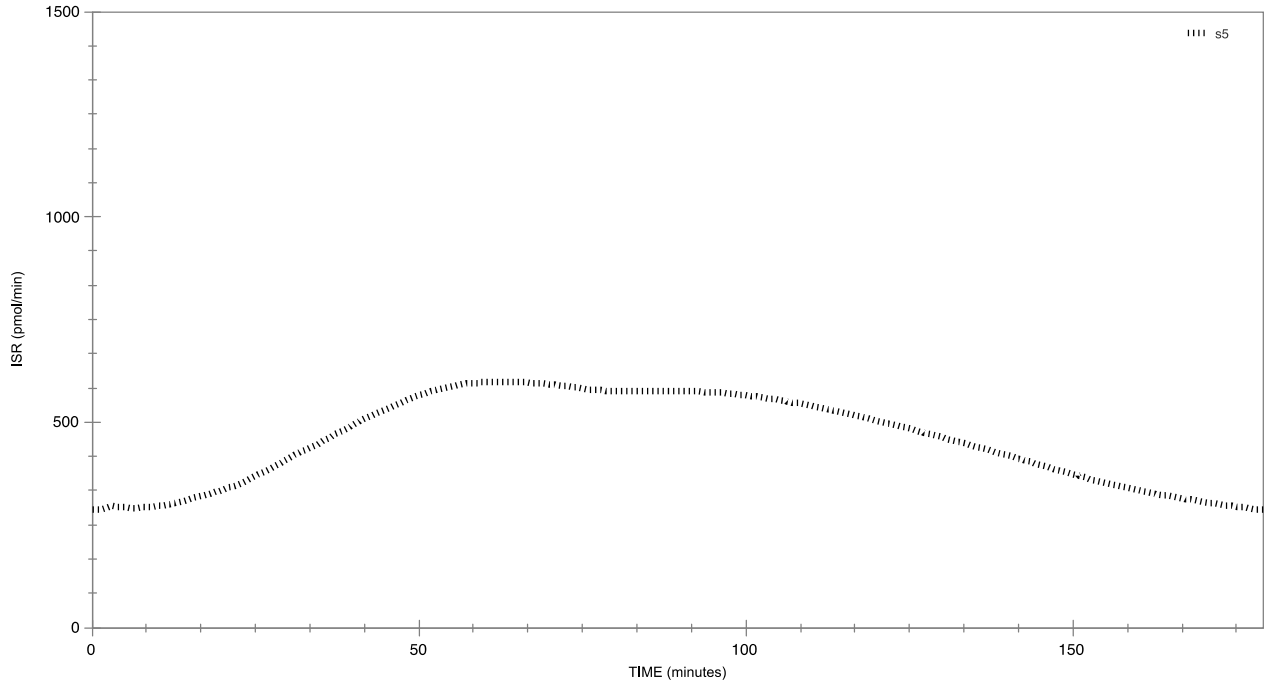

Fig. S7. Insulin secretion rate (dotted line) computed by the model of beta cell function reconstructed during the hyperglycemic clamp, when the plasma glucose input function is the one of the MMTT (fig. S2), not the one of the hyperglycemic clamp (fig. S5), in the index case SDGS003.

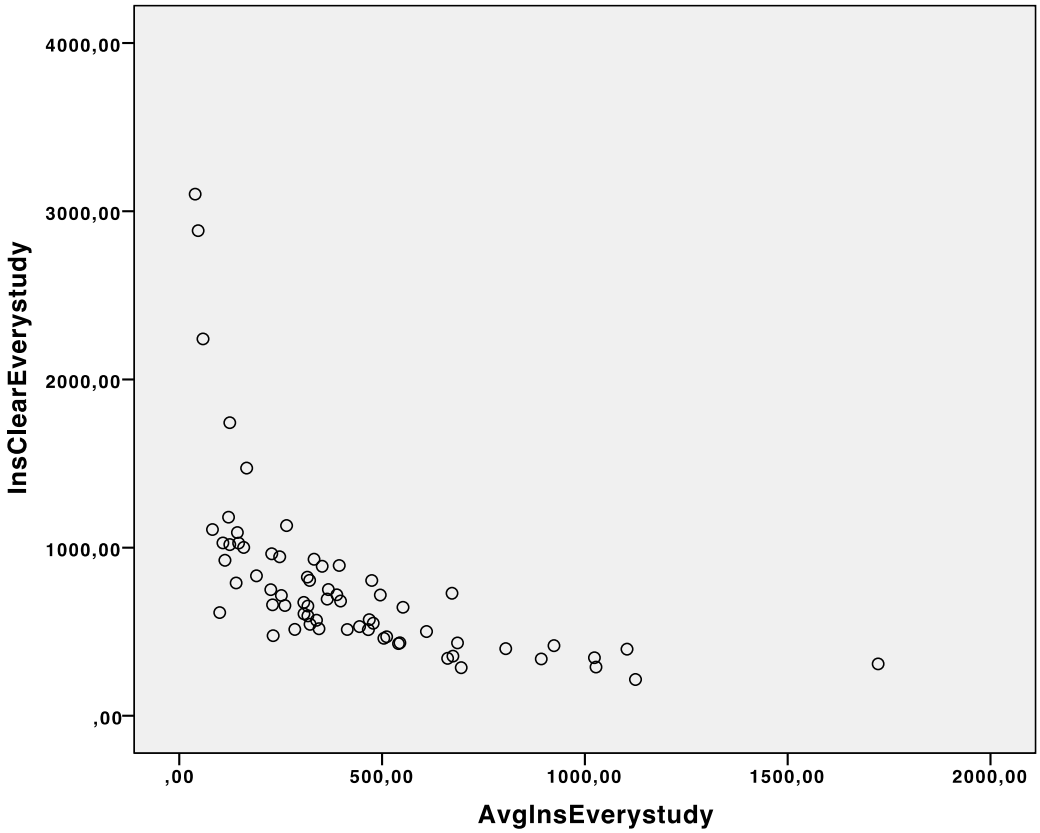

Fig S8. Average insulin concentration ($pmol \cdot l^{-1}$, x axis) and average insulin clearance ($pmol \cdot min^{-1} \cdot m^{-2}$ BSA, y axis) in all the tests (meal tolerance test and hyperglycemic clamps) reported in the present paper.

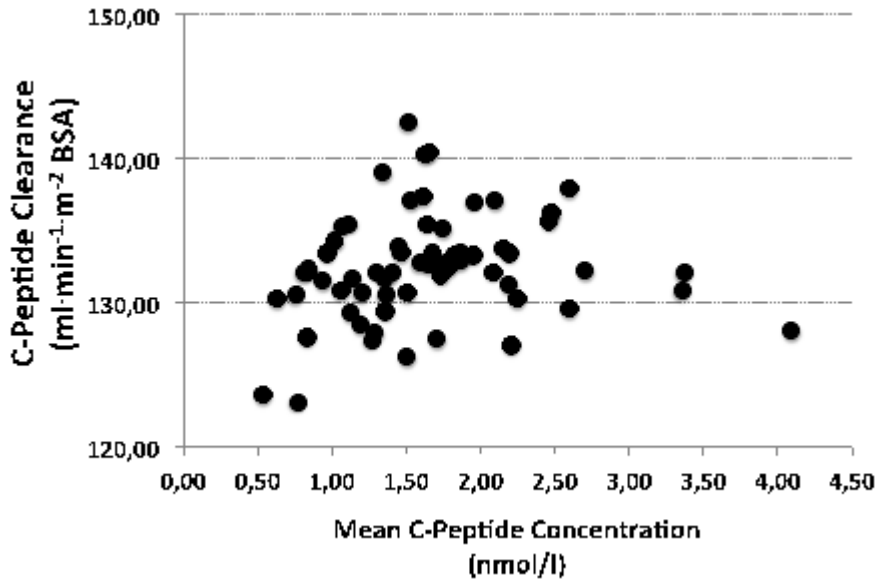

Fig S9. Average c-peptide concentration (nmol·l^{-1}, x axis) and average c-peptide clearance (ml·min^{-1}·m^{-2} BSA, y axis) in all the tests (meal tolerance test and hyperglycemic clamps) reported in the present paper.