Time Course of Normalization of Functional β-Cell Capacity in the Diabetes Remission Clinical Trial After Weight Loss in Type 2 Diabetes

Sviatlana V. Zhyzhneuskaya,[1] Ahmad Al-Mrabeh,[1] Carl Peters,[1] Alison Barnes,[2] Benjamin Aribisala,[3] Kieren G. Hollingsworth,[1] Alex McConnachie,[4] Naveed Sattar,[5] Michael E.J. Lean,[6] and Roy Taylor[1]

Diabetes Care 2020;43:813–820 | https://doi.org/10.2337/dc19-0371

OBJECTIVE

To assess functional β-cell capacity in type 2 diabetes during 2 years of remission induced by dietary weight loss.

RESEARCH DESIGN AND METHODS

A Stepped Insulin Secretion Test with Arginine was used to quantify functional β-cell capacity by hyperglycemia and arginine stimulation. Thirty-nine of 57 participants initially achieved remission (HbA_{1c} <6.5% [<48 mmol/mol] and fasting plasma glucose <7 mmol/L on no antidiabetic drug therapy) with a 16.4 ± 7.7 kg weight loss and were followed up with supportive advice on avoidance of weight regain. At 2 years, 20 participants remained in remission in the study. A nondiabetic control (NDC) group, matched for age, sex, and weight after weight loss with the intervention group, was studied once.

RESULTS

During remission, median (interquartile range) maximal rate of insulin secretion increased from 581 (480–811) pmol/min/m^2 at baseline to 736 (542–998) pmol/min/m^2 at 5 months, 942 (565–1,240) pmol/min/m^2 at 12 months ($P = 0.028$ from baseline), and 936 (635–1,435) pmol/min/m^2 at 24 months ($P = 0.023$ from baseline; $n = 20$ of 39 of those initially in remission). This was comparable to the NDC group (1,016 [857–1,507] pmol/min/m^2) by 12 ($P = 0.064$) and 24 ($P = 0.244$) months. Median first-phase insulin response increased from baseline to 5 months (42 [4–67] to 107 [59–163] pmol/min/m^2; $P < 0.0001$) and then remained stable at 12 and 24 months (110 [59–201] and 125 [65–166] pmol/min/m^2, respectively; $P < 0.0001$ vs. baseline) but lower than that of the NDC group (250 [226–429] pmol/min/m^2; $P < 0.0001$).

CONCLUSIONS

A gradual increase in assessed functional β-cell capacity occurred after weight loss, becoming similar to that of NDC group participants by 12 months. This result was unchanged at 2 years with continuing remission of type 2 diabetes.

[1]Magnetic Resonance Centre, Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, U.K.
[2]Human Nutrition Research Centre, Population Health Sciences Institute, Newcastle University, Newcastle upon Tyne, U.K.
[3]Computer Science Department, Lagos State University, Lagos, Nigeria
[4]Robertson Centre for Biostatistics, University of Glasgow, Glasgow, U.K.
[5]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, U.K.
[6]School of Medicine, Dentistry and Nursing, University of Glasgow, Glasgow, U.K.

Corresponding author: Roy Taylor, roy.taylor@ncl.ac.uk

Received 21 February 2019 and accepted 29 December 2019

This article contains Supplementary Data online at https://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc19-0371/-/DC1.

S.V.Z. and A.A.-M. are joint first authors.

This article is featured in a podcast available at https://www.diabetesjournals.org/content/diabetes-core-update-podcasts.

Type 2 diabetes has long been known to be associated with decreased functional β-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 a decreased number of islets able to be isolated from the pancreas of people with type 2 diabetes (2) was followed by histological studies that reported a 24–65% decrease in β-cell number compared with weight-matched subjects without diabetes (3,4). However, the functional deficit has been reported to be greater than expected, with a 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 β-cell function has been conventionally ascribed to β-cell death or apoptosis (3,9,10). This has been challenged by studies that have identified loss of β-cell insulin secretory function as a result of 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 nondiabetic glucose control following dietary weight loss (15–18). The seminal question is now how closely back to normal the functional β-cell capacity returns during 2 years' remission of type 2 diabetes.

The Diabetes Remission Clinical Trial (DiRECT) reported return to nondiabetic glucose control in more than one-third of a large primary care population of people with type 2 diabetes at 2 years using a simple, effective dietary method (17,19,20). Few in vivo studies have assessed functional β-cell capacity in type 2 diabetes and none after reversal of type 2 diabetes (14,21). We now report on functional β-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 HbA_{1c} <6.5% (<48 mmol/mol) and fasting plasma glucose (FPG) <7.0 mmol/L with those who remained in the diabetic range. For comparison, an additional nondiabetic control (NDC) group was studied.

RESEARCH DESIGN AND METHODS

Participants

Participants in DiRECT had diabetes duration of <6 years, were 20–65 years of age, had a BMI between 27 and 45 kg/m^2, and had an HbA_{1c} ≥6.5% (≥48 mmol/mol) if on diet alone or HbA_{1c} ≥6.1% (≥43 mmol/mol) if on treatment with oral hypoglycemic agents. Tyneside participants were randomized to intervention (n = 64) and control (n = 26) as previously reported (17,19,20). Main exclusion criteria were current insulin use, recent routine HbA_{1c} ≥12% (≥108 mmol/mol), and weight loss of >5 kg within the past 6 months. The Tyneside subgroup of DiRECT underwent insulin secretion studies. An NDC group (n = 25) of participants 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 sex, and an oral glucose tolerance test was performed to exclude any degree of glucose intolerance. Data on the DiRECT control group of participants randomized to conventional therapy for type 2 diabetes are presented for completeness, although the main comparison is between responders and nonresponders, with the NDC 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 program (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 reintroduction 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 participants were continued on their usual diabetes management per National Institute for Clinical Excellence guidelines. β-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 the intervention and the 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, U.K.), and body weight was measured in the fasting state using calibrated digital scales (Seca Ltd., Birmingham, U.K.) immediately before studies at each time point.

To interpret the β-cell function data, the intervention group was divided into responders who achieved nondiabetic glucose control (HbA_{1c} <6.5% [<48 mmol/mol] and FPG <7.0 mmol/L) while off all antidiabetic agents and nonresponders who did not fit these current criteria (19,20,22,23). The Consolidated Standards of Reporting Trials diagram (Supplementary Fig. 1) shows that the criteria were applied at the post–weight loss visit, with responders and nonresponders analyzed separately. At 5 months, 40 of the 64 intervention group participants achieved nondiabetic plasma glucose control after the weight loss intervention, and 39 responders underwent the Stepped Insulin Secretion Test with Arginine (SISTA) (Supplementary Fig. 1). One subject declined all the SISTA tests after baseline and was counted as withdrawn. At 12 and 24 months, insulin secretion data were available on 28 and 20 responders and 16 and 12 nonresponders, respectively. To allow clear description of time course, those who achieved nondiabetic levels of HbA_{1c} and FPG but subsequently relapsed were not added to the defined nonresponder 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 NDC group (n = 25) was studied on one occasion. Ethical approval was obtained from the West of Scotland Research Ethics Committee (Glasgow, U.K.) (reference number: 13/WS/0314).

SISTA

After an overnight fast of at least 10 h, participants were transported to the Magnetic Resonance Centre. Only water was allowed to maintain hydration. Any diabetes medications were withdrawn on the evening before the test. The functional capacity of the β-cells was assessed using SISTA (24), as modified by Lim et al. (15). The schematic and details of performing the SISTA are shown in Supplementary Fig. 2. 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 arterialized blood. After basal samples had been taken, a glucose bolus followed by 20% glucose infusion (Supplementary Appendix) achieved a square-wave step increase in plasma glucose level 2.8 mmol/L above basal during the first 30 min. A repeat bolus at 30 min 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 s at 60 min.

Insulin secretion rates were estimated by deconvolution from C-peptide concentrations. The ISEC computer program, which applies a regularization method of deconvolution, was used, giving an output of insulin secretion rate (pmol/min/m^2 of body surface area) (15,25). This took account of sex, age, diabetes status, height, weight, body surface area, and BMI. Data are reported on the assessed functional β-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 analyzer; Yellow Springs Instrument Company, Yellow Springs, OH) and serum insulin and C-peptide by ELISA (Mercodia, Uppsala, Sweden). HbA_{1c} was measured centrally at the Clinical Pathology Laboratory of the Institute of Cardiovascular & Medical Sciences (Glasgow, U.K.). Total fasting plasma triglyceride (TG) was quantified (Roche Diagnostics, West Sussex, U.K.) at the Department of Clinical Biochemistry, The Newcastle upon Tyne Hospitals NHS Foundation Trust.

Statistical Analysis

Data were analyzed by using SPSS statistical software (IBM Corporation) using independent samples *t* test, Mann-Whitney *U* test, and Spearman rank correlation test, as appropriate. The primary comparison between responder and nonresponder groups was the insulin secretion data. These data were skewed in both groups and analyzed nonparametrically. Data are presented as median and interquartile range (IQR) (25th and 75th centiles) or mean ± SD, as appropriate.

RESULTS

Baseline Comparison of Responders and Nonresponders

At baseline, both responders and nonresponders had similar weights (100.4 ± 16.6 vs. 102.1 ± 18.8 kg). Fasting plasma insulin (FPI) in responders was 86.4 (IQR 55.5–145.5) pmol/L and 71.4 (51.5–100.6) pmol/L in nonresponders ($P = 0.094$). There were no significant differences between insulin secretion rates in responders and nonresponders at baseline, although median rates of both maximal insulin secretion (581 [480–811] vs. 451 [296–691] pmol/min/m^2; $P = 0.081$) and first-phase insulin response (42 [4–67] vs. 23 [10–36] pmol/min/m^2; $P = 0.299$) were higher in responders. Responders and nonresponders differed significantly in HbA_{1c} (7.4 ± 1.0% [57.5 ± 10.6 mmol/mol] vs. 7.9 ± 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 nonresponders 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 NDC group 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 vs. 6.6 ± 4.3 kg; $P = 0.036$). There was no change in weight in the diabetic control group from baseline.

HbA_{1c} remained in the nondiabetic range in responders ($n = 20$) at 24 months, having remained steady, although significantly higher, than in the NDC group (6.0 ± 0.3% [41.7 ± 3.5 mmol/mol] vs. 5.4 ± 0.3% [35.2 ± 3.4 mmol/mol]; $P < 0.0001$) (Table 1). At 24 months, mean HbA_{1c} in nonresponders was 8.1 ± 1.3% (65.3 ± 14.4 mmol/mol) ($P < 0.0001$ compared with responders) despite their use of antidiabetes medications. Control participants and nonresponders showed no change in HbA_{1c} from baseline to 24 months. FPG level exhibited comparable changes to HbA_{1c} in all groups (Table 1).

FPI and C-Peptide

FPI decreased to similar levels in responders and nonresponders at 5 months (32.6 [IQR 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 the NDC group (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; $P < 0.0001$) and nonresponders (34.4 [16.5–49.5] pmol/L; $P = 0.001$). FPI in control participants 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 nonresponders, reduction in C-peptide was also observed after weight loss (Table 1).

Assessment of Functional β-Cell Capacity

The changes in plasma glucose for responders versus nonresponders are shown in Fig. 1*A–D*. The intended rapid increase of 2.8 mmol/L was achieved in all groups for each of the two 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 (Fig. 1*E–H*) and nonresponders (Fig. 1*I–L*).

For responders, maximal rates of insulin secretion in response to arginine bolus during hyperglycemia were 581 (IQR 480–811) pmol/min/m^2 at baseline and 736 (542–1,020) pmol/min/m^2 at 5 months. These two points were not significantly different ($P = 0.160$), but an almost linear increase was observed across 12 months after weight loss such that rates increased to 942 (565–1,240) pmol/min/m^2 ($P = 0.028$ vs. baseline). This improvement was maintained at

Table 1—Summary of weight change and fasting metabolic parameters for responder, nonresponder, diabetic control, and NDC groups at baseline and 5, 12, and 24 months

	Baseline	5 months	12 months	24 months
Participants, *n*				
Responders	39	39	28	20
Nonresponders	18	18	16	12
Control	26	23	20	19
NDC	—	25	—	—
Weight (kg)				
Responders	100.4 ± 16.6	84.0 ± 13.4; $P < 0.0001$	85.5 ± 16.2; $P < 0.0001$	88.8 ± 17.7; $P = 0.016$
Nonresponders	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 (%)				
Responders	—	16.0 ± 6.1; $P < 0.0001$	14.3 ± 6.9; $P < 0.0001$	10.5 ± 6.1; $P = 0.016$
Nonresponders	—	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	—	—	—	—
HbA_{1c} (%)				
Responders	7.4 ± 1.0	5.9 ± 0.4; $P < 0.0001$	5.8 ± 0.4; $P < 0.0001$	6.0 ± 0.3; $P < 0.0001$
Nonresponders	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)				
Responders	8.3 ± 2.4	5.7 ± 0.8; $P < 0.0001$	5.6 ± 0.6; $P < 0.0001$	5.6 ± 0.7; $P < 0.0001$
Nonresponders	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)				
Responders	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); $P < 0.0001$
Nonresponders	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)				
Responders	0.99 ± 0.32	0.59 ± 0.23; $P < 0.0001$	0.59 ± 0.21; $P < 0.0001$	0.65 ± 0.25; $P < 0.0001$
Nonresponders	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)				
Responders	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); $P < 0.0001$
Nonresponders	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)	—	—

Data are mean ± SD and median (IQR) unless otherwise indicated. Data for the NDC group are shown in the 5 months column because these participants were recruited to have weight equivalent to the intervention group after weight loss. Comparisons are shown for baseline to 5 months, baseline to 12 months, and baseline to 24 months. The percentage of weight loss is shown for each group at that particular time point vs. the same number of participants at the baseline. *Responders vs. nonresponders at each time point.

24 months (936 [635–1,435] pmol/min/m^2; $P = 0.023$ vs. baseline) (Fig. 2*A*). The maximal rate of insulin secretion for the NDC group was 1,016 (857–1,509) pmol/min/m^2, comparable to that for the responders at 12 and 24 months ($P = 0.064$ and $P = 0.244$, respectively) (Fig. 2*A*). Nonresponders showed no change in median maximal insulin responses (baseline 461 [296–691] pmol/min/m^2, 5 months 491 [388–629] pmol/min/m^2, 12 months 485 [387–568] pmol/min/m^2, 24 months 452 [347–616] pmol/min/m^2). The difference between responders and nonresponders 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 (530 [453–844], 567 [472–867], 559 [429–835], and 488 [378–720] pmol/min/m^2, respectively) (Fig. 2*A* and Supplementary Fig. 3). Maximal insulin response to arginine in the intervention group at 12 months correlated with first-phase insulin response ($r = 0.6$; $P < 0.0001$) and FPG and HbA_{1c} ($r = -0.4$; $P = 0.003$ 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 (IQR 4–67) pmol/min/m^2 at baseline to 107 (59–163) pmol/min/m^2 ($P < 0.0001$) at 5 months, remaining constant at 12 months (110 [59–201] pmol/min/m^2; $P < 0.0001$). At 24 months, this level was maintained in responders (125 [65–166] pmol/min/m^2; $P < 0.0001$) (Fig. 2*B*). The first-phase

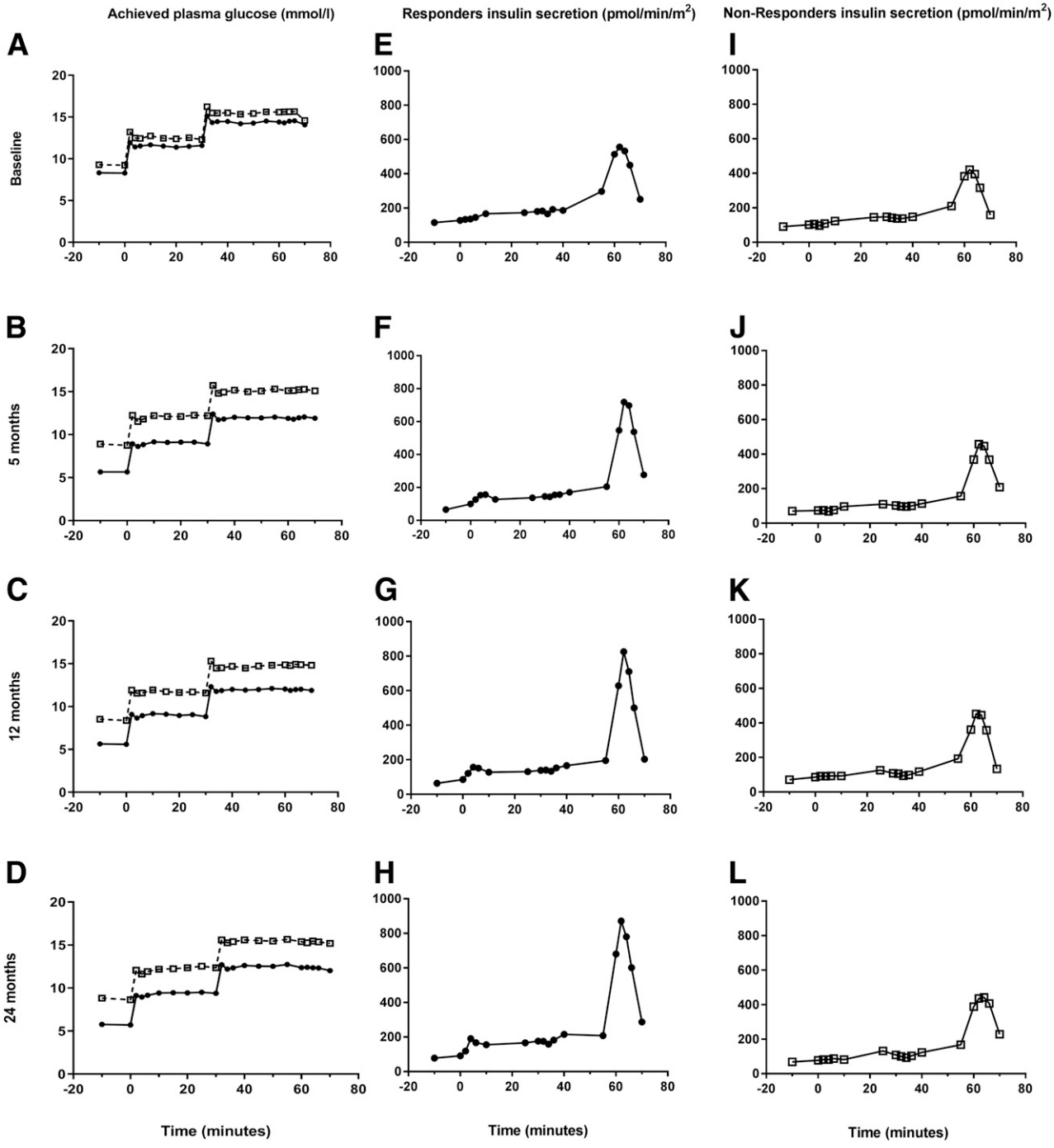

Figure 1—Stepped insulin secretion test. *A–D*: Mean plasma glucose during SISTA in responders (●) and nonresponders (□) at baseline, 5 months, 12 months, and 24 months, respectively. *E–H*: Median insulin secretion rates in responders at baseline, 5 months, 12 months, and 24 months, respectively. *I–L*: Median insulin secretion rates in nonresponders at baseline, 5 months, 12 months, and 24 months, respectively.

insulin response remained substantially lower than in the NDC group (250 [226–429] pmol/min/m^2; $P < 0.0001$) compared with responders at each time point after weight loss. There was no change in nonresponders from baseline (23 [10–36] pmol/min/m^2) either after weight loss at 5 months (14 [7–33] pmol/min/m^2; $P = 0.864$), at 12 months (19 [−7 to 36] pmol/min/m^2; $P = 0.746$) (Fig. 2*B*), or at 24 months (17 [6–33] pmol/min/m^2; $P = 1.000$). The first-phase insulin response of the diabetic control group remained low and unchanged (25 [−5 to 61], 28 [3–43], 29 [−11 to 60], and 38 [4–71] pmol/min/m^2, respectively) (Fig. 2*B* and Supplementary Fig. 3).

The median first-phase insulin response was associated with ambient FPG, being 89 (IQR 59–188) pmol/min/m^2 with FPG ≤6.0 mmol/L, 70 (45–81) with FPG 6.1–6.9 mmol/L, and 35 (−3 to 45) pmol/min/m^2 with FPG ≥7 mmol/L. The relationship between first-phase insulin response and FPG is shown in Fig. 3. First-phase insulin response correlated significantly in the intervention group with FPG ($r = -0.9$; $P < 0.0001$), HbA_{1c} ($r = -0.8$; $P < 0.0001$), maximal insulin secretion ($r = 0.6$; $P < 0.0001$), and

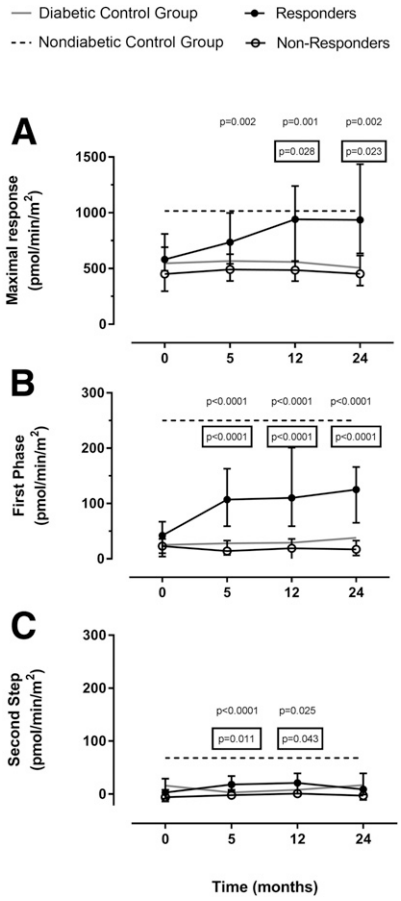

Figure 2—β-Cell response during SISTA. Comparison of the median maximal insulin response (*A*), median first-phase insulin response (*B*), and median second-step insulin secretion (*C*) in responders, nonresponders, and NDC group participants at baseline and 5, 12, and 24 months. *P* values in boxes indicate responders at each time point vs. baseline, and *P* values without boxes indicate responders vs. nonresponders at each time point.

second-step insulin response ($r = 0.4$; $P = 0.022$) at 12 months. 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 (Supplementary Fig. 3).

Second-Step Insulin Response

There was a small, but significant increase in median second-step insulin response in responders (3 [IQR −9 to 29] to 18 [8–34] pmol/min/m^2; $P = 0.011$) at 5 months (Fig. 2*C*), remaining steady to 12 months (21 [1–39] pmol/min/m^2; $P = 0.043$) and with no significant change by 24 months (9 [−1 to 39] pmol/min/m^2; $P = 0.256$) versus baseline. Median second-step insulin response did not change significantly either in nonresponders (baseline −6 [−14 to 8] pmol/min/m^2, 5 months −2 [−8 to 6] pmol/min/m^2, 12 months 1 [−1 to 7] pmol/min/m^2, 24 months −3 [−11 to 9] pmol/min/m^2) or in control participants (16 [−2 to 33], 3 [−13 to 15], 8 [−7 to 50], and 17 [1–23] pmol/min/m^2, respectively). There was a significant difference between second-step insulin response in responders and nonresponders at 5 months ($P < 0.0001$) and 12 months ($P = 0.025$) but not at 24 months ($P = 0.070$). The median second-step insulin response in the NDC group was 68 (42–135) pmol/min/m^2, greater than that of the responders at every time point ($P < 0.0001$).

CONCLUSIONS

We report the gradual increase in assessed functional β-cell capacity over a 24-month 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 participants returning to nondiabetic plasma glucose control exhibiting an early increase followed by stability from 5 to 24 months. Unlike the assessed β-cell capacity, first-phase insulin response improved significantly but remained around one-half of that of the NDC group participants.

Studies of rodent β-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 β-cells during middle age or more advanced years. This may account for the prolonged phase of recovery reported here following weight loss and restoration of glucose control in type 2 diabetes. Given the recognized heterogeneity of β-cells (26), it is possible that some cells are at a more advanced stage of dedifferentiation, and the slow return to normal functional β-cell capacity reflects different rates of redifferentiation (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 β-cell number or function during maintained or increased body weight (1,3,4). No comparable observations have been made in previous studies, and the pathophysiological mechanism underlying this observation requires investigation. GLP-1 responsiveness was not examined in this study, and dietary weight loss has previously been shown not to change it after return to nondiabetic glucose control (28,29).

The first-phase insulin response did not completely normalize, although the degree of recovery was compatible with maintaining nondiabetic blood glucose control. Not all β-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 subnormal response during the postprandial 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 nondiabetic 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 a continued low-calorie diet that was more restricted (~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 antidiabetes agents but not insulin (17). Subjects with prediabetes and first-degree relatives of people with type 2 diabetes have a subnormal first-phase response sufficient to permit overall control of plasma glucose before the onset of type 2 diabetes (30,31), so it is possible that we may have observed a return to the premorbid levels in those achieving remission.

Metabolic stress on the β-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 is likely to be through fat. Although the early sharp decrease in FPG levels will enhance the first-phase response, this typically happens within hours, with

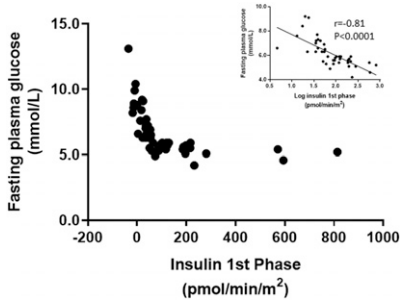

Figure 3—Relationship between FPI and first-phase insulin secretion. FPG and first-phase insulin secretion in the whole intervention group at 12 months ($n = 48$: responders = 28, nonresponders = 16, relapsers = 4) were plotted. The inset shows the log-transformed data (seven individuals omitted because of negative first-phase insulin secretion values unable to be logged).

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 β-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 after weight loss (16). The conditions of the insulin secretion test used permit assessment of β-cell function largely independent of tissue insulin sensitivity. As an indication of whole-body insulin sensitivity, FPI, and hence its inverse, was not different between responders and nonresponders at any time point (Table 1).

The present studies were conducted in people with <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 β-cell function in response to the metabolic stress. At 12 months, responders had a significantly lower duration of diabetes than nonresponders (17). This durability over time to withstand the β-cell stress induced by the combination of high-glucose and high-fat exposure suggests that exploration of the genetic basis of this β-cell behavior is required. The majority of discovered genes associated with type 2 diabetes code for β-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 the demonstration of complete resistance to fat-induced stress of islets isolated by Lee et al. (38) from ZDF rats not predisposed to develop diabetes upon high-fat feeding. It is possible that novel therapeutic targets may be identified to protect the β-cells of susceptible individuals, guided by genotyping.

Attempts to study β-cell mass have evolved from postmortem histological studies (3,4,39), through techniques of fresh pancreas slice histology (40) and incubation, to imaging of β-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 β-cells that are functional can be assessed indirectly by metabolic tests (42,43). An arginine bolus during hyperglycemia elicits a large spike in insulin secretion that depends on functional β-cell mass. There is a tight correlation between the response seen at different levels of plasma glucose in type 2 diabetic and nondiabetic 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. SISTA uses this defined response to permit an assessment of the functional β-cell capacity in vivo and observation of the time course of recovery of function.

In summary, the functional β-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 that weight regain was minimized, both functional β-cell capacity and first-phase insulin response remained stable at least up to 2 years, with no evidence of any time-dependent decrease in β-cell function.

Acknowledgments. The authors thank Helen Pilkington for research nurse support and Louise Burnip, Alison Younghusband, and Susan McLellen for clinical biochemistry, all of The Newcastle upon Tyne Hospitals NHS Foundation Trust. The authors also are grateful to the volunteers for participation.

Funding and Duality of Interest. This study was funded by Diabetes UK as a Strategic Research Initiative (award number 13/0004691) and was supported by facilities of the National Institute for Health Research Newcastle Biomedical Research Centre. A.A.-M. reports a grant from Diabetes UK to conduct the Re-TUNE study. N.S. reports grants and personal fees from Boehringer Ingelheim and personal fees from AstraZeneca, Eli Lilly, Sanofi, and Novo Nordisk. M.E.J.L. reports personal fees from Counterweight and Cambridge Weight Plan not related to the present work. R.T. reports grants from Diabetes UK and lecture fees from Novartis, Eli Lilly, and Jansen during the conduct of the study. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. S.V.Z. performed the clinical work and cowrote the manuscript. A.A.-M. analyzed the data and cowrote the manuscript. C.P. performed the clinical work and edited the manuscript. A.B. trained and mentored practice nurses and edited the manuscript. B.A. contributed to the data analysis and edited the manuscript. K.G.H. developed methodologies and edited the manuscript. A.M. oversaw the statistical analysis and edited the manuscript. N.S. and M.E.J.L. contributed to the discussion and reviewed the manuscript. R.T. oversaw the data analysis and cowrote the manuscript. R.T. is the guarantor of this work 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.

References

1. U.K. Prospective Diabetes Study Group. U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group [published correction appears in Diabetes 1996; 45:1655]. Diabetes 1995;44:1249–1258

2. Deng S, Vatamaniuk M, Huang X, et al. Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. Diabetes 2004;53:624–632

3. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003;52:102–110

4. Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC. Pancreatic beta-cell mass in European subjects with type 2 diabetes. Diabetes Obes Metab 2008;10(Suppl. 4):32–42

5. Gastaldelli A, Ferrannini E, Miyazaki Y, Matsuda M, DeFronzo RA; San Antonio metabolism study. Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study. Diabetologia 2004;47:31–39
6. Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA. β-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. J Clin Endocrinol Metab 2005;90:493–500
7. Jensen CC, Cnop M, Hull RL, Fujimoto WY, Kahn SE; American Diabetes Association GENNID Study Group. Beta-cell function is a major contributor to oral glucose tolerance in high-risk relatives of four ethnic groups in the U.S. Diabetes 2002;51:2170–2178
8. Kendall DM, Sutherland DE, Najarian JS, Goetz FC, Robertson RP. Effects of hemipancreatectomy on insulin secretion and glucose tolerance in healthy humans. N Engl J Med 1990;322:898–903
9. Marchetti P, Bugliani M, Lupi R, et al. The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients. Diabetologia 2007;50:2486–2494
10. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. Proc Natl Acad Sci U S A 1998;95:2498–2502
11. Pinnick K, Neville M, Clark A, Fielding B. Reversibility of metabolic and morphological changes associated with chronic exposure of pancreatic islet beta-cells to fatty acids. J Cell Biochem 2010;109:683–692
12. Cinti F, Bouchi R, Kim-Muller JY, et al. Evidence of β-cell dedifferentiation in human type 2 diabetes. J Clin Endocrinol Metab 2016;101:1044–1054
13. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. Cell 2012;150:1223–1234
14. Weir GC, Bonner-Weir S. Islet β cell mass in diabetes and how it relates to function, birth, and death. Ann N Y Acad Sci 2013;1281:92–105
15. Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. Diabetologia 2011;54:2506–2514
16. Steven S, Hollingsworth KG, Al-Mrabeh A, et al. Very low-calorie diet and 6 months of weight stability in type 2 diabetes: pathophysiological changes in responders and nonresponders. Diabetes Care 2016;39:808–815
17. Taylor R, Al-Mrabeh A, Zhyzhneuskaya S, et al. Remission of human type 2 diabetes requires decrease in liver and pancreas fat content but is dependent upon capacity for β cell recovery. Cell Metab 2018;28:547–556.e3
18. White MG, Shaw JAM, Taylor R. Type 2 diabetes: the pathologic basis of reversible β-cell dysfunction. Diabetes Care 2016;39:2080–2088
19. Lean ME, Leslie WS, Barnes AC, et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. Lancet 2018;391:541–551
20. Lean MEJ, Leslie WS, Barnes AC, et al. Durability of a primary care-led weight-management intervention for remission of type 2 diabetes: 2-year results of the DiRECT open-label, cluster-randomised trial. Lancet Diabetes Endocrinol 2019;7:344–355
21. Pipeleers D, Chintinne M, Denys B, Martens G, Keymeulen B, Gorus F. Restoring a functional beta-cell mass in diabetes. Diabetes Obes Metab 2008;10(Suppl. 4):54–62
22. Taylor R, Barnes A. From new understanding of type 2 diabetes to practical management. Diabetologia 2018;61:273–283
23. Nagi D, Hambling C, Taylor R. Remission of type 2 diabetes: a position statement from the Association of British Clinical Diabetologists (ABCD) and the Primary Care Diabetes Society (PCDS) [Internet], 2019. Available from https://www.pcdsociety.org/news/details/acbd-and-pcds-release-joint-position-statement-on-remission-of-type-2-diabetes. Accessed 17 January 2020
24. Toschi E, Camastra S, Sironi AM, et al. Effect of acute hyperglycemia on insulin secretion in humans. Diabetes 2002;51(Suppl. 1):S130–S133
25. Hovorka R, Soons PA, Young MA. ISEC: a program to calculate insulin secretion. Comput Methods Programs Biomed 1996;50:253–264
26. Bonner-Weir S, Aguayo-Mazzucato C. Physiology: pancreatic β-cell heterogeneity revisited. Nature 2016;535:365–366
27. Weir GC, Aguayo-Mazzucato C, Bonner-Weir S. β-Cell dedifferentiation in diabetes is important, but what is it? Islets 2013;5:233–237
28. Lingvay I, Guth E, Islam A, Livingston E. Rapid improvement in diabetes after gastric bypass surgery: is it the diet or surgery? Diabetes Care 2013;36:2741–2747
29. Steven S, Hollingsworth KG, Small PK, et al. Calorie restriction and not glucagon-like peptide-1 explains the acute improvement in glucose control after gastric bypass in type 2 diabetes. Diabet Med 2016;33:1723–1731
30. Elbein SC, Hasstedt SJ, Wegner K, Kahn SE. Heritability of pancreatic beta-cell function among nondiabetic members of Caucasian familial type 2 diabetic kindreds. J Clin Endocrinol Metab 1999;84:1398–1403
31. Lundgren H, Bengtsson C, Blohmé G, Lapidus L, Waldenström J. Fasting serum insulin concentration and early insulin response as risk determinants for developing diabetes. Diabet Med 1990;7:407–413
32. Boucher A, Lu D, Burgess SC, et al. Biochemical mechanism of lipid-induced impairment of glucose-stimulated insulin secretion and reversal with a malate analogue. J Biol Chem 2004;279:27263–27271
33. Nolan CJ, Prentki M. The islet beta-cell: fuel responsive and vulnerable. Trends Endocrinol Metab 2008;19:285–291
34. Ferner RE, Ashworth L, Tronier B, Alberti KGMM. Effects of short-term hyperglycemia on insulin secretion in normal humans. Am J Physiol 1986;250:E655–E661
35. Boland BB, Brown C Jr., Boland ML, et al. Pancreatic β-cell rest replenishes insulin secretory capacity and attenuates diabetes in an extreme model of obese type 2 diabetes. Diabetes 2019;68:131–140
36. Taneera J, Lang S, Sharma A, et al. A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. Cell Metab 2012;16:122–134
37. Xin Y, Kim J, Okamoto H, et al. RNA sequencing of single human islet cells reveals type 2 diabetes genes. Cell Metab 2016;24:608–615
38. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. Proc Natl Acad Sci U S A 1994;91:10878–10882
39. Rahier J, Goebbels RM, Henquin JC. Cellular composition of the human diabetic pancreas. Diabetologia 1983;24:366–371
40. Marciniak A, Cohrs CM, Tsata V, et al. Using pancreas tissue slices for in situ studies of islet of Langerhans and acinar cell biology. Nat Protoc 2014;9:2809–2822
41. Chen C, Cohrs CM, Stertmann J, Bozsak R, Speier S. Human beta cell mass and function in diabetes: recent advances in knowledge and technologies to understand disease pathogenesis. Mol Metab 2017;6:943–957
42. Nano R, Melzi R, Mercalli A, et al. Islet volume and indexes of β-cell function in humans. Cell Transplant 2016;25:491–501
43. Meier JJ, Breuer TG, Bonadonna RC, et al. Pancreatic diabetes manifests when beta cell area declines by approximately 65% in humans. Diabetologia 2012;55:1346–1354
44. Fritsche A, Stefan N, Hardt E, Schützenauer S, Häring H, Stumvoll M. A novel hyperglycaemic clamp for characterization of islet function in humans: assessment of three different secretagogues, maximal insulin response and reproducibility. Eur J Clin Invest 2000;30:411–418
45. Ward WK, Beard JC, Halter JB, Pfeifer MA, Porte D Jr. Pathophysiology of insulin secretion in non-insulin-dependent diabetes mellitus. Diabetes Care 1984;7:491–502