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

3

4 **Short title: Ethnicity and type 2 diabetes pathophysiology**

5

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21

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25 **ABSTRACT**

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

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

35 RESULTS. With comparable post-challenge glucose responses, BAM exhibited lower second  
36 phase c-peptide response to intravenous (BAM 70.6 vs WEM 115.1nmol/l min<sup>-1</sup> (ratio of  
37 geometric mean 0.55, 95%CI 0.37,0.83)  $p=0.006$ ) and oral (BAM 65.4 vs WEM 88.5nmol/l  
38 min<sup>-1</sup> (mean difference -23.2 (95%CI -40.0,-6.3)  $p=0.009$ ) glucose. BAM peripheral insulin  
39 response to oral glucose was preserved (BAM 47.4 vs WEM 59.4nmol/l min<sup>-1</sup> (ratio of  
40 geometric mean 0.89 (95%CI 0.59,1.35)  $p=0.566$ ), with relative reductions in insulin clearance  
41 (BAM 506.2 vs WEM 630.1 mL/m<sup>2</sup> BSA min<sup>-1</sup> (mean difference -123.9 (95%CI -270.5, 22.6)  
42  $p=0.095$ ), associated with enhanced incretin responses (GIP iAUC: BAM 46.8 vs WEM  
43 33.9 $\mu$ g/l min<sup>-1</sup> (mean difference 12.9 (95%CI 2.1,23.7)  $p=0.021$ ).

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

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

50 **Abbreviations:**

51 BAM: Black West African men

52 CRF: Clinical research facility

53 MRI: Magnetic resonance imaging

54 NEFA: Non-esterified fatty acids

55 VAT: Visceral adipose tissue

56 WC: Waist circumference

57 WEM: White European men

58

59

## 60 INTRODUCTION

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

63 The main pathophysiological processes of insulin secretory failure and insulin resistance that  
64 underlie T2D are well documented (4) but differences in the pathogenesis based on ethnicity  
65 are increasingly recognised. There is a growing literature examining metabolism in non-  
66 diabetic Black populations, with studies in non-diabetic African-American children and  
67 adolescents describing marked hyperinsulinaemia compared to other ethnicities (5-11) and  
68 extensive reports that Black populations, both indigenous (12) and diasporic (13-19), exhibit a  
69 hyperinsulinaemic response to glucose. Conventionally hyperinsulinaemia is understood to  
70 occur in response to heightening insulin resistance, however this does not fully explain the  
71 response in Black populations (6, 9, 11). Studies in children measuring c-peptide have  
72 described a combination of increased insulin secretion and reduced hepatic insulin clearance  
73 (7, 9). Studies in healthy and prediabetic adults have shown lower rates of insulin clearance  
74 (13, 15, 19) but heterogeneity in the populations has made independence from insulin resistance  
75 and body weight/composition differences difficult to ascertain. If intensified hyperinsulinaemia  
76 represents greater insulin secretion, it may predispose to earlier beta-cell exhaustion in the  
77 development of T2D. To date no studies have undertaken comparisons of beta-cell function in  
78 Black African and White European populations with recent-onset T2D. As this may be the time  
79 people first present to health services, this is an important phase to understand.

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

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

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

92

## 93 **MATERIALS & METHODS**

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

### 98 **Participants**

99 Men of Black West African or White European ethnicity (self-declared, confirmed by  
100 grandparental birthplace), aged 18-65 years, BMI 25-35 kg/m<sup>2</sup>, with a documented diagnosis  
101 of T2D within 5 years, treated with lifestyle advice ± metformin, and HbA<sub>1c</sub> ≤63·9 mmol/mol  
102 (<8%) were recruited from South London General Practices taking part in an early detection  
103 T2D screening programme (20). Participants were deemed ineligible if: treated with other  
104 diabetes medications, chronic oral steroids, beta-blockers; serum creatinine >150 mmol/l;  
105 serum alanine transaminase level >2.5-fold above the upper limit of the reference range;  
106 positive auto-antibodies for anti-insulin, anti-GAD or anti-A2; sickle cell disease (trait  
107 permitted); or medications believed to affect the outcome measures. Participants completed a  
108 medical screening before study entry. BAM were matched with WEM for age (± 5 years) and  
109 BMI (± 3 kg/m<sup>2</sup>).

### 110 **Study design**

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

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

119 *Hyperglycemic clamp assessment of first and second phase insulin secretory function.* A two-  
120 hour hyperglycaemic clamp was conducted (21). Participants were weighed in light clothing  
121 and their body surface area (BSA) calculated (22). An antecubital fossa vein was cannulated  
122 for administration of intravenous glucose; a second cannula was inserted retrogradely into the  
123 dorsum of the hand, and placed in a warming unit, to achieve arterialised venous blood samples.  
124 Three fasting samples (-20, -10 and 0 minutes) were collected before starting the glucose  
125 infusion (20% glucose) at time 0 minutes; a priming regimen, based on BSA (23), was used  
126 for the first 15 minutes to increase rapidly the plasma glucose to 6.9 mmol/l above fasting. The  
127 glucose infusion rate was then adjusted to maintain plasma glucose at 6.9 mmol/l above fasting  
128 for a further 105 minutes. Blood sampling occurred every 2 minutes for the first 10 minutes  
129 and every 5 minutes thereafter to inform adjustment of the glucose infusion rate for ‘clamping’  
130 the plasma glucose. Blood samples were drawn at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 75, 90,  
131 105 and 120 minutes for the assessment of plasma glucose, and serum insulin and c-peptide.

132 *Mixed meal tolerance test assessment of insulin and incretin secretion.* A three-hour meal  
133 tolerance test was conducted using a liquid milkshake (Ensure Plus, Abbott Nutrition, UK),  
134 providing 6 kcal/kg body weight. An antecubital fossa vein was cannulated for blood  
135 sampling. Following the collection of fasting samples at time -10 and 0 minutes the participants  
136 consumed the drink within 5 minutes. Blood was collected at 10, 20, 30, 40, 50, 60, 75, 90,  
137 120, 150 and 180 minutes for the assessment of glucose, non-esterified fatty acids (NEFA),  
138 insulin and c-peptide, and for GLP-1 and GIP at 30, 60 and 120 minutes.

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



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

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

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

158 Model-based measurement of beta-cell function: the glucose, insulin and c-peptide curves  
159 during the hyperglycaemic clamp and meal tolerance test were modelled using methods  
160 previously described (24-26) (SAAM-II 1.2 software; SAAM Institute, Seattle, WA). The main  
161 outputs of the hyperglycaemic clamp model are: glucose sensitivity of first-phase secretion  
162 ( $\sigma^1$ ), expressed as the amount of insulin secreted in response to a rate of increase in glucose of  
163 1 mmol/l between time 0 and 1 min of the study, in  $(pmol \cdot m^{-2} BSA)/(mmol \cdot l^{-1} \cdot min^{-1})$ ;  
164 glucose sensitivity of second-phase secretion ( $\sigma^2$ ), expressed as the steady state insulin

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

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

$$175 \quad \text{Clearance}_{Ins} = \frac{AUC_{ISR}}{AUC_I + (I_{Final} - I_{Basal}) \cdot MRT_{Ins}}$$

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

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

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

$$189 \text{ Meal effect} = \frac{AUC\ ISR_{Meal} - AUC\ ISR_{Intravenous}}{AUC\ ISR_{Meal}}$$

190 Further details of the computation of the ‘meal effect’ are provided in *Supplementary Material*.

## 191 **Statistics**

192 All datasets were tested for normality (Shapiro-Wilks test) and non-normally distributed  
193 variables were transformed (log 10) for analysis. Normally-distributed data are expressed as  
194 mean ± standard deviation, and log-normal data were back transformed to give geometric mean  
195 and 95% CI for the ratio of the geometric mean. Differences between ethnic groups were  
196 determined by independent samples t-test using the raw data where they were normally  
197 distributed or logarithmic-transformed data where not.  $p \leq 0.05$  was considered statistically  
198 significant. Note that for the data analysed on the natural scale, the null value is 0 and so where  
199  $p < 0.05$ , the 95% CI will exclude 0 but for the data analysed on the log scale and back-  
200 transformed to give the ratio of geometric means, the null value is 1 and so where  $p < 0.05$ , the  
201 95% CI will exclude 1. The relationship between average insulin clearance and average insulin  
202 concentration was analysed by linear and nonlinear regression analysis, as described in  
203 *Supplementary Material*. Analyses were performed using SPSS software, version 24 (IBM  
204 Analytics, NY).

205

## 206 RESULTS

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

### 215 *Beta-cell insulin secretory function*

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

223 During the meal tolerance test the two ethnic groups exhibited the same glucose response,  
224 however, mean c-peptide iAUC was significantly lower in BAM. The meal insulin iAUC was  
225 not significantly different between ethnic groups (Table 3, Figure 2). The modelled data from  
226 the meal tolerance test showed no significant ethnic differences in first-phase insulin secretory  
227 function (Table 3) but second-phase secretory function was lower in BAM ( $p=0.01$ ). The  
228 insulin secretion rate was lower amongst BAM at 4 ( $p=0.019$ ) and 5.5 mmol/l ( $p=0.02$ ). This

229 difference was lost at higher glucose concentrations of 8 mmol/l ( $p=0.112$ ), 11 ( $p=0.199$ ) and  
230 15 mmol/l ( $p=0.247$ ) (Figure 3).

### 231 *Insulin clearance*

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

### 239 *Incretin responses*

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

243

244

## 245 **DISCUSSION**

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

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

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

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

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

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

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

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



319 induction of hyperglycaemia (38-40). Reduction in insulin clearance upon increasing levels of  
320 insulin secretion is proposed to be due to the saturable nature of hepatocellular insulin receptors  
321 (41, 42). However there is also evidence that incretin hormones affect insulin clearance (39,  
322 40, 43). There has been very little study of incretin hormones and how these vary according to  
323 ethnicity. In the current study, BAM exhibited significantly higher postprandial GIP  
324 concentrations, which may have contributed to the non-significant trend for lower average  
325 insulin clearance that was observed. The effect of GIP on insulin clearance is unclear; some  
326 authors have demonstrated an insulin clearance reducing effect of GIP (44, 45), whilst others  
327 have shown no effect (42, 46). Some of the conflict in these findings may have occurred  
328 because insulin clearance appears to adapt to insulin resistance and glucose intolerance, a  
329 potential mechanism by which beta-cell function is preserved in the progression to T2D (47,  
330 48). There has been very little investigation of incretin hormones within Black populations and  
331 in those which have the focus has been on the role of incretins in the upregulation of insulin  
332 secretion; African-American children have been reported to have lower GLP-1, but similar GIP  
333 secretion compared to European-American children (49) whereas in a study of Black and White  
334 obese adolescents, Michaliszyn *et al* (2017) reported no difference in GLP-1 or GIP amongst  
335 Blacks (36), and Velasquez-Mieyer *et al.* (2003) found higher GLP-1 in obese African-  
336 American adults compared with European-Americans, with no measurement of GIP (50). We  
337 modelled the impact of the mixed meal, including, but not limited to, the incretin response on  
338 insulin secretory function ('meal effect'), but detected no ethnic differences. Michaliszyn *et al*  
339 (2017) modelled the 'potentiation factor', which describes the modulation of the relationship  
340 between glucose concentration and insulin secretion and comprises several mechanisms  
341 including the release of endogenous incretin hormones. In contrast to our data they found no  
342 differences in incretin concentrations in response to an oral glucose challenge but report a  
343 significantly higher early potentiation factor in Blacks (36). Our data suggest that by the time

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

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

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

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

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

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

410 **Author contributions**

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

417

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

	<b>BAM (n = 19)</b>	<b>WEM (n = 15)</b>	<b>Mean difference (95% CI)</b>	<b>p-value</b>
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 <sup>2</sup> )	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 <sup>2</sup> ) <sup>†</sup>	130.8 (54.1)	189.0 (75.7)	-58.2 (-104.2 to -12.2)	<b>0.015</b>
Thigh skeletal muscle area (cm <sup>2</sup> ) <sup>†</sup>	434.2 (49.6)	379.2 (57.2)	55.0 (17.0 to 93.0)	<b>0.006</b>
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. <sup>†</sup>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).

**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) <sup>#</sup>	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 <sup>-1</sup> ) <sup>#</sup>	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 <sup>-1</sup> ) <sup>#</sup>	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)	<b>0.004</b>
C-peptide iAUC 0-10 mins (nmol/l min <sup>-1</sup> ) <sup>#</sup>	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 <sup>-1</sup> ) <sup>#</sup>	70.6 (52.5 to 94.8)	115.1 (84.8 to 156.3)	0.55 (0.37 to 0.83)	<b>0.006</b>
$\sigma^1$ [(pmol/m <sup>2</sup> BSA)/(mmol/l min <sup>-1</sup> )] <sup>#</sup>	65.6 (27.0 to 159.2)	95.3 (42.5 to 213.8)	0.69 (0.21 to 2.20)	0.507
$\sigma^2$ [(pmol min <sup>-1</sup> m <sup>2</sup> BSA)/mmol/l] <sup>#</sup>	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 <sup>2</sup> BSA min <sup>-1</sup> )	167.2 (38.4)	185.4 (37.0)	-18.2 (-44.7 to 8.4)	0.173
Average insulin clearance (mL/m <sup>2</sup> BSA min <sup>-1</sup> ) <sup>#</sup>	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<sup>#</sup>. Positively skewed data were transformed ( $\log^{10}$ ) prior to statistical testing. Differences between ethnic groups tested using independent samples t-test.  $\sigma^1$ , glucose sensitivity of  $\beta$  cell during first-phase insulin secretion;  $\sigma^2$ , glucose sensitivity of  $\beta$  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.

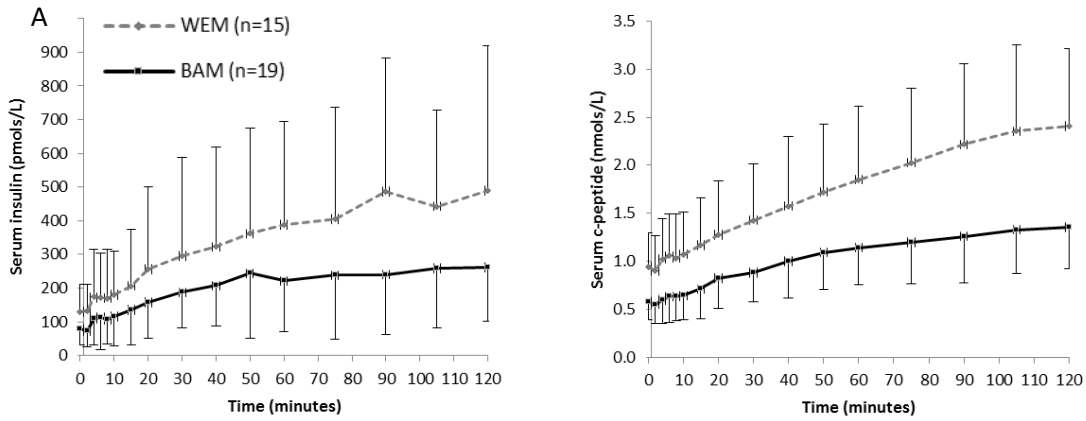
**Table 3. Meal tolerance test assessment of insulin secretory function in Black African and White European participants**

	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 <sup>-1</sup> ) <sup>#</sup>	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) <sup>#</sup>	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 <sup>-1</sup> ) <sup>#</sup>	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)	<b>0.008</b>
c-peptide iAUC (nmol/l min <sup>-1</sup> )	65.4 (17.7)	88.5 (29.4)	-23.2 (-40.0 to -6.3)	<b>0.009</b>
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 <sup>-1</sup> ) <sup>#</sup>	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 <sup>-1</sup> )	46.8 (17.4)	33.9 (12.0)	12.9 (2.1 to 23.7)	<b>0.021</b>
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 <sup>-1</sup> )	-52576 (26325)	-61337 (26442)	8760 (-10047 to 27568)	0.349
σ <sup>1</sup> [(pmol/m <sup>2</sup> BSA)/(mmol/l min <sup>-1</sup> )]	1420.1 (1184.2)	1134.6 (710.7)	285.5 (-447.2 to 1018.2)	0.432
Average insulin clearance (mL/m <sup>2</sup> BSA min <sup>-1</sup> )	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<sup>#</sup>. Positively skewed data transformed (log<sup>10</sup>) prior to statistical testing. Differences between ethnic groups tested using independent samples t-test. σ<sup>1</sup>, 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.



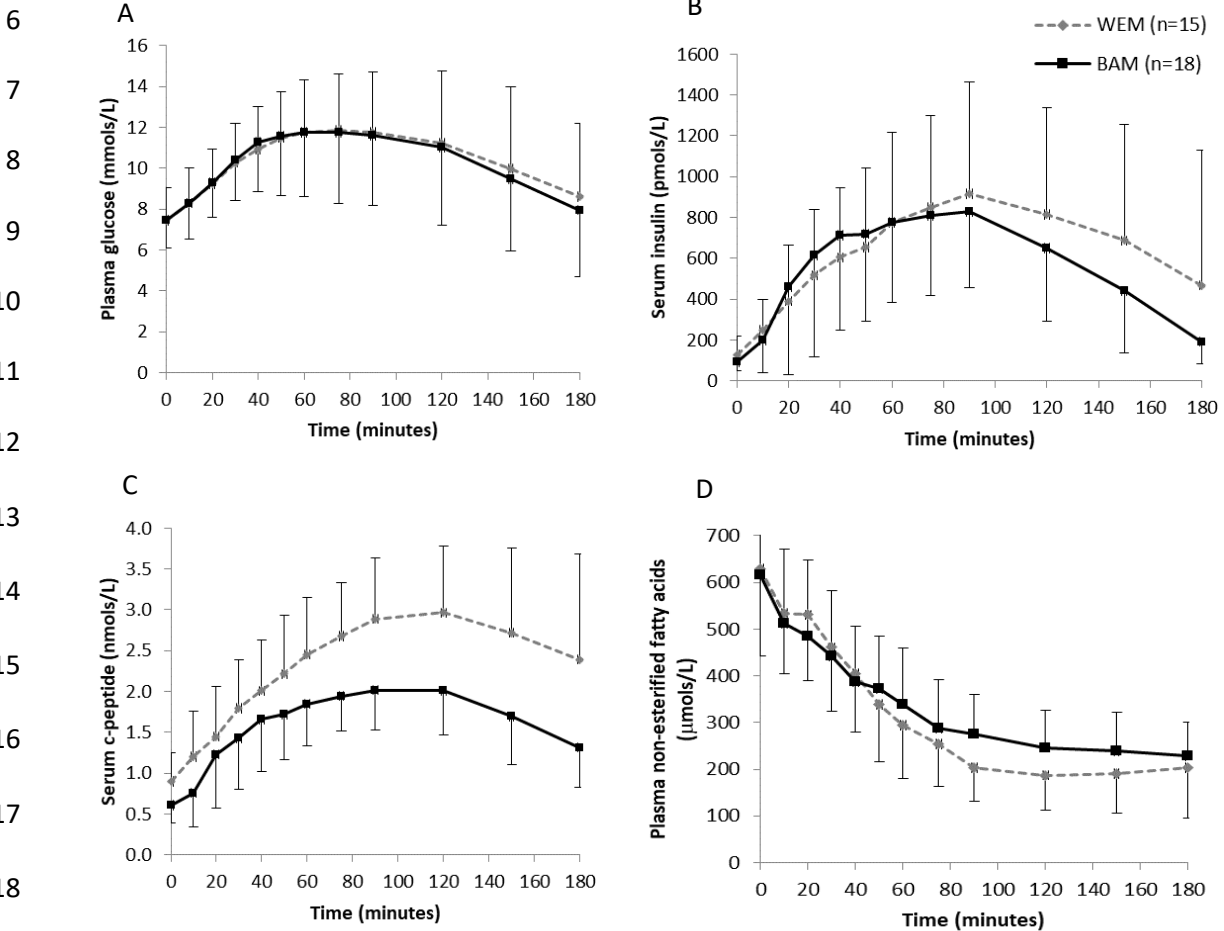
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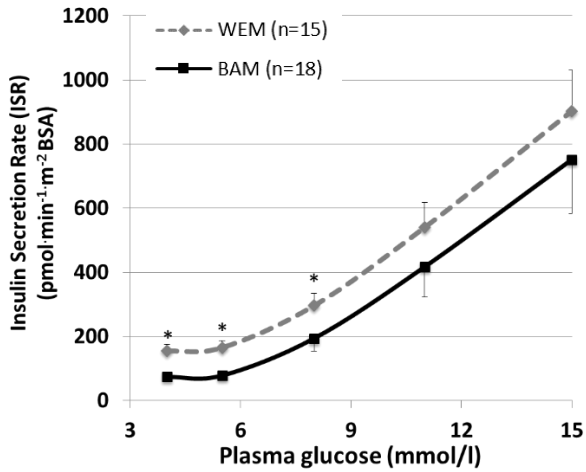
3 Figure 1. Serum insulin (A) and c-peptide (B) responses in the hyperglycaemic clamp in BAM  
4 and WEM

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19  
20 Figure 2. Plasma glucose (A), serum insulin (B), c-peptide (C), and non-esterified fatty acid  
21 (D) responses to a mixed meal tolerance test in BAM and WEM

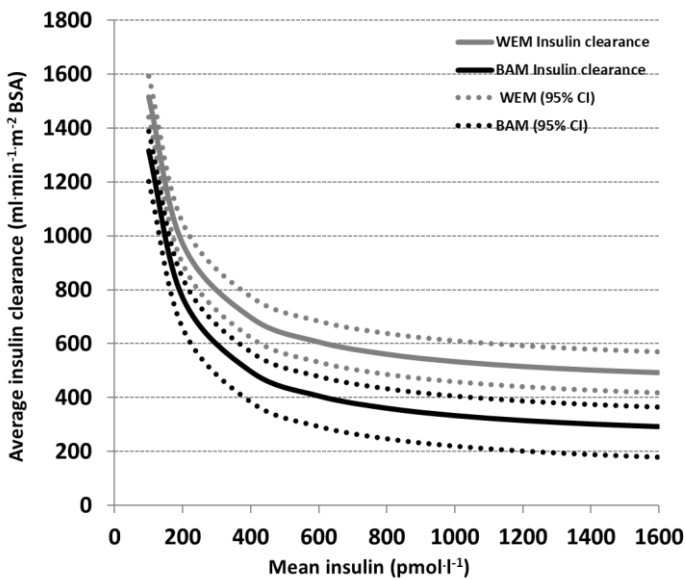
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24 Figure 3. Insulin secretion rates at 5 increasing plasma glucose concentrations during the meal  
 25 tolerance test, as reconstructed by mathematical modelling of beta cell function, in BAM and  
 26 WEM.

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30 Figure 4. Relationship between average insulin clearance and average insulin concentration  
 31 during the hyperglycemic clamp and the mixed meal test in BAM and WEM. Average insulin  
 32 clearance (ml·min⁻¹·m⁻²·BSA): BAM = 224 + (109151/average insulin concentration), WEM =  
 33 425 + (109151/average insulin concentration).

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179

180

## Supplementary material

181

182

### 183 Derivation of the formula to compute insulin clearance

184

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

$$186 \quad \text{Clearance} = \frac{\text{Outflux}}{\text{Concentration}}$$

187

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

$$191 \quad \text{Clearance}_{Ins} = \frac{AUC_{Outflux}}{AUC_I}$$

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

$$194 \quad AUC_{Outflux} = AUC_{ISR} - (I_{Final} - I_{Basal}) \cdot DV_{Ins}$$

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

197 Since:

$$198 \quad DV = \text{Clearance} \cdot \text{MRT}$$

199 in which  $\text{MRT}$  is the mean residence time of insulin, it follows:

200

$$201 \quad AUC_I \cdot \text{Clearance}_{Ins} = AUC_{ISR} - (I_{Final} - I_{Basal}) \cdot \text{MRT}_{Ins} \cdot \text{Clearance}_{Ins}$$

202

203 The final formula becomes:

$$204 \quad \text{Clearance}_{Ins} = \frac{AUC_{ISR}}{AUC_I + (I_{Final} - I_{Basal}) \cdot \text{MRT}_{Ins}}$$

205

206 As to the value used for  $MRT_{Ins}$  in the present paper, we used the values reported by Navalesi R *et al.*  
207 (1978) J Clin Invest;61(1):197-208; in that paper, the average MRT in people with type 2 diabetes  
208 was  $\approx 27$  min, whereas it was  $\approx 18$  min in healthy controls.

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

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

220

#### 221 **Computation of the “meal effect” on the beta cell response to glucose**

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

226 The plasma glucose curve of the meal test of SDGS003 is shown in Figure S1. Data modelling generated  
227 a mathematical reconstruction of the beta cell response to glucose during a mixed meal test which fitted  
228 the C-peptide experimental points as shown in Figure S2 with the corresponding insulin secretion rate  
229 shown in Figure S3.

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

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

238

239 
$$\text{Meal effect} = \frac{AUC\ ISR_{Meal} - AUC\ ISR_{Intravenous}}{AUC\ ISR_{Meal}}$$

240

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

243

244 **Nonlinear regression analysis of the relationship between insulin clearance and insulin**  
245 **concentration**

246 Figure S8 shows the plot of mean insulin concentration during either the hyperglycemic clamp or the  
247 meal tolerance test (x axis) and average insulin clearance (y axis). The relationship is strongly nonlinear  
248 and apparently hyperbolic, as confirmed by regression analysis (linear regression:  $R^2$  0.338; hyperbolic  
249 relationship:  $R^2$  0.834;  $p < 0.01$ ); in agreement with existing evidence (Cobelli C. & Pacini G. (1988)  
250 Diabetes; 37(2):223-31. Van Cauter E *et al.* (1992) Diabetes; 41(3):368-77), we did not find this to be  
251 the case when we investigated the relationship between c-peptide concentration and c-peptide clearance,  
252 no inverse relationship was evident ( $p > 0.05$ ; Figure S9). For the insulin data the best fitting hyperbola  
253 was found by identifying the unknown parameters  $b_1$  and  $b_2$  of the following equation:

254 
$$y = b_1 + (b_2/x)$$

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

258 
$$y = (b_1 + b_3 * \text{Ethnicity}) + [(b_2 + b_4 * \text{Ethnicity})/x]$$

259 in which Ethnicity takes the value 0 or 1 if the individual is WEM or BAM, respectively. If the same  
260 hyperbola can describe WEM and BAM together,  $b_3$  and  $b_4$  will not be statistically different from 0.  
261 The unknown parameter  $b_3$ , but not  $b_4$ , turned out to be statistically different from 0. Thus, two different  
262 hyperbolas (fig 4 of the main paper) are needed to best describe the relationship between average insulin  
263 clearance and average insulin concentration in WEM and BAM.

264

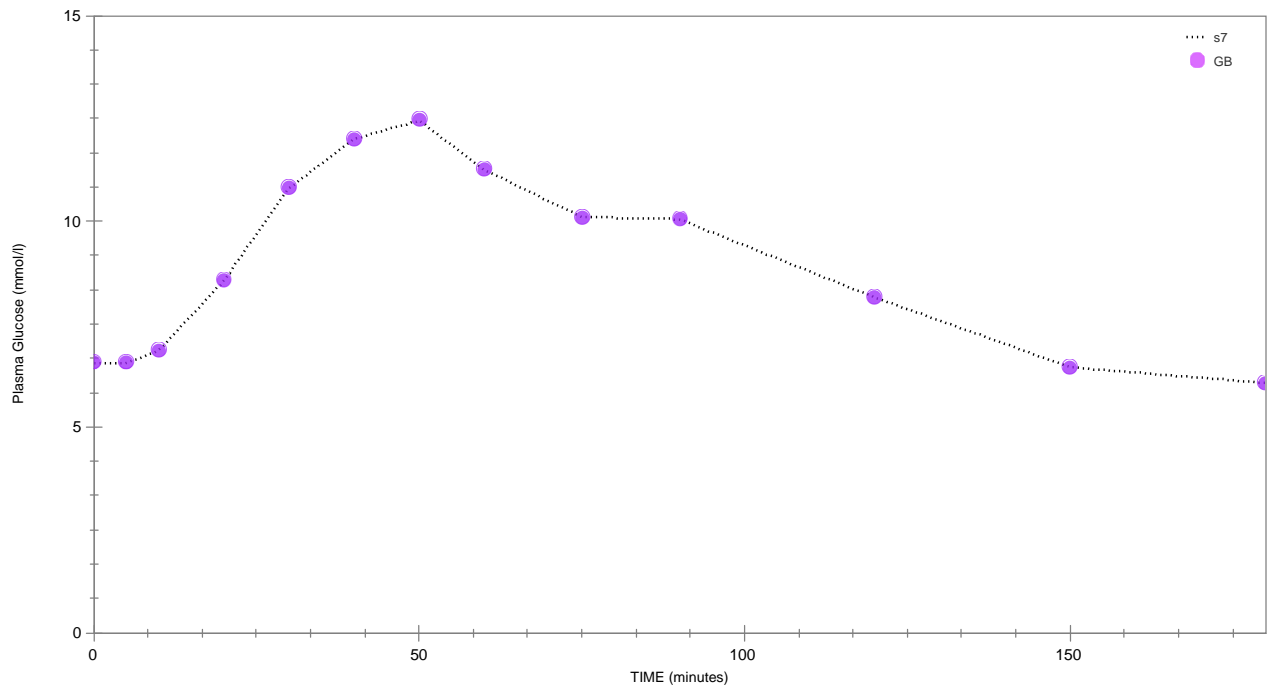
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### Figures

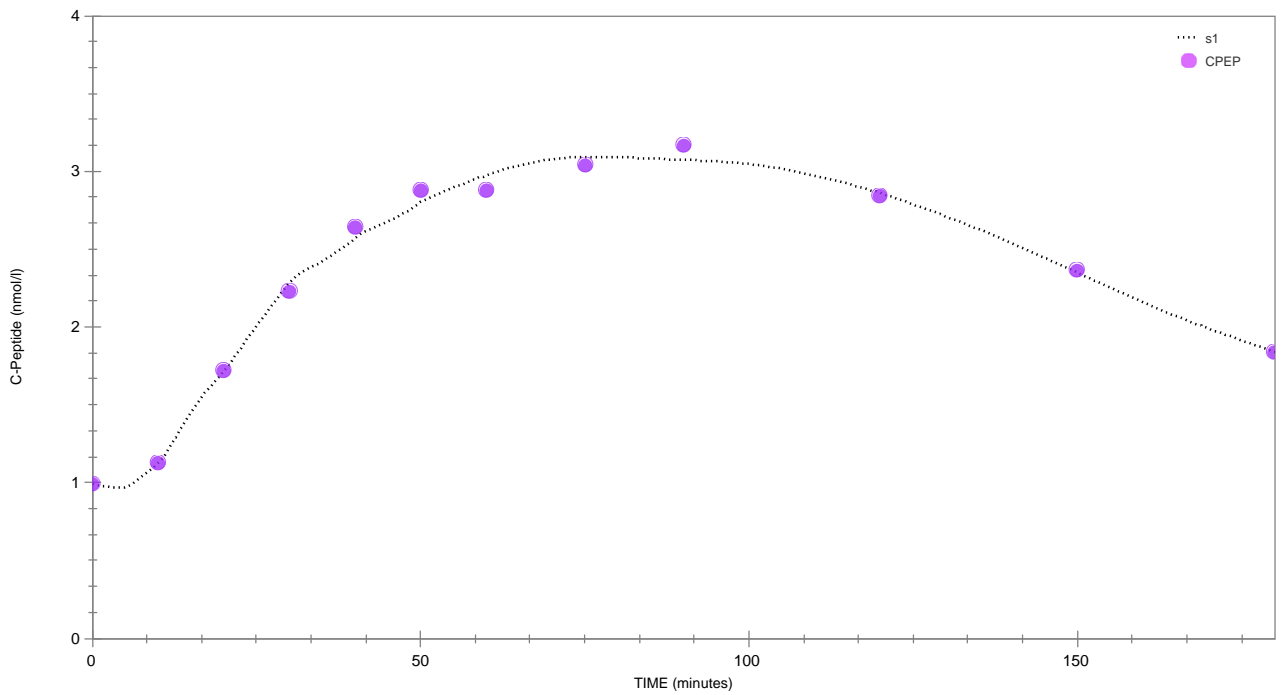
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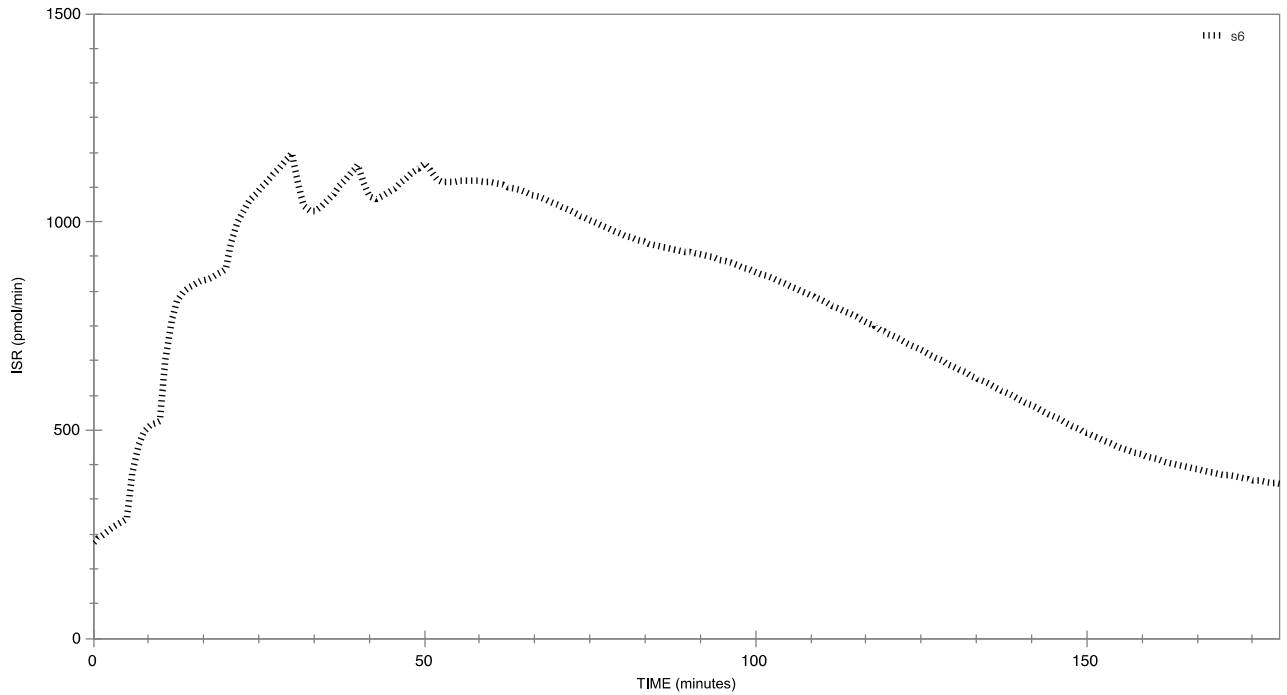
269 Fig. S1. Plasma glucose experimental points (filled circles) and input function (dotted line) of the  
270 mathematical model of beta cell function during the MMTT in the index case SDGS003

271



272

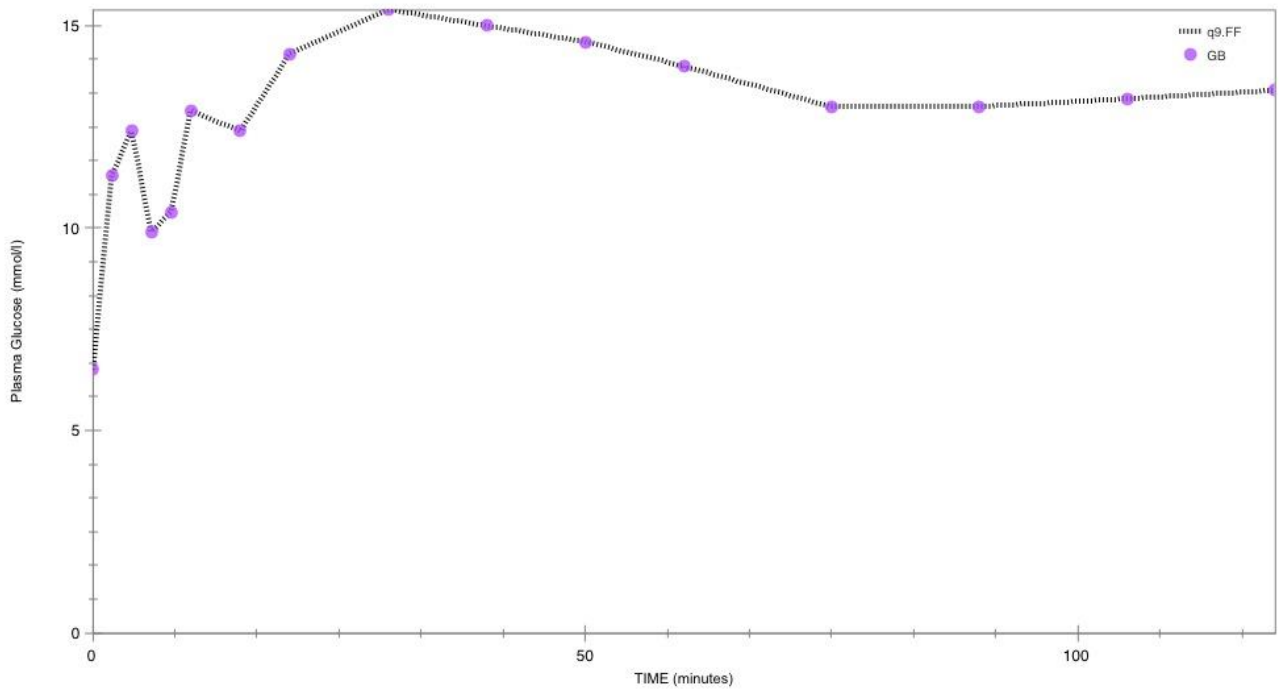
273 Fig. S2. Plasma C-peptide experimental points (filled circles) and model fit (dotted line) to the data of  
274 the MMTT in the index case SDGS003



275

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

278

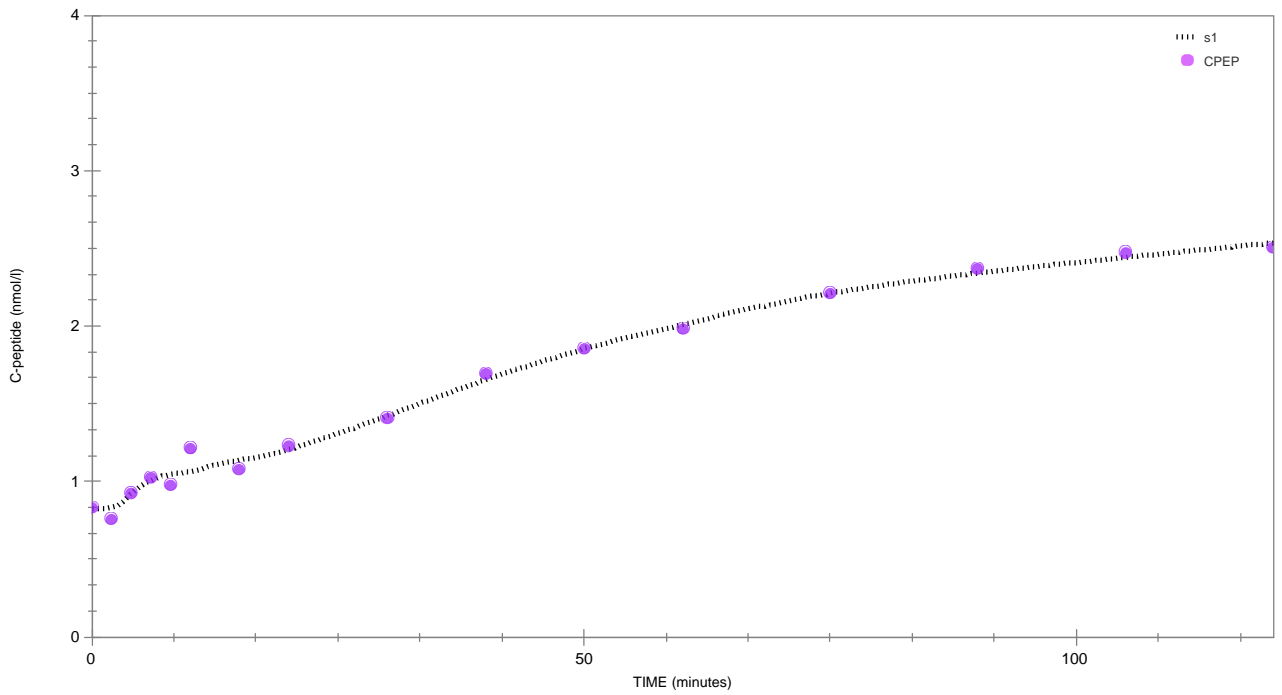


279

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

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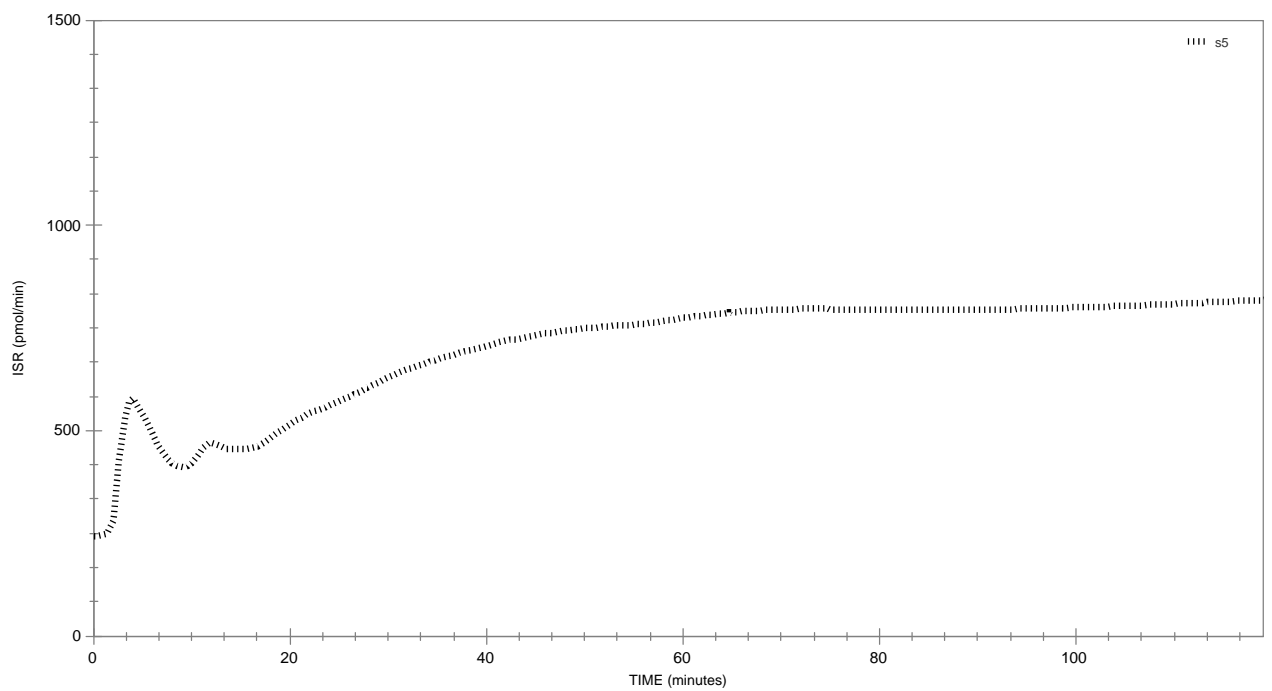
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285 Fig. S5. Plasma C-peptide experimental points (filled circles) and model fit (dotted line) to the data of  
 286 the MMTT in the index case SDGS003.

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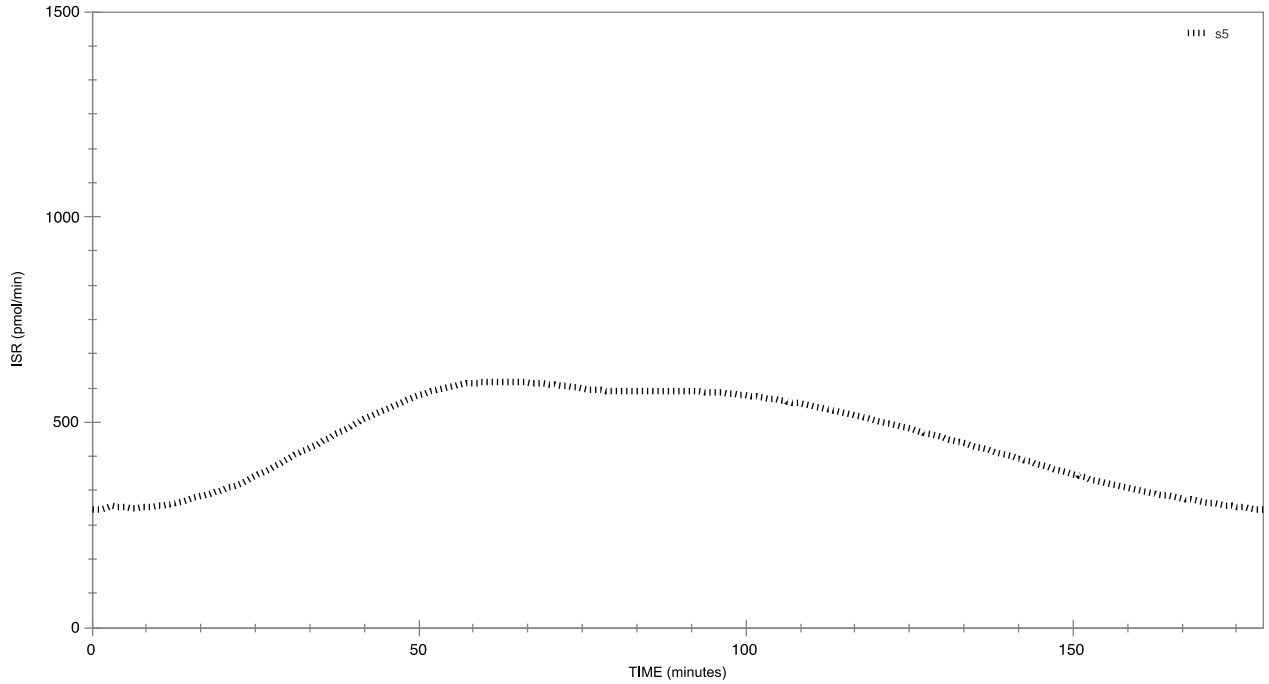


288

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

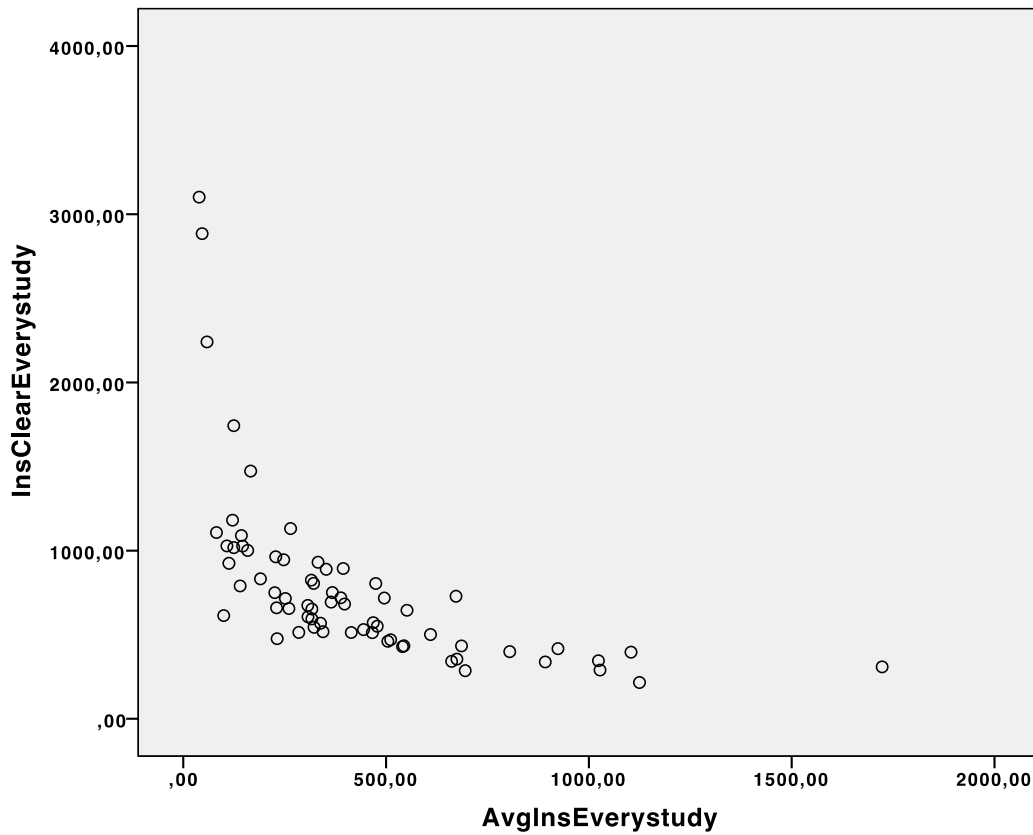
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293

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



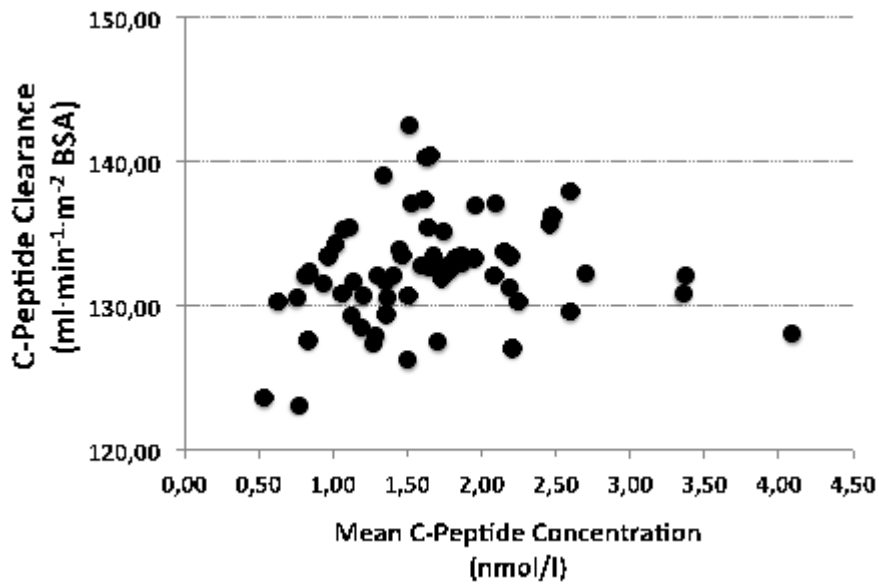
297

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

300

Fig S8.

301



302

303

304 Fig S9. Average c-peptide concentration (nmol·l<sup>-1</sup>, x axis) and average c-peptide clearance (ml·min<sup>-1</sup>·m<sup>2</sup> BSA, y axis) in all the tests (meal tolerance test and hyperglycemic clamps) reported in the  
305 present paper.  
306

307

308

309