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Time course of normalisation of functional beta cell capacity in DiRECT after weight loss in type 2 diabetes

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Abstract

Aim: To assess functional beta cell capacity in type 2 diabetes during 2 years of remission induced by dietary weight loss.

Methods: A Stepped Insulin Secretion Test with Arginine (SISTA) was used to quantify functional beta cell capacity by hyperglycemia and arginine stimulation. 39/57 subjects initially achieved remission (HbA1c<6.5% (<48mmol/mol) and FPG <7 mmol/l on no anti-diabetic drug therapy) with 16.4±7.7 kg weight loss, and were followed up with supportive advice on avoidance of weight regain. At 2 years, 20 subjects remained in remission in the study. A Non-Diabetic Control (NDC) group, matched for age/sex/weight after weight loss with the intervention group, was studied once.

Results: During remission, median [IQ range] maximal rate of insulin secretion increased from 581 [480-811] at baseline to 736 [542-998] at 5 months, 942 [565-1240] at 12 months (p=0.028 from baseline), and 936 [635-1435] pmol/min/m² at 24 months (p=0.023 from baseline; n=20/39 of those initially in remission). This was comparable to NDC (1016 [857-1507] pmol/min/m²) by 12 (p=0.064) and 24 months (p=0.244).

Median first phase insulin response increased from baseline to 5 months (42 [4-67] to 107 [59-163] pmol/min/m²; p<0.0001), then remained stable at 12 and 24 months (110 [59-201] and 125 [65-166] pmol/min/m² respectively; p<0.0001 vs. baseline) but lower than that of the NDC group (250 [226-429] pmol/min/m²; p<0.0001).

Conclusion: A gradual increase in assessed functional beta cell capacity occurred after weight loss, becoming similar to Non-Diabetic Controls by 12 months. This was unchanged at 2 years with continuing remission of type 2 diabetes.

Introduction

Type 2 diabetes has long been known to be associated with decreased functional beta cell capacity. This has appeared to be progressive, resulting in requirement for insulin therapy in 50% of people within 10 years of diagnosis (1). The early observation of decreased number of islets able to be isolated from the pancreas of people with type 2 diabetes (2) was followed by histological studies which reported 24-65% decrease in beta cell number compared with weight matched non-diabetic subjects (3, 4). However, the functional deficit has been reported to be greater than expected, with 50-97% decrease (5-7). Consistent with this, a 50% hemipancreatectomy does not bring about type 2 diabetes in most people (8).

The decrease in overall beta cell function has been conventionally ascribed to beta cell death or apoptosis (3, 9, 10). This has been challenged by recent studies which have identified loss of beta cell insulin secretory function due to suppression of relevant genes (11-14). This dedifferentiation is potentially reversible. Our previous shorter term studies have shown that type 2 diabetes can be returned to non-diabetic glucose control following dietary weight loss (15-18). The seminal question is now how closely back to normal the functional beta cell capacity returns during two years remission of type 2 diabetes.

DiRECT (the Diabetes Remission Clinical Trial) reported return to non-diabetic glucose control in over one third of a large Primary Care population of people with type 2 diabetes at two years using a simple, effective dietary method (17, 19, 20). There are few *in vivo* studies assessing functional beta cell capacity in type 2 diabetes, and none after reversal of type 2 diabetes (14, 21). We now report upon functional beta cell capacity as assessed by stepped hyperglycemic clamps plus arginine bolus in a geographically defined cohort of DiRECT participants. This was quantified together with first phase insulin secretion before and up to 24 months after substantial weight loss in type 2 diabetes, comparing those who achieved HbA1c < 6.5% (< 48 mmol/mol) and fasting plasma glucose < 7.0 mmol/l) with those who remained in the diabetic range. For comparison, an additional group of Non-Diabetic Controls were studied.

Methods

Participants

Participants in DiRECT had diabetes duration of less than 6 years, 20-65 years of age, BMI between 27 and 45 kg/m², HbA1c \geq 6.5% (\geq 48 mmol/mol) if on diet alone or HbA1c \geq 6.1% (\geq 43 mmol/mol) if on treatment with oral hypoglycaemic agents. Tyneside participants were randomised to Intervention (n=64) and Control (n=26) as previously reported (17, 19, 20). Main exclusion criteria were: current insulin use, recent routine HbA1c \geq 12% (\geq 108 mmol/mol) and weight loss of $>$ 5 kg within the last 6 months. The Tyneside subgroup of DiRECT underwent insulin secretion studies. A Non-Diabetic Control group (n=25) with no known first-degree history of type 2 diabetes was recruited to match the Intervention group at the post-weight loss time point for BMI, age, and gender, and an oral glucose tolerance test was performed to exclude any degree of glucose intolerance. Data on the DiRECT Control group of participants randomised to conventional therapy for type 2 diabetes are presented for completeness although the main comparison is between Responders and Non-Responders with the Non-Diabetic Control group as reference.

Study protocol

Weight loss and supportive weight maintenance was managed by Primary Care nurses with specific training on an integrated, structured, weight management programme (Counterweight-Plus)(19). All metabolic tests were performed at the Newcastle Magnetic Resonance Centre. After baseline tests, the Intervention group commenced a liquid formula diet (825–853 kcal/day) for an average of 16 weeks with continuation of all everyday activities. This was followed by a stepped food re-introduction phase. Thereafter, all participants were advised to follow a diet of normal foodstuffs to taste, but with individual advice and support to prevent weight regain. All oral hypoglycemic agents were withdrawn on Day 1 of the liquid formula diet. The Control group were continued on their usual diabetes management as per National Institute for Clinical Excellence guidelines. Beta cell function was assessed at baseline, after weight loss and return to normal eating at 5 months, at 12 months and at 24 months for both Intervention and Control groups. Tests were carried out on the NDC group (n=25) on one occasion only. Height was measured using a portable stadiometer (Chasmors Ltd, London) and body weight was measured in the fasting state using calibrated digital scales (Seca Ltd., Birmingham, UK) immediately prior to studies at each time point.

In order to interpret the beta cell function data, the Intervention group was divided into Responders who achieved non-diabetic glucose control (HbA1c < 6.5% (< 48 mmol/mol) and fasting plasma glucose < 7.0 mmol/l) off all anti-diabetic agents and Non-Responders who did not fit these current criteria (19, 20, 22, 23). The Consort diagram (Figure S1) shows that the criteria were applied at the post-weight loss visit with Responders and Non-Responders being analysed separately. At 5 months, 40 of the 64 Intervention subjects achieved non-diabetic plasma glucose control after the weight loss intervention and 39 Responders underwent the SISTA (Figure S1). One subject declined all the SISTA tests after baseline and counted as withdrawn. At 12 and 24 months, insulin secretion data were available on 28 and 20 Responders and 16 and 12 Non-Responders respectively. To allow clear description of time course, those who achieved non-diabetic levels of HbA1c and fasting plasma glucose but subsequently relapsed were not added to the defined Non-Responder group. In the diabetic Control group data were available on 23 participants at baseline and 5 months, 20 at 12 months, and 19 at 24 months. The Non-Diabetic Control group (n=25) was studied on one occasion.

Ethical approval was obtained from the West of Scotland Research Ethics Committee (reference number: 13/WS/0314).

Stepped Insulin Secretion Test with Arginine (SISTA)

After an overnight fast of at least 10 hours, participants were transported to the Magnetic Resonance Centre. Only water was allowed to maintain hydration. Any diabetes medications were withdrawn on the evening prior to the test. The functional capacity of the beta cells was assessed using the Stepped Insulin Secretion Test with Arginine (SISTA) (24), as modified by Lim and colleagues (15). The schematic and details of performing the SISTA are shown in Figure S2. First phase insulin response is assessed as usual and the maximal capacity for insulin response under the conditions of the test is assessed following hyperglycemic clamping plus a bolus of arginine.

Two large bore (18G) cannulae were inserted into forearm veins. Heat packs were used to allow sampling of arterialised blood. After basal samples had been taken, a glucose bolus followed by 20% Glucose infusion (Appendix) achieved a square wave step increase in

plasma glucose level 2.8 mmol/l above basal during the first 30 minutes. A repeat bolus at 30 minutes and increased infusion rate achieved steady state plasma glucose 5.6 mmol/l above fasting blood glucose for the rest of the test. A bolus of arginine (5 g) was administered over 30 seconds at 60 minutes.

Insulin secretion rates were estimated by deconvolution from C-peptide concentrations. The ISEC computer program was used which applies a regularisation method of deconvolution giving an output of insulin secretion rate in pmol/min/m² of body surface area (15, 25). This took account of gender, age, diabetes status, height, weight, body surface area and BMI. Data are reported on the assessed functional beta cell capacity (maximal rates of insulin secretion under the test conditions) and first phase response. Data are also reported for the insulin response to the second rapid increment in plasma glucose ('second step response') although the *in vivo* relevance of this is not yet established.

Analytical Procedures

Plasma glucose was measured by the glucose oxidase method (YSI glucose analyser, Yellow Springs Instrument Company, Yellow Springs, OH) and serum insulin and C-peptide by ELISA (Merckodia, Uppsala, Sweden). HbA1c was measured centrally at the Institute of Cardiovascular and Medical Sciences at Clinical Pathology Laboratory in Glasgow. Total fasting plasma triglyceride (TG) was quantified (Roche Diagnostics, West Sussex, U.K.) at the Department of Clinical Biochemistry, Newcastle upon Tyne Hospital National Health Service Foundation Trust.

Statistical analysis

Data were analysed by using IBM SPSS statistical software (www.ibm.com) using Independent Samples T test, Mann Whitney U test, and Spearman Rank correlation test as appropriate. The primary comparison between Responder and Non-Responder groups was the insulin secretion data. These were skewed in both groups and analysed non-parametrically. Data are presented as median and interquartile range (25th and 75th centiles) or mean \pm SD as appropriate.

Results

Baseline comparison of Responders and Non-Responders

At baseline, both Responders and Non-Responders had similar weight (100.4 ± 16.6 vs 102.1 ± 18.8 kg). Fasting plasma insulin (FPI) in Responders was 86.4 [55.5-145.5] pmol/l and 71.4 [51.5-100.6] pmol/l in Non-Responders ($p=0.094$). There were no significant differences between insulin secretion rates in Responders and Non-Responders at baseline although median rates of both maximal insulin secretion (581 [480-811] vs. 451 [296-691] pmol/min/m²; $p=0.081$) and first phase (42 [4-67] vs. 23 [10-36] pmol/min/m²; $p=0.299$) were higher in Responders. Responders and Non-Responders differed significantly in HbA1c ($7.4 \pm 1.0\%$ (57.5 ± 10.6 mmol/mol) vs. $7.9 \pm 0.8\%$ (62.5 ± 8.8 mmol/mol); $p=0.041$) and duration of diabetes (2.7 ± 1.6 vs. 3.8 ± 1.6 years; $p=0.026$) respectively.

Changes in weight and glucose control

Responders and Non-Responders lost weight similarly at 5 months (Table 1). Weight increased in Responders by 3.2 ± 4.2 kg between 5 and 12 months and by 6.6 ± 4.3 kg between 5 and 24 months. By design, weight of the Non-Diabetic Controls was similar to that of both study groups at 5 months (86.6 ± 14.9 vs. 84.0 ± 13.4 kg and 88.7 ± 18.8 kg respectively) and remained similar at both 12 months (86.6 ± 14.9 vs. 85.5 ± 16.2 kg; $p=0.845$ and 92.5 ± 18.3 kg; $p=0.552$ respectively) and 24 months (88.8 ± 17.7 kg; $p=0.648$ and 90.3 ± 14.6 kg; $p=0.649$ respectively). Those who failed to maintain remission between 5 and 24 months were characterized by more weight regain (11.3 ± 6.7 kg vs. 6.6 ± 4.3 kg; $p=0.036$). There was no change in weight in the Control group from baseline.

HbA1c remained in the non-diabetic range in Responders ($n=20$) at 24 months having remained steady although significantly higher than in NDC ($6.0 \pm 0.3\%$ (41.7 ± 3.5 mmol/mol) vs. $5.4 \pm 0.3\%$ (35.2 ± 3.4 mmol/mol); $p < 0.0001$, Table 1). At 24 months mean HbA1c in Responders remained $6.0 \pm 0.3\%$ (41.7 ± 3.5 mmol/mol; $p < 0.0001$ vs. NDC) and higher in Non-Responders $8.1 \pm 1.3\%$ (65.3 ± 14.4 mmol/mol; $p < 0.0001$) despite their use of anti-diabetes medications. Controls and Non-Responders showed no change in HbA1c from baseline to 24 months. Fasting plasma glucose level exhibited comparable changes to HbA1c in all groups (Table 1).

Fasting plasma insulin and C-peptide

Fasting plasma insulin (FPI) decreased to similar levels in Responders and Non-Responders at 5 months (32.6 [19.6-53.3] vs. 33.8 [20.7-43.3] pmol/l respectively) and stayed similar at 12 months (Table 1). The newly achieved level in both groups was similar to that of NDC (16.4 [10.0-37.2] pmol/l). At 24 months FPI stayed significantly lower than baseline in Responders (43.5 [18.4-61.6] pmol/l; $p < 0.0001$) as well as in Non-Responders (34.4 [16.5-49.5] pmol/l; $p = 0.001$). FPI in Controls did not change significantly from baseline to 24 months (70.1 [44.7-122.5] to 44.7 [31.3-64.5] pmol/l; $p = 0.050$). In all groups, fasting C-peptide exhibited a similar pattern of change to FPI (Table 1). Fasting C-peptide in Responders decreased from baseline (baseline 0.99 ± 0.32 nmol/l, 5 months 0.59 ± 0.23 nmol/l, 12 months 0.59 ± 0.21 nmol/l, 24 months 0.65 ± 0.25 nmol/l; $p < 0.0001$ for each vs. baseline). Following similar weight loss in the Non-Responder group reduction in C-peptide was also observed after weight loss (Table 1).

Assessment of functional beta cell capacity

The changes in plasma glucose for Responders vs. Non-Responders are shown in Figure 1 (A, B, C & D). The intended rapid increase of 2.8mmol/l was achieved in all groups for each of the 2 steps of the SISTA, followed in each case by stable hyperglycemia. The profile of insulin secretion rate at baseline, 5 months and 12 months is shown for Responders (Figure 1, panels E, F, G,&H) and Non-Responders (panels I, J,K,&L).

For Responders, maximal rates of insulin secretion in response to arginine bolus during hyperglycemia were 581 [480-811] pmol/min/m² at baseline and 736 [542-998] pmol/min/m² at 5 months. These two points were not significantly different ($p = 0.160$), but an almost linear increase was observed across 12 months following weight loss such that rates increased to 942 [565-1240] pmol/min/m² ($p = 0.028$ compared with baseline). This improvement was maintained at 24 months (936 [635-1435] pmol/min/m²; $p = 0.023$ vs. baseline) (Figure 2A). The maximal rate of insulin secretion for NDC was 1016 [857-1509]

pmol/min/m², comparable to that for the Responders at 12 and 24 months (p=0.064 and p=0.244 respectively) (Figure 2A). Non-Responders showed no change in median maximal insulin responses (baseline 451 [296-691], 5 months 491 [388-629], 12 months 485 [387-568], and 24 months 452 [347-616] pmol/min/m²). The difference between Responders and Non-Responders was significant by 5 months (p=0.002), and remained so at 12 months (p=0.001) and 24 months (p=0.002). The maximal insulin response in the diabetic Control group remained unchanged during 2 years follow up (546 [453-844], 567 [472-867], 559 [429-835], and 488 [378-720] pmol/min/m² respectively)(Figure 2A and Appendix Figure S3). Maximal insulin response to arginine in the Intervention group at 12 months correlated with first phase insulin response (r=0.6; p<0.0001), FPG and HbA1c (r=-0.4; p=0.003 for both). There was no correlation between maximal insulin response and total fasting TG at 12 months.

First phase insulin secretion

First phase insulin secretion increased in Responders from 42 [4-67] pmol/min/m² at baseline to 107 [59-163] pmol/min/m² (p<0.0001) at 5 months, remaining constant at 12 months (110 [59-201] pmol/min/m²; p<0.0001). At 24 month this was maintained in Responders (125 [65-166] pmol/min/m²; p<0.0001) (Figure 2B). The first phase response remained substantially lower than in the NDC (250 [226-429] pmol/min/m²; p<0.0001) compared with Responders at each time point after weight loss. There was no change in Non-Responders from baseline (23 [10-36] pmol/min/m²) either after weight loss at 5 months (14 [7-33] pmol/min/m²; p=0.864), at 12 months (19 [-7-36] pmol/min/m²; p=0.746) (Figure 2B), or at 24 months (17 [6-33] pmol/min/m²; p=1.000). The first phase insulin response of the diabetic Control group remained low and unchanged (25 [-5-61], 28 [3-43], 29 [-11-60] and 35 [4-5] pmol/min/m² respectively (Figure 2B and Appendix Figure S3).

The median first phase insulin response was associated with ambient fasting plasma glucose, being 89 [59-188] pmol/min/m² with FPG≤6.0mmol/l, 70 [45-81] with FPG 6.1-6.9mmol/l, and 35 [-3-45] pmol/min/m² with FPG≥7mmol/l. The relationship between first phase response and fasting plasma glucose is shown in Figure 3. First phase insulin response correlated significantly in Intervention group with FPG (r=-0.9; p<0.0001), HbA1c (r=-0.8;

$p < 0.0001$), maximal insulin secretion ($r = 0.6$; $p < 0.0001$) and second step insulin response ($r = 0.4$; $p = 0.022$) at 12 month. There was no significant correlation with total fasting TG ($r = 0.3$; $p = 0.098$). In the diabetic Control group first phase insulin response did not change during the study (Figure S3).

Second step insulin response

There was a small but significant increase in median second step insulin response in Responders 3 [-9-29] pmol/min/m² to 18 [8-34] pmol/min/m² ($p = 0.011$) at 5 months (Figure 2C), remaining steady to 12 months (21 [1-39] pmol/min/m²; $p = 0.043$), and with no significant change by 24 months (9 [-1-39] pmol/min/m²; $p = 0.256$) vs. baseline. Median second step did not change significantly either in Non-Responders (baseline -6 [-14-8], 5 months -2 [-8-6], 12 months 1 [-1-7], 24 months -3 [-11-9] pmol/min/m²) or in Controls (16 [-2-33], 3 [-13-15], 8 [-7-50], and 17 [1-23] pmol/min/m² respectively). There was a significant difference between second step in Responders and Non-Responders at 5 months ($p < 0.0001$) and at 12 months ($p = 0.025$) but not at 24 months ($p = 0.070$).

The median second step insulin response in NDC group was 68 [42-135] pmol/min/m², greater than that of the Responders at every time point ($p < 0.0001$).

Discussion

We report the gradual increase in assessed functional beta cell capacity over a 24 months period following weight loss-induced reversal of type 2 diabetes up to 6 years duration. The first phase insulin response followed a different time course, with those returning to non-diabetic plasma glucose control exhibiting an early increase followed by stability from 5 to 24 months. Unlike the assessed beta cell capacity, first phase insulin response improved significantly but remained around half of that of the non-diabetic controls.

Studies of rodent beta cells *in vitro* suggest a relatively rapid resumption of insulin secretory function after removal of a metabolic stress (11). However, these studies typically involve cells from young animals and exposure to metabolic stress for a relatively short time. In human type 2 diabetes, exposure to excess lipid supply has been present for years or decades, affecting beta cells during middle age or more advanced years. This may account

for the prolonged phase of recovery here reported following weight loss and restoration of glucose control in type 2 diabetes. Given the recognised heterogeneity of beta cells (26), it is possible that some cells are at a more advanced stage of de-differentiation and the slow return to normal functional beta cell capacity reflects different rates of re-differentiation (27). It is of considerable interest that this process continues for at least 12 months after commencing negative calorie balance, in sharp contrast to the previously reported inevitable steady decline in beta cell number or function during maintained or increased body weight (1, 3, 4). No comparable observations have been made in previous studies, and the pathophysiologic mechanism underlying this requires investigation. GLP-1 responsiveness was not examined in this study and dietary weight loss has previously been shown not to change this after return to non-diabetic glucose control (28, 29).

The first phase insulin response did not completely normalise, although the degree of recovery was compatible with maintaining of non-diabetic blood glucose control. Not all beta cells are required to achieve a normal first phase insulin response, which can be achieved by rapid degranulation of some proportion of the whole. More detailed studies are required to determine the effect of an apparently adequate but sub-normal response during the post-prandial period. During 8 weeks of negative calorie balance in the Counterpoint study, we originally observed a gradually improving first phase insulin response over 8 weeks in step with a gradually decreasing pancreas fat content (15). The extent of recovery appeared to be within the non-diabetic range but all the participants were in the first 4 years after diagnosis, had previously been treated with diet or metformin only and were studied during continued low calorie diet which was more restricted (approximately 700 kcal/day). The present data extend these findings, demonstrating that the recovery during the first few months is maximal in a group with duration of type 2 diabetes up to 6 years and previously treated with any number of anti-diabetes agents but not insulin (17). Subjects with pre-diabetes and first-degree relatives of people with type 2 diabetes have a subnormal first phase response sufficient to permit overall control of plasma glucose prior to onset of type 2 diabetes (30, 31) so it is possible that we may have observed return to the premorbid levels in those achieving remission.

Metabolic stress on the beta cell can be produced by exposure to excess glucose or excess fat (11, 32, 33). In human type 2 diabetes it is almost certain that both contribute, although

initiation of the metabolic stress during normoglycemia likely to be via fat. Although the early sharp decrease in fasting plasma glucose levels will enhance the first phase response, this typically happens within hours with rapid recovery (34, 35). This was evident within 7 days of commencing a very low calorie diet (15) although the subsequent return to near normal first phase insulin response to a glucose stimulus was observed to develop steadily over 8 weeks, in step with the gradual decrease to normal of the excess lipid exposure of the beta cells. In contrast, there was no change in muscle insulin sensitivity over 2 months following remission in Counterpoint (15). These observations were extended in the Counterbalance study which demonstrated continued normal hepatic insulin sensitivity but minor improvement only in muscle insulin sensitivity by 6 months following weight loss (16). The conditions of the insulin secretion test used permit assessment of beta cell function largely independent of tissue insulin sensitivity. As an indication of whole body insulin sensitivity, fasting plasma insulin, and hence its inverse, was not different between responders and non-responders at any time point (Table 1).

The present studies were conducted on people with less than 6 years duration of diagnosed type 2 diabetes at the time of recruitment. The actual duration of diabetes will have been longer, and many participants reported delay in seeking medical advice after symptom onset. However, this will be common to all primary care populations with type 2 diabetes and time from diagnosis remains the practical yardstick. The protocol for DiRECT was informed by the Counterpoint study and the early results of the Counterbalance study which showed no return of first phase insulin secretion beyond 11 years of diagnosis (15, 16). Even within 6 years of diagnosis of type 2 diabetes, it is apparent that there are some individuals who are susceptible to a more rapid loss of beta cell function in response to the metabolic stress. At 12 months, Responders had a significantly lower duration of diabetes than Non-Responders (17). This durability over time to withstand the beta cell stress induced by the combination of high glucose and high fat exposure, suggests that exploration of the genetic basis of this beta cell behaviour is required. The majority of discovered genes associated with type 2 diabetes code for beta cell processes (36, 37) and this information on phenotypic heterogeneity between individuals offers a route to linking specific genes with durability under metabolic stress. An early indication of this phenomenon was provided by Unger's demonstration of complete resistance to fat induced stress of islets isolated from

ZDF rats not predisposed to develop diabetes upon high fat feeding (38). It is possible that novel therapeutic targets may be identified to protect the beta cells of susceptible individuals, guided by genotyping.

Attempts to study beta cell mass have evolved from post-mortem histological studies (3, 4, 39), through techniques of fresh pancreas slice histology (40) and incubation, to imaging of beta cells *in vivo* (41). Although conceptually attractive, the latter lack precision and at present there is no practical method of quantifying *in vivo*. In contrast, the mass of beta cells which are functional can be assessed indirectly by metabolic tests (42, 43). An arginine bolus during hyperglycemia elicits a large spike in insulin secretion dependent upon functional beta cell mass. There is a tight correlation between the response seen at different levels of plasma glucose in type 2 diabetic and non-diabetic groups (44, 45). Although a true maximal response may be obtained at 25 mmol/l plasma glucose, the relative differences would be expected to remain. The SISTA test utilizes this defined response to permit an assessment of the functional beta cell capacity *in vivo*, and observation of the time course of recovery of function.

In summary, the functional beta cell capacity as assessed in this study appeared to return to normal over 12 months in those who maintained weight loss-induced reversal of type 2 diabetes. First phase insulin response improves more rapidly, but does not return to normal. Provided weight regain was minimised, both functional beta cell capacity and first phase insulin response remained stable at least up to 2 years with no evidence of any time-dependent decrease in beta cell function.

Author Contributions

SZ performed the clinical work and co-wrote the manuscript. AA-M analysed the data and co-wrote the manuscript. AB trained and mentored practice nurses and edited the manuscript. CP performed the clinical work and edited the manuscript. BA contributed to data analysis and edited the manuscript. KGH developed methodologies and edited the manuscript. AM oversaw statistical analysis and edited the manuscript. NS contributed to discussion and reviewed the manuscript. MEJL contributed to discussion and reviewed the manuscript. RT oversaw data analysis and co-wrote the manuscript.

Guarantor's statement

Professor Roy Taylor is the guarantor for the study and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

Disclosure statement

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		Baseline	5 months	12 months	24 months
		Resp=39 Non-Resp=18 Control=26 -	Resp=39 Non-Resp=18 Control=23 NDC=25	Resp=28 Non-Resp=16 Control=20 -	Resp=20 Non-Resp=12 Control=19 -
Weight (kg)	Resp	100.4±16.6	84.0±13.4 p<0.0001	85.5±16.2 p<0.0001	88.8±17.7 p=0.016
	Non-Resp	102.1±18.8	88.7±18.8 p=0.008	92.5±18.3 p=0.050	90.3±14.6 p=0.028
	Control	96.7±11.8	95.8±12.8	95.2±14.4	95.0±13.9
	NDC	-	86.6±14.9	-	-
Weight loss (%)	Resp	-	16.0±6.1 p<0.0001	14.3±6.9 p<0.0001	10.5±6.1 p=0.016
	Non-Resp	-	13.2±6.1 p=0.008	9.4±5.2 p=0.050	8.3±4.5 p=0.028
	Control	-	1.1±3.1	0.8±4.4	1.7±5.5
	NDC	-	-	-	-
HbA1c (%)	Resp	7.4±1.0	5.9±0.4 p<0.0001	5.8±0.3 p<0.0001	6.0±0.3 p<0.0001
	Non-Resp	7.9±0.8 *p=0.041	8.0±1.7 *p<0.0001	7.6±0.7 *p<0.0001	8.1±1.3 *p<0.0001
	Control	7.3±1.0	7.8±1.5	8.5±2.2	7.2±1.0
	NDC	-	5.4±0.3	-	-
FPG (mmol/l)	Resp	8.3±2.4	5.7±0.8 p<0.0001	5.6±0.6 p<0.0001	5.6±0.7 p<0.0001
	Non-Resp	9.3±2.8	8.8±2.6 *p<0.0001	8.5±1.8 *p<0.0001	9.3±4.0 *p<0.0001
	Control	8.3±2.0	8.4±2.3	8.5±2.2	8.0±2.0
	NDC	-	5.1±0.4	-	-
FPI (pmol/l)	Resp	86.4[55.5-145.5]	32.6[19.6-53.3] p<0.0001	28.9[17.6-65.7] p<0.0001	43.5[18.4- 61.6]p<0.0001
	Non-Resp	71.4[51.5-100.6]	33.8[20.7-43.3] p<0.0001	32.7[23.7-61.7] p=0.006	34.4[16.5-49.5]p=0.001
	Control	70.1[44.7-122.5]	58.4[36.2-83.0]	65.5[39.9-76.9]	44.7[31.3-64.5] p=0.050
	NDC	-	16.4[10.0-37.2]	-	-
Fasting C-peptide (nmol/l)	Resp	0.99±0.32	0.59±0.23 p<0.0001	0.59±0.21 p<0.0001	0.65±0.25 p<0.0001
	Non-Resp	0.84±0.28	0.61±0.20 p=0.003	0.65±0.24 p=0.042	0.59±0.23 p=0.022
	Control	0.92±0.41	0.87±0.34	0.87±0.41	0.78±0.22
	NDC	-	0.53±0.32	-	-
Total fasting TG (mmol/l)	Resp	1.71[1.15-2.29]	1.05[0.73-1.53] p<0.0001	1.09[0.75-1.62] p<0.0001	1.00[0.79-1.46] p<0.0001
	Non-Resp	1.57[1.25-1.99]	1.15[0.90-1.40] p=0.009	1.24[0.92-1.47] p=0.048	1.29[0.95-1.53]
	Control	1.06[0.81-1.58]	1.20[1.00-1.50]	1.21[1.00-1.91]	1.05[0.89-1.63]

	NDC	-	1.10[0.80-1.50]	-	-
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Table 1. Summary of weight change and fasting metabolic parameters for Responder, Non-Responder, Diabetic Control and Non-Diabetic Control (NDC) groups at baseline, 5, 12, and 24 months. Data for the NDC group are shown in the 5 month column as this group was recruited to have weight equivalent to the Intervention group after weight loss. Data shown as mean \pm SD or median [IQ range]. Comparisons are shown for baseline to 5 months, baseline to 12 months, and baseline to 24 months (p value) and for Responders vs. Non-Responders at each time point (*p value). N.B. The percentage of weight loss is shown for each group at that particular time point vs. the same number of participants at the baseline.

Legends to Figures

Figure 1. Stepped insulin secretion test

A, B, C, D: mean plasma glucose during SISTA in Responders —●— and Non-Responders □
---- at baseline, 5 months, 12 months, and 24 months respectively.

E, F, G, H: median insulin secretion rates (ISR) in Responders at baseline, 5 months, 12 months, and 24 months respectively.

I, J, K, L: median insulin secretion rates (ISR) in Non-Responders at baseline, 5 months, 12 months, and 24 months respectively.

Figure 2. Beta cell response during SISTA

Comparison of the median maximal insulin response (A), median first phase insulin response (B), and median second step insulin secretion (C) in Responders, Non-Responders, Non-Diabetic Controls (NDC) at baseline, 5, 12, and 24 months (p value in a box for Responders at each time point vs. baseline; p value without a box for Responders vs. Non-Responders at each time point).

Figure 3. Relationship between fasting plasma insulin and first phase insulin secretion

Fasting plasma glucose and first phase insulin secretion in the whole intervention group at 12 months (n=48: Responders=28, Non-Responders=16, Relapsers=4) were plotted. The inset graph shows the log transformed data (7 individuals omitted due to negative 1st phase insulin secretion values unable to be logged).

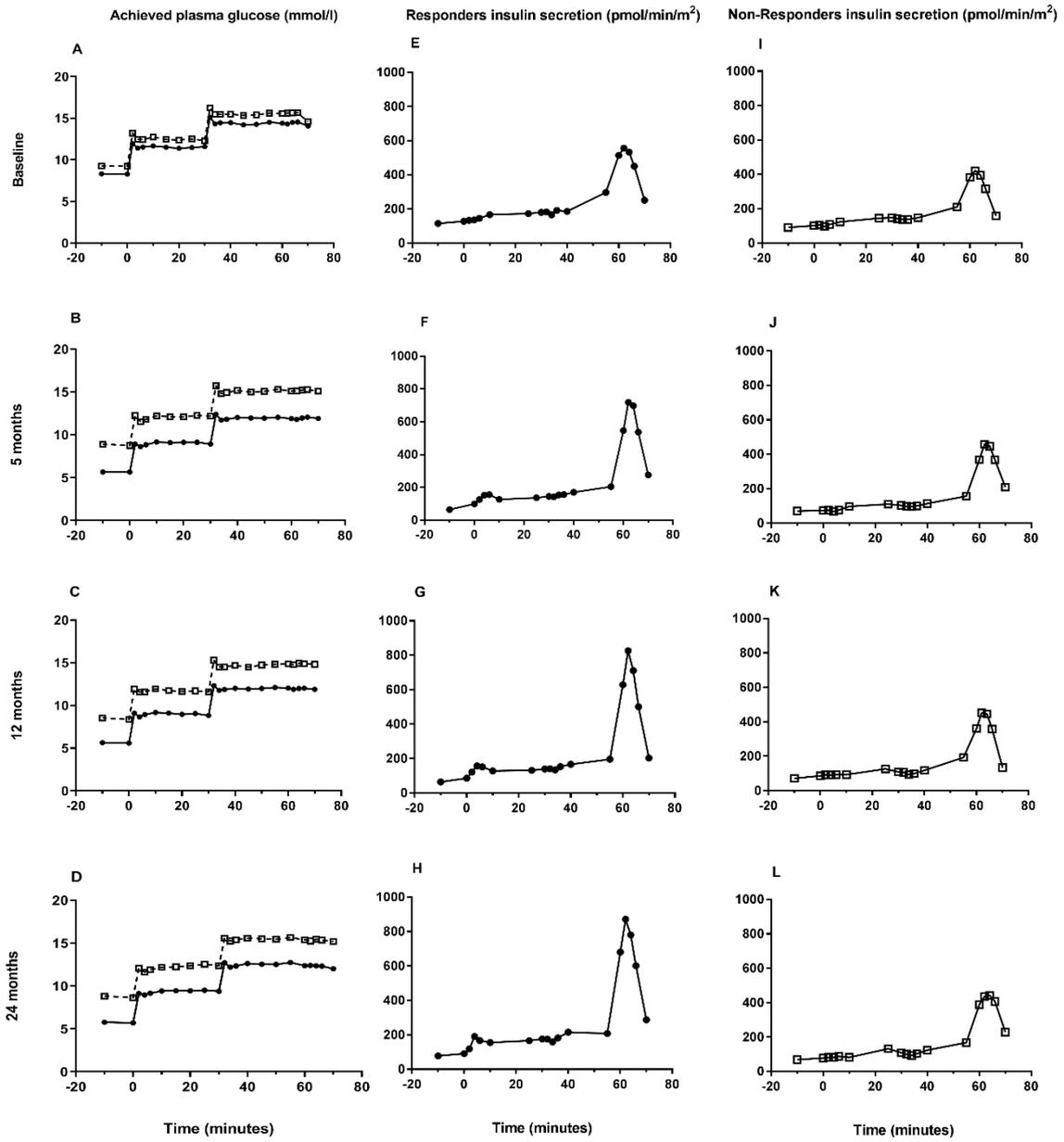


Figure 1

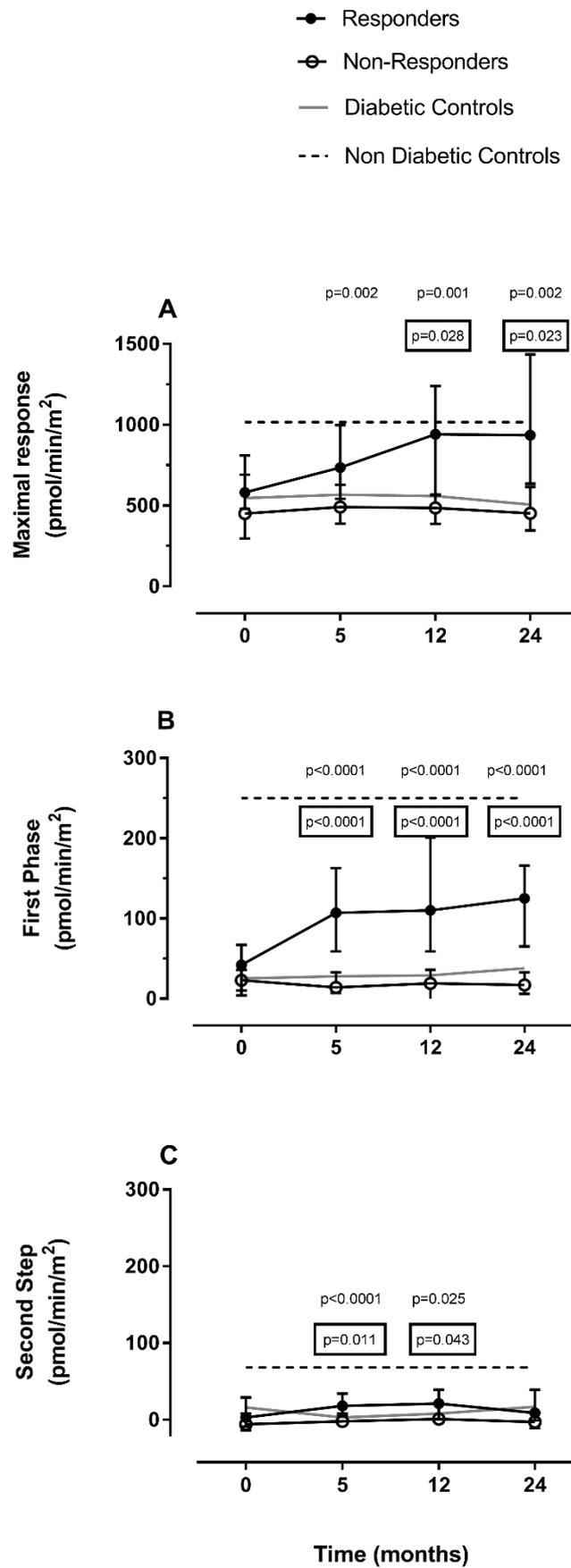


Figure 2

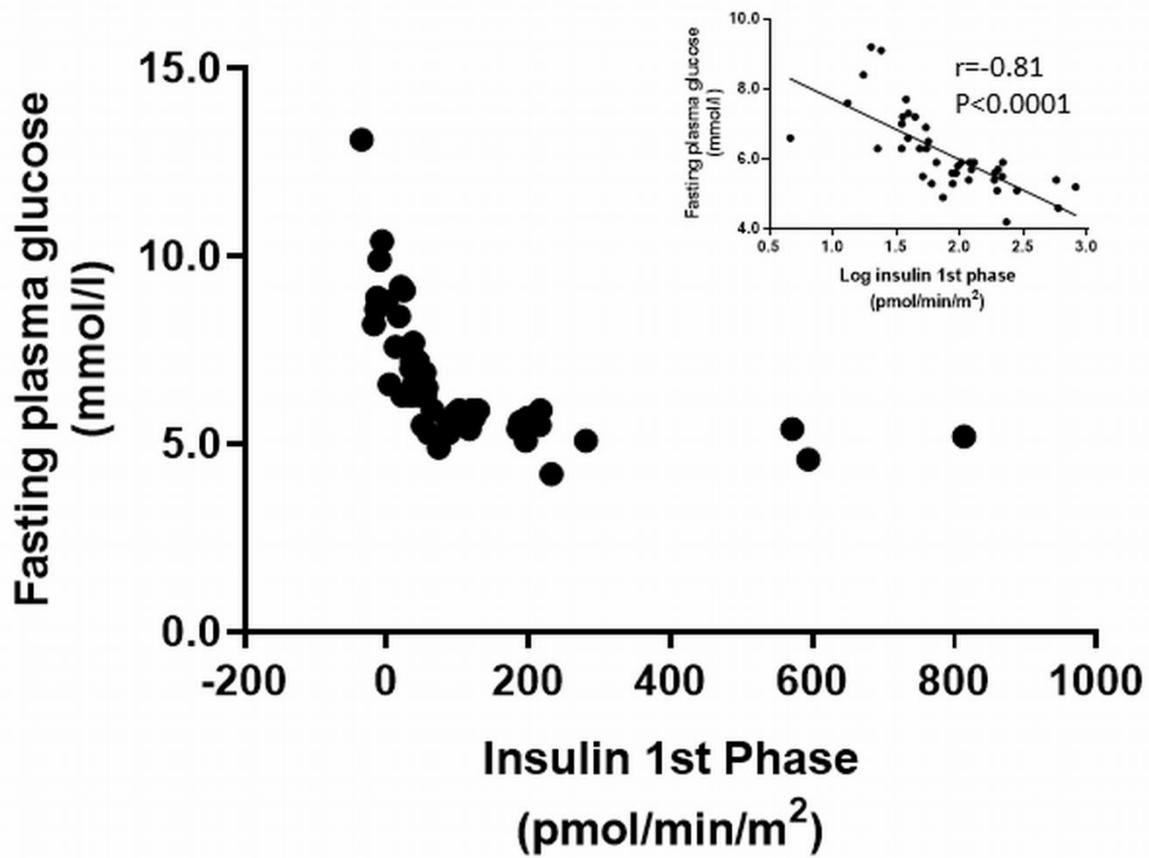


Figure 3

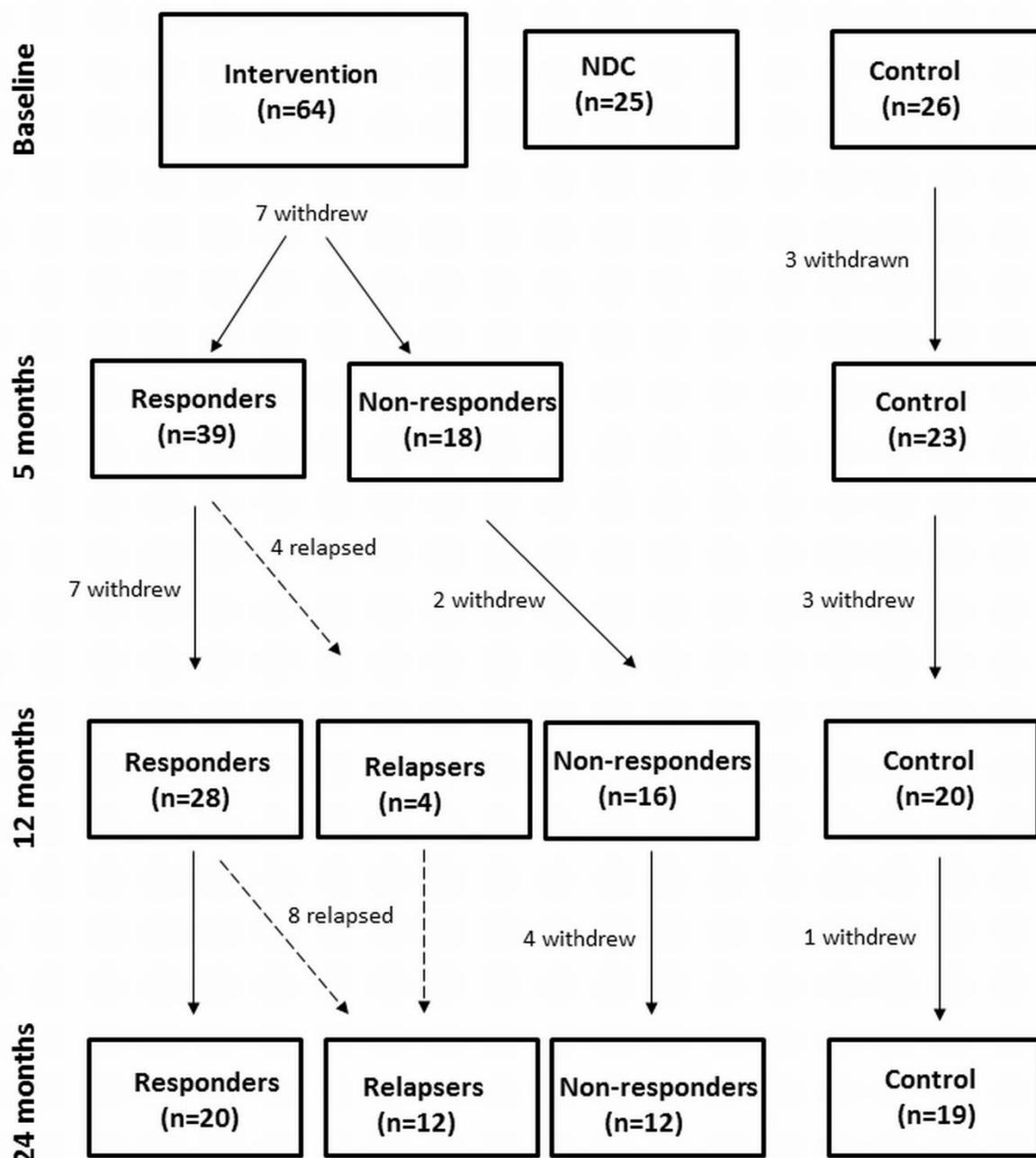


Fig S1

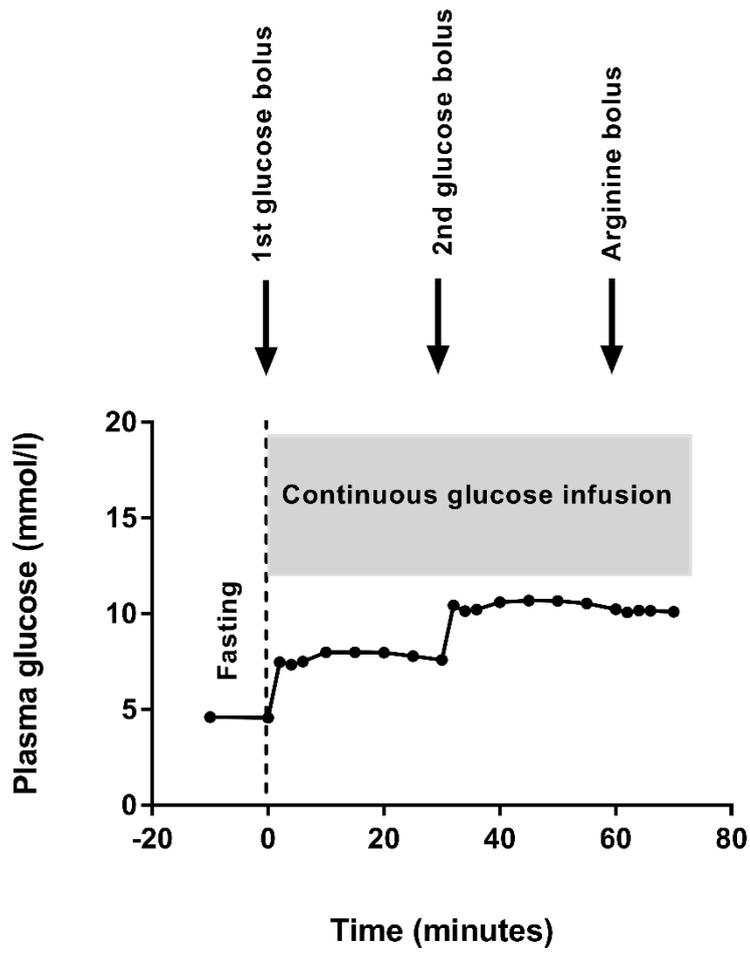


Fig S2

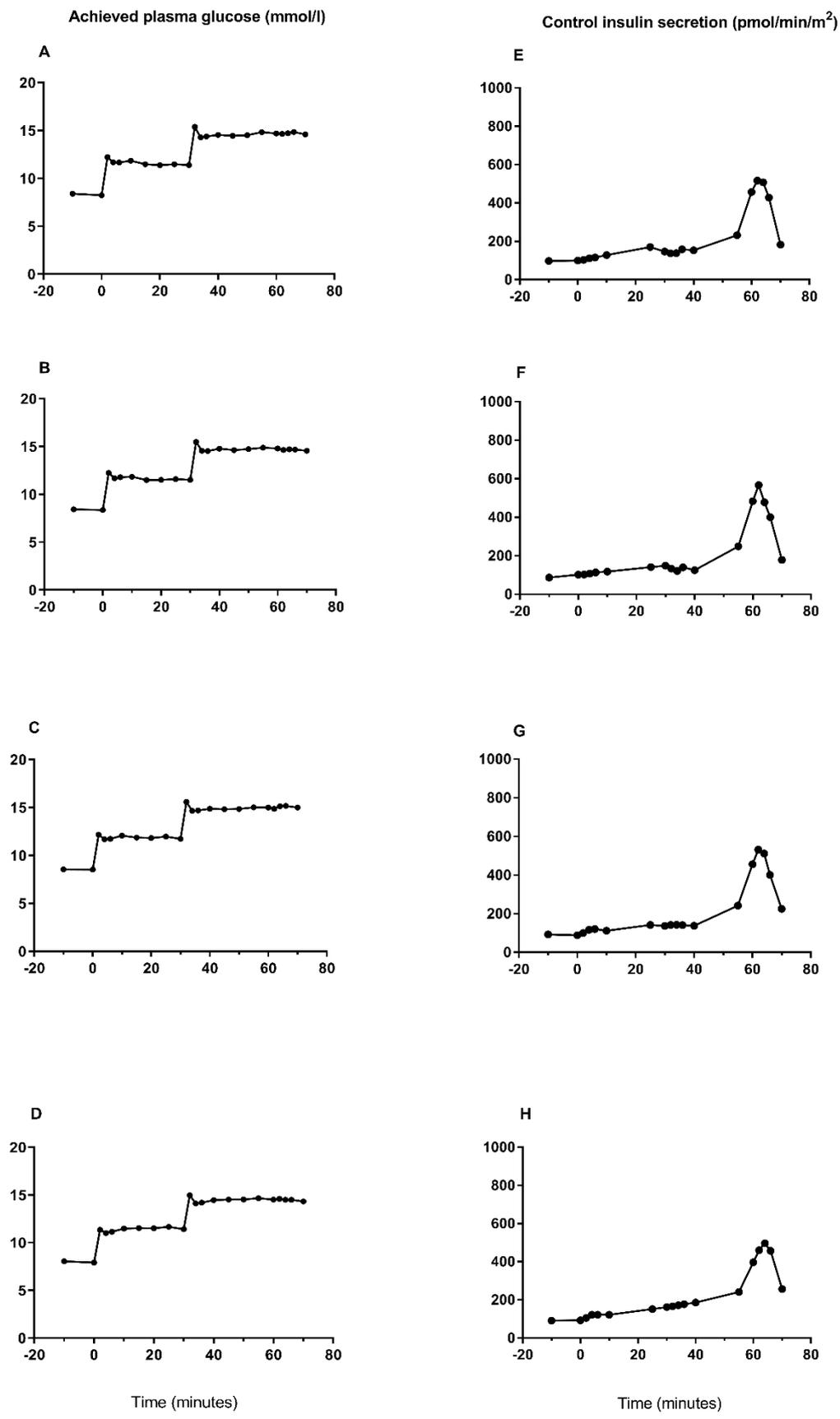


Figure S3