2-year remission of type 2 diabetes and pancreas morphology: a post-hoc analysis

Ahmad Al-Mrabeh PhD[1]; Kieren G Hollingsworth PhD[1]; Prof James AM Shaw MD[2]; Prof Alex McConnach
FMedSci[4]; Prof Michael EJ Lean MD[5]; Prof Roy Taylor MD[1*]

[1]Magnetic Resonance Centre, Translational and Clinical Research Institute, Newcastle University, Newcastle

[2]Regenerative Medicine, Translational and Clinical Research Institute, Newcastle University, Newcastle upo

[3]Robertson Centre for Biostatistics, University of Glasgow, Glasgow, UK

[4]Institute of Cardiovascular and Medical Science, University of Glasgow, Glasgow, UK

[5]School of Medicine, Dentistry and Nursing, University of Glasgow, Glasgow, UK

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*Senior Author; Correspondence:
Roy Taylor or Ahmad Al-Mrabeh

Newcastle Magnetic Resonance Centre
Campus for Ageing and Vitality
Newcastle upon Tyne NE4 5PL
United Kingdom
Telephone: +44 (0)191 222 1172
Fax: +44 (0)191 222 1151
Email: Roy.Taylor@ncl.ac.uk or Ahmad.al-Mrabeh2@ncl.ac.uk

Summary

Background: The pancreas is small and irregular in shape in type 2 diabetes (T2DM). If these abnormalities are caused by the disease state itself rather than being a predisposing factor, remission of T2DM should restore normal morphology. The objective of this study was to determine whether such changes occurred during two years of remission.

Methods: intervention group participants in the Diabetes Remission Clinical Trial were divided into 'Responders' (HbA1c <6.5%; 48 mmol/mol and fasting blood glucose <7.0 mmol/l off all anti-diabetes medication; n=39) and non-responders (n=16) who remained diabetic. The main statistical analyses were carried out using ANOVA with correction for potential confounding. Novel magnetic resonance techniques were employed to quantify pancreas volume, the irregularity of the pancreas borders, and intra-pancreatic fat content. β-cell function and biomarkers of tissue growth were also measured. Data were compared with matched non-diabetic controls.

Findings: At baseline, pancreas volume was 61.7(SD 16.0)cm^3 in T2DM and 79.8 (14.3) cm^3 in non-diabetic controls (p<0.0001). At 24 months, pancreas volume had increased by 9.4(95% CI: 6.1,12.8)cm^3 in responders compared with 6.4(2.5,10.3) cm^3 in non-responders (p=0.001) . The pancreas borders were more irregular in T2DM at baseline compared with non-diabetic controls (Fractal Dimension 1.138(0.027) vs 1.097(0.025), p<0.0001) and normalised by 24 months in responders only (1.099(0.028)). Intra-pancreatic fat declined by -1.02 (-1.51, -0.53)% in responders and -0.51(-1.19, 0.17)% in non-responders (p=0.23).
Interpretation: These data demonstrate for the first time, to our knowledge, reversibility of the abnormal pancreas morphology of T2DM by weight loss-induced remission.

Funding: Diabetes UK.

Research in context

Evidence before this study

In type 2 diabetes (T2DM), pancreas volume is around 30% lower than normal with irregularity of shape. It is not known whether T2DM develops more readily in those with a smaller pancreas at birth, or whether diminished size is secondary to the disease process. Insulin is a potent growth hormone at the high post-prandial concentration to which pancreas parenchyma is normally exposed. Loss of these peaks in insulin secretion in T2DM could lead to pancreatic involution. A PubMed search using keywords “type 2 diabetes” and "pancreas volume" returned only 15 results. Most of the published studies were cross-sectional in design. None investigated the effect of remission due to weight loss on pancreas volume in T2DM except one paper from our group reporting no short term change in a different cohort.

Added value of this study

This study demonstrates for the first time normalisation of the gross morphology of the pancreas and underpins a frame-shift of understanding of the nature of T2DM. In people with established T2DM, long-term return to non-diabetic blood glucose control is well documented but is not yet universally accepted. Concurrence of morphological and functional restoration of the pancreas provides a solid basis for the concept. The increase in pancreas volume is associated with return of β-cell function, as well as decrease in intra-pancreatic fat content and change in circulating plasma growth factors. The pancreas, predominantly composed of acinar cells, remains one of the least studied organs in diabetes despite its central importance to metabolic control. Studies in diabetes have focused on islet function with little attention to the whole pancreas, largely due to lack of adequate techniques for *in vivo* study related to its inaccessible position.

Implications of all the available evidence

We observed a substantial increase in pancreas volume and decrease in the irregularity of shape of the organ during two years of restored post-meal insulin secretion after weight loss induced remission of T2DM. The increased pancreas volume was associated with both return of insulin secretion and fall in intra-pancreatic fat content. These data demonstrate that T2DM is a potentially reversible disease affecting the whole pancreas with gradual morphological and functional recovery during remission.

Introduction

Despite its importance for whole body metabolism, the pancreas remains one of the least studied organs. This is largely due to its retroperitoneal position and elongated form. Magnetic resonance imaging can be used to measure pancreas volume (1), and the organ is 20-30% smaller in T2DM (2-6). We have reported the associated irregular shape of the pancreas using novel techniques (3). These observations raise the question of whether T2DM develops more readily in those born with a smaller, dysmorphic pancreas, or whether this is secondary to the disease process.

Pancreas volume is also decreased in people with type 1 diabetes within the first year after diagnosis (7-8). Deficiency of endocrine function may be driving the change in both types of diabetes. Insulin is a potent growth hormone at peak concentrations (9). Given that plasma levels of insulin increase normally by 10-15 fold after a meal and that interstitial concentration of insulin around an islet is likely to increase even more, loss of a paracrine action of insulin might explain a decline in pancreas volume in T2DM. Other growth-related circulating factors, particularly GDF-15, FGF-21 and IGF-1 could contribute (6). Also, excess fat within the pancreas is associated with acinar cell fibrosis and potentially with decrease in pancreas volume (10, 11).

Studies over the last decade have revealed the mechanisms underlying weight loss induced remission of T2DM (12,15). In a previous short term study, restoration of non-diabetic blood glucose control did not cause increase in pancreas volume over 6 months (3). We hypothesised that a prolonged period of remission would permit normalisation of gross morphology of the pancreas. The Diabetes Remission Clinical Trial (DiRECT) achieved non-diabetic blood glucose control for 2 years in 36% of the intervention group (16) and permitted testing of this hypothesis.

Methods

Study design

This study involved the subset of participants in the randomised controlled DiRECT able to access the Newcastle University Magnetic Resonance Centre. Baseline characteristics, inclusion criteria, and major clinical outputs of DiRECT have been published (16, 17). Ethical approval for DiRECT (clinical trial registration ISRCTN03267836) was obtained from the West of Scotland Ethics Committee, and all participants gave informed written consent. The Ethics permission was amended in 2016 to permit study of the non-diabetic comparator (NDC) which was recruited by advertisement to match the intervention group after weight loss to provide a guide of how closely to non-diabetic normal pancreas parameters may be restored.

The primary analysis compared data on pancreas volume and irregularity of pancreas border from responders (HbA1c < 48 mmol/mol (6.5%) and FPG<7 mmol/l off all anti-diabetes medications since baseline) with non-responders who remained diabetic. All participants with pancreas volume measurement by MR were included in the analysis. Data were compared with those from NDC group, studied on one occasion, to determine the extent of any normalisation.

By design, the NDC group was matched for age, sex and the post-weight loss weight of the intervention group (86.6±3.0 vs. 85.8±2.0 kg, p=0.55; BMI 29.7±0.8 vs. 29.9±0.6kg/m^2, p=0.96) (Table 1). The randomised T2DM control group was well matched with the intervention group (Table S1).

Clinical and metabolic studies

Participants in the intervention group received the Counterweight-Plus weight management programme, followed by stepped food reintroduction and a low intensity weight maintenance phase up to 24 months (Figure 1). Studies were conducted in intervention and control groups at baseline, 5 months, 12 months, and 24 months.

At baseline, clinical characteristics of responders and non-responders were well matched although the former had shorter duration of diabetes, lower HbA1c, higher fasting insulin and HOMA-IR (Table 1). By 24 months, 13 of the 45 remaining participants had relapsed but are included given the prolonged non-diabetic state and lack of short term change in pancreas volume following change in metabolic state (3).

All studies were carried out after an overnight fast. Insulin secretion was assessed by the stepped insulin secretion test with arginine (SISTA)(12) . MR studies were carried out on a separate day following the metabolic studies. Two participants underwent MR scans, but did not wish to have blood sampling after baseline.

Magnetic Resonance acquisition

MR data were acquired using a 3.0 Tesla Philips scanner with six-channel cardiac array (Philips, Netherlands). A three point Dixon acquisition for fat fraction image measurements was carried out with gradient-echo scans during one breath hold. Two balanced turbo field echo (BTFE) anatomical scans for localization purposes and to assess pancreas morphology were also acquired (4, 12).

Quantification of intra-pancreatic and abdominal fat

Intra-pancreatic fat was measured using the MR-opsy method, applying a threshold of fat content on the acquired fat fraction image to exclude any contribution of interlobular visceral fat to that of the parenchymal tissues of the pancreas (4). All pancreas fat data were analysed by a single

observer (AAM) who originally validated this advanced image processing technique (4). Scans were anonymised immediately after acquisition using non-sequential randomly generated letters/numbers. The key to the blinding was devised and held by a different team member (KGH). The scorer remained blinded until data were finalised.. Subcutaneous and visceral fat areas (SAT/VAT) were measured by another observer blinded as above, using three-point Dixon imaging at the L4–L5 vertebral level (4, 15).

Volume rendering and fractal analysis

Pancreas volume was measured with the above blinding method by surface rendering of the BTFE acquired MR image (3). Briefly, the BTFE image was processed in Drishti (Australian National University) to derive the 3D volume of the whole pancreas, then total pancreas volume was measured using the 'Get volume' function of Drishti's volume exploration and presentation tool. For the Fractal Dimension (FD) analysis, two-dimensional projections of the total extracted volume were generated in Drishti then analysed by FracLac in ImageJ (National Institutes of Health, USA) to assess the complexity of pancreas borders (coefficient of variation 0.88%) (3). The higher the FD, the more irregular the border.

Biochemical analysis

HbA1c (Tina-quant gen 3 method, Roche) and C-reactive protein (CRP)(Roche, Burgess Hill, UK) were measured at the Clinical Pathology Laboratory (University of Glasgow). Total plasma triglyceride was measured by standard methods (Roche Diagnostics, Burgess Hill, UK) at the Clinical Pathology Laboratory (Newcastle upon Tyne Hospital). Plasma glucose was measured by Yellow Springs glucose analyser (YSI, Ohio, USA). Plasma insulin and glucagon were measured by ELISA kits (Mercodia, Uppsala, Sweden). Plasma insulin like growth factor-1 (IGF-1), growth and development factor-15 (GDF-15), and fibroblast growth factor-21 (FGF-21) were analysed by appropriate ELISA kits (R&D systems, Minneapolis, USA) with coefficients of variation of 5.3%, 3.1% and 4.3% respectively. Homeostasis Model Assessment (HOMA) was calculated using HOMA2 Calculator (Oxford University, Oxford, UK).

Statistical analysis

This paper describes a post hoc analysis of the DiRECT dataset. A mixed effects regression model based on repeated-measures ANOVA with Bonferroni's post hoc analysis was used to assess the change in pancreas volume and fractal dimension in each group. This was done after adjusting for age, sex, diabetes duration, and baseline body weight or baseline pancreas volume/FD. All models included a random participant effect, and assumed a compound symmetric correlation structure over time. Examination of change over time was carried out using paired analysis. For absolute values, data are presented as mean (SD) or median and interquartile range (25^{th} and 75^{th}) based on data distribution. For changes from baseline, data were presented as mean (95% CI). For paired data, Student's paired t test or Wilcoxon Signed Rank test were employed based on data distribution. For unpaired analysis, Mann–Whitney U test or two-sample t test was used. To test correlation, Pearson's

or Spearman's rank were used as appropriate. Minitab (version 17) and SPSS (version 26) were used for all statistical analyses. P <0.05 was considered statistically significant.

Role of the Funding source
The funder had no input into study design, conduct, analysis nor writing.

Results

Baseline characteristics

Pancreas volume was lower in the diabetic group than the NDC group (61.7(16.0) vs. 79.8 (14.3) cm^3, p<0.0001). The pancreas volumes of responders and non-responders were similar (62.8(16.8) vs. 59.0 (14.0) cm^3, p=0.39).

The pancreas borders were more irregular in diabetes (FD 1.138(0.027) vs. NDC 1.097(0.025), p<0.0001). The degree of irregularity was similar in responders (1.137(0.025) and non-responders (1.138(0.030), p=0.85).

There was no difference in intra-pancreatic fat at baseline between the responders (8.7(2.4) and non-responders (7.8(2.3)%, p=0.24) and both were higher than the NDC group (6.2 (2.1)%, p<0.0001 and p<0.031 respectively). Intra-pancreatic fat did not differ between intervention and diabetic control groups at baseline (8.5(2.4) vs. 8.2 (2.2)%, p=0.65). GDF-15 levels were higher in T2DM than NDC (0.93[0.72-1.34]vs. 0.67[0.51-0.76] ng/ml, p=0.0001). FGF-21 and IGF-1 levels were similar in diabetic and NDC groups (0.58 [0.42-0.85] vs. 0.57 [0.19-1.20] ng/ml, p=0.89 and 72.3(30.7) vs. 77.0 (24.8) ng/ml, p=0.32, respectively). Comparisons between responder and non-responder groups are shown in Table 1.

Responders and non-responders differed at baseline only in respect of diabetes duration (2.7(1.6) vs. 3.8(1.7) years, p=0.04), HbA1c (57.5(10.6) vs. 62.6(9.2) mmol/mol, p=0.05), fasting insulin (85.0[55.5-143.5) vs. 65.5[48.2-86.9] pmol/l, p= 0.04), HOMA2-IR% (1.86[1.25-3.03] vs. 1.33[1.10-2.00], p=0.008)(Table 1).

Change in pancreas volume

Change in pancreas volume was significant over time in responders even after adjusting for body weight/age/diabetes duration/gender (main ANOVA effect, p=0.001). There was a significant effect of baseline pancreas volume (p=0.01). However, there was no effect of age (p=0.06), weight (p=0.26), gender (p=0.19), and diabetes duration(p=0.83).

Table 2 shows data on pancreatic volume for each group at each time point. It remained unchanged 5 months after commencement of weight loss irrespective of remission (responders: 62.8 (16.8) to 62.3 (4.0) cm^3, p=0.84; non-responders: 58.9 (13.9) to 62.1(4.0)cm^3, p=0.39; Table 2). Figure 2 shows the change from baseline for each group. At 12 months, there was no significant change in responders (n=32, to 67.6 (5.6) cm^3, p=0.13) and in non-responders (n=13, to 64.5(5.7) cm^3, p=0.13). By 24 months, pancreas volume had increased in responders (n=32, to 72.2 (5.4) cm^3; p=0.02 vs. baseline) with a smaller increase in non-responders (n=13: to 65.4 (5.5) cm^3, p=0.10 vs. baseline; Table 2).
The overall change of volume in responders was greater than non-responders (9.4(6.1, 12.8) cm^3 vs. 6.4(2.5, 10.3) cm^3, p=0.001; Figure 2, Table 2). There was a small increase in the T2DM control group (2.6(-1.0, 6.4)cm^3; p=0.10) which was significantly less than responders (p=0.001 for controls vs. responders). The extent of change towards normal in the responder group is shown in Figure 3B. By 24 months, pancreas volume in responders was still lower than the NDC group (71.1 (14.7) vs. 79.8(14.3)cm^3, p=0.03).

Change in pancreas irregularity

FD changed over time after weight loss (main ANOVA effect, p=0.01), and this change was related to remission (p=0.04 for time*remission interaction). If adjusted for diabetes duration/baseline FD/ body weight/age/ and gender significance was lost (p=0.11 for time*remission interaction).

There was no significant effect of baseline parameters on change in FD except for sex (p=0.030). After adjusting for baseline, post hoc pairwise comparisons showed no significant difference between responders and non-responders at 24 months (1.099 (0.028) vs. (1.121 (0.025), p=0.06), but there was a difference between responders and controls 1.099(0.028) vs. 1.125(0.028), p=0.01).
Figure 3 shows FD at each time point in responders. There was a steady improvement in volume with FD being decreased at 12 months (1.104(0.03), p=0.02 vs. baseline) and at 24 months (1.099 (0.028), p=0.003 vs. baseline, Figure 3C). Mean FD of responders at 24 months became similar to that of NDC (1.099(0.028) vs. 1.097(0.025), p=0.48; Figure 3C). The difference in change from baseline between responders and non-responders was significant (-0.038(-0.039,-0.036) vs. -0.017(-0.017,-0,018); p=0.05). Non-responders showed no significant change from baseline (1.138(0.030) to 1.122(0.026), p=0.12). In view of the modest numerical decrease in FD of the non-responder group, the effect of improvement in glucose control within the diabetic range was examined. The upper tertile of improvement in FD in non-responders compared with the lower tertile showed a greater decrease in HbA1c (-9.3 (-17.9,-0.7) vs. +5.2 (-2.2, 12.6) mmol/mol, p=0.03). FD of the diabetic control group was unchanged between baseline and 24 months (1.126(0.032) to 1.125(0.028), p=0.23).

Change in endocrine function

Beta cell

At 5 months after weight loss, first phase insulin secretion increased only in responders (to 0.11 [0.060 to 0.157] nmol/ml/min, p<0.0001 vs. baseline), maintained at 24 months (0.06 [0.038 to 0.131] nmol/ml/min, p=0.001 vs. baseline). The change in first phase insulin secretion correlated with change in pancreas volume at 24 months (R=0.33, p=0.03; Figure S1A). Maximally stimulated insulin response in responders did not increase by 5 months (0.74 [0.530 to 0.990] nmol/ml/min, p=0.21 vs. baseline) but rose steadily over the whole period (0.98 [0.609 to 1.514] nmol/ml/min, p=0.005 vs. baseline), becoming similar to NDC (Figure 3D). Consistent with the persistent improvement in pancreas volume and morphology, maximal insulin secretion remained high in relapsers, and was not different to responders at 2 years (1.00 [0.618-1.384] vs. 0.942[0.60-1.474] nmol/min/m^2, p= 1.0*).*

Alpha cell

Plasma glucagon levels at baseline were similar between responders and non-responders (22.0 [19.0-32.8] vs. 24.5[18.5-31.5] pg/ml, p=0.75) and both were higher than the NDC (14.0 [8.0-21.0] pg/ml, p<0.0001, and p=0.0008, respectively). After weight loss, fasting glucagon decreased in both responders (to 11.5[15.0-31.24]pg/ml, p<0.0001 vs. baseline) and non-responders (to 12.5[7.0-21.0] pg/ml, p=0.009 vs. baseline). Over 24 months, there was no change in in glucagon level within the diabetic control group.

HOMA index

HOMA2-IR decreased in both groups after weight loss (responder: 1.86[1.25-3.03] to 0. 60[0.38-1.03], p<0.0001; non-responders: 1.33[1.10-2.00] to 0.61[0.43-0.82], p<0.0001). At 12 months, HOMA2-IR remained low in responders and non-responders (068[0.39-1.14] vs. 0.58[0.48-0.98], p=0.79).

Change in pancreas fat

The initial fall in fat content of the pancreas was similar in responders and non-responders but after 24 months only that of the responders was lower than baseline (p<0.0001). Responders decreased by -1.02 (-1.51,-0.53)% compared to -0.51(-1.19, 0.17)% for non-responders (p=0.23), but levels remained higher than in NDC (8.0(2.5) vs. 6.2 (2.1) p= 0.02). There was a negative correlation between change in pancreas fat and change in pancreas volume (R=-0.39, p=0.008; Figure S1B). Over 24 months, there was no change in pancreas fat within the diabetic control group (Table S1).

Change in plasma biomarkers

At 5 months, after weight loss, GDF-15 decreased in responders (0.92 [0.71-1.48] to 0.75[0.57-0.96] ng/ml, p=0.002) with no significant change in non-responders (0.99[0.73-1.16] to 0.75[0.70-1.00] ng/ml, p=0.33) (Table 1). FGF-21 decreased similarly in responders and non-responders after weight and IGF-1 increased in both responders and non-responders (Table 1). . The subsequent time course of change is shown in Table 1 and lack of change within the diabetic control group (Table S1).

CRP decreased after weight loss in the whole intervention group (2.7[1.0-4.6] to 1.1[0.49-1.8] mg/l, p<0.0001). By 12 months, CRP was lower than baseline in responders (to 2.4[1.0-5.4] mg/l, p=0.001), and in non-responders (to 1.5[1.0-1.8] mg/l, p=0.01) and at 24 months, there was no significant difference between responders and non-responders (1.3[0.4-4.3] vs. 1.6[0.4-2.2] mg/l, p=0.71).

Discussion

This study describes for the first time, to our knowledge, changes in the gross morphology of the pancreas in T2DM after weight loss-induced remission. After 24 months of remission, the small, irregular pancreas typical of T2DM had increased in volume to 89% of that of a matched non-diabetic group. This was associated with a complete return to normal of the irregularity of pancreas border in contrast to no change in non-responders. Both of these changes were correlated with decrease in intra-pancreatic fat and recovery of first phase insulin secretion. The low pancreas volume and irregularity in shape in T2DM is likely to be a consequence rather than a cause of the disease state, which may be related to deficiency of post-prandial peaks of insulin secretion over many years of disease progression. Weight loss was associated with normalisation of the raised GDF-15 levels only in those who became non-diabetic, whereas decrease in FGF-21 and increase in IGF-1 occurred irrespective of whether or not non-diabetic glucose control was regained.

Total pancreas volume reflects acinar cells mass with islet and ducts contributing only ~5% (18). Low pancreas volume could be a risk factor for T2DM development or secondary to the disease process. The decline in pancreas volume is evident soon after diagnosis, and appears to decrease further with increasing duration of diabetes (3, 15, 19). Restoration of normal insulin secretion for 6 months following dietary reversal of T2DM did not significantly increase pancreas volume (3, 15). However, insulin deficiency is likely to develop over many years during and after T2DM onset, and any recovery in insulin production may take years to impact on size. We hypothesised that longer-term observation would be required to detect a consequent increase in volume. Plasticity and regeneration of acinar cells are well-documented Clinical and observational studies in chronic pancreatitis have previously suggested that pancreatic acinar cell mass is related to islet function (20). *In vitro* studies on cultured rodent

acinar cells have demonstrated the expected trophic effects of insulin on increased cell number, DNA, and protein content (21). The present data provide insight into the physiological associations of acinar cell mass regeneration.

Loss of β-cell function is associated with acinar cell fibrosis in humans (11) and fat replacement of the acinar cells is associated with fibrosis in the ZDF diabetic rat (10). Advanced fibrosis may lead to destruction of the islets and cause further β-cell dysfunction (10). Trans-differentiation of the acinar cells into adipocytes occurs during ageing, regulated through c-Myc transcription factor (22). Whether these acinar cell changes in rodents exist in humans is uncertain and the cellular and molecular basis of the changes of human T2DM requires to be elucidated. The role of intra-pancreatic fat on pancreas tissues, directly or indirectly through β-cell function, is an important question yet to be answered. Fat replacement of acinar cells in the pancreas is associated with ageing, obesity, and diabetes. In several studies we have demonstrated the association of raised intra-pancreatic fat with T2DM, and lowering of this with remission (12, 15). In a previous cohort with longer duration of diabetes (3) we have demonstrated a negative relationship between intra-pancreatic fat content and pancreas volume and it is notable that decrease in volume was demonstrated in the present study with diabetes of duration only up to 6 years. Insulin resistance in peripheral tissue does not change after weight loss and in liver changes identically in responders and non-responders (12, 23).

The present data demonstrate that recovery of acinar cell mass is associated with smoothing of the pancreatic borders. This simplest explanation could be that this reflects the re-expansion of the whole organ and at least partial correction of the atrophied state associated with diabetes. The processes underlying T2DM operate for at least a decade before diagnosis (24), possibly including a gradual decrease in pancreas volume, and this time course may also apply to resolution of the morphological changes. This is supported by the present observation in those who put on weight and re-developed diabetes by 24 months but there was no loss of volume or change in irregularity of pancreas border despite loss of acute first phase insulin response and increase in hepatic-derived triglycerides (14).

Regulation of cellular growth is a major physiological process involving several hormones. IGF-1 exhibits affinity for the insulin receptor and mimics its function at high concentration (9). However, there are no IGF-I receptors on acinar cells (21). Given the known cross-reactivity of IGF-1 and insulin with the heterologous receptors at around 10% of sensitivity to the homologous receptor, it is likely that insulin has a predominant trophic effect. IGF-1 has been reported to decrease in association with diabetes and ageing (25), where pancreas volume was also reported to be sub-normal (2, 7, 26). GDF-15 is involved in cellular processes related to cell stress and apoptosis and is associated with T2DM and cardiovascular disease (27). We found GDF-15 level to be high in T2DM compared with non-diabetic controls. In addition, FGF-21 was reported to be strongly associated with obesity and T2DM and a regulator of lipid and glucose metabolism (28). Secreted by the liver and highly expressed in the acinar cells of the pancreas (29), it has a protective role against islet inflammation through regulation of lipid accumulation (30). However, FGF-21 levels decreased equally after weight loss irrespective of remission or change in pancreas volume.

Limitations of the present study require to be considered. Firstly, the slow, gradual return towards normal in pancreas volume suggests that even the 2 year time course of the present study may has been insufficient to observe the full impact of the return to the non-diabetic state. Secondly,

although a clear difference in recovery of pancreas volume was demonstrated between responders and non-responders, a statistically significant difference in change of border irregularity between responders and non-responders was not observed. This was so even though irregularity of pancreas borders improved in responders only, and the observed improvement in HbA1c within the diabetic range by some within the non-responder group may have accounted for this phenomenon. Thirdly, although plasticity and regeneration of acinar cells are well documented in medical literature (31, 32) we did not measure faecal elastase and future studies should measure this to assess pancreas insufficiency. Fourthly, although the cohort of people with T2DM were typical for the condition, 98% were of Caucasian ethnicity (16). Intra-pancreatic fat levels are typically lower in Black than Causcasian populations although it is unlikely that this disease, which behaves similarly in different populations in response to over- or under-nutrition (33,34), would exhibit differences in basic pathogenesis. Direct study of other ethnicities is required. Finally, as the circulating plasma biomarkers reflect whole body rather than pancreas stress, further mechanistic studies in animal models are required to determine how fat, insulin, IGF-1, GDF-15, and FGF-21 affect atrophy of acinar cells.

Contribution Statement: AAM carried out morphometric, biochemical & statistical analyses, and drafted the manuscript. KGH and AAM developed MR methodologies for data acquisition and analysis. JAMS & NS contributed substantially to data analysis and interpretation. AM is the chief biostatistician of DiRECT, who supervised all the statistical analyses. RT & MEJL are the principal investigators of DiRECT. All authors critically reviewed and revised the manuscript, and have read and approved the final version.

Conflict of Interest: RT reports lecture fees from Lilly and Novartis, consultancy fees from Wilmington Healthcare and is author of the book 'Life Without Diabetes'. MEJL reports personal fees from Roche, Novo Nordisk & Eli Lilly. NS reports personal fees from Amgen, personal fees from AstraZeneca, grants and personal fees from Boehringer Ingelheim, personal fees from Eli Lilly, personal fees from Janssen, personal fees from NAPP Pharmaceuticals, personal fees from Novo Nordisk, personal fees from Sanofi, outside the submitted work. JAMS reports lecture fee and travel support for attendance at an international diabetes conference from Novo Nordisk in addition to honoraria for participating in Novo Nordisk and Medtronic Scientific Advisory Boards. AAM report grant from Diabetes UK to carry out the Re-TUNE study, and EASD Lilly scholarship to attend 2019 EASD meeting. All other authors report no conflict of interest.

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References

1. Szczepaniak EW, Malliaras K, Nelson MD, Szczepaniak LS. Measurement of pancreatic volume by abdominal MRI: a validation study. PLoS One. 2013;8(2):e55991.
2. Macauley M, Percival K, Thelwall PE, Hollingsworth KG, Taylor R. Altered volume, morphology and composition of the pancreas in type 2 diabetes. PLoS One. 2015;10(5):e0126825.
3. Al-Mrabeh A, Hollingsworth KG, Steven S, Taylor R. Morphology of the pancreas in type 2 diabetes: effect of weight loss with or without normalisation of insulin secretory capacity. Diabetologia. 2016;59(8):1753-9.
4. Al-Mrabeh A, Hollingsworth KG, Steven S, Tiniakos D, Taylor R. Quantification of intrapancreatic fat in type 2 diabetes by MRI. PLoS One. 2017;12(4):e0174660.
5. Yagihashi S. Diabetes and pancreas size, does it matter? J Diabetes Investig. 2017;8(4):413-5.
6. Al-Mrabeh A. Pathogenesis and remission of type 2 diabetes: what has the twin cycle hypothesis taught us? Cardiovascular Endocrinology and Metabolism. 2020;In press.
7. Williams AJ, Thrower SL, Sequeiros IM, Ward A, Bickerton AS, Triay JM, et al. Pancreatic volume is reduced in adult patients with recently diagnosed type 1 diabetes. The Journal of clinical endocrinology and metabolism. 2012;97(11):E2109-13.
8. Virostko J, Williams J, Hilmes M, Bowman C, Wright JJ, Du L, et al. Pancreas Volume Declines During the First Year After Diagnosis of Type 1 Diabetes and Exhibits Altered Diffusion at Disease Onset. Diabetes Care. 2019;42(2):248-57.
9. Poggi C, Le Marchand-Brustel Y, Zapf J, Froesch ER, Freychet P. Effects and binding of insulin-like growth factor I in the isolated soleus muscle of lean and obese mice: comparison with insulin. Endocrinology. 1979;105(3):723-30.
10. Matsuda A, Makino N, Tozawa T, Shirahata N, Honda T, Ikeda Y, et al. Pancreatic fat accumulation, fibrosis, and acinar cell injury in the Zucker diabetic fatty rat fed a chronic high-fat diet. Pancreas. 2014;43(5):735-43.
11. Clark A, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI, Matthews DR, et al. Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. Diabetes Res. 1988;9(4):151-9.
12. 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;54:2506-14.
13. Taylor R, Al-Mrabeh A, Zhyzhneuskaya S, Peters C, Barnes AC, Aribisala BS, et al. Remission of human type 2 diabetes requires decrease in liver and pancreas fat content but is dependent upon capacity for beta cell recovery. Cell Metab. 2018;28(4):547-56.e3.
14. Al-Mrabeh A, Zhyzhneuskaya SV, Peters C, Barnes AC, Melhem S, Jesuthasan A, et al. Hepatic Lipoprotein Export and Remission of Human Type 2 Diabetes after Weight Loss. Cell Metab. 2020;31(2):233-49 e4.
15. Steven S, Hollingsworth KG, Al-Mrabeh A, Avery L, Aribisala BS, Caslake M, 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;39:158-65.
16. Lean MEJ, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, 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. The Lancet Diabetes & Endocrinology. 2019;7(5):344-55.

17. Taylor R, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, et al. Clinical and metabolic features of the randomised controlled Diabetes Remission Clinical Trial (DiRECT) cohort. Diabetologia. 2018;61(3):589-98.
18. Longnecker DS. Anatomy and Histology of the Pancreas. Pancreapedia: The Exocrine Pancreas Knowledge Base. 2014.
19. Lim S, Bae JH, Chun EJ, Kim H, Kim SY, Kim KM, et al. Differences in pancreatic volume, fat content, and fat density measured by multidetector-row computed tomography according to the duration of diabetes. Acta diabetologica. 2014;51(5):739-48.
20. Bateman AC, Turner SM, Thomas KS, McCrudden PR, Fine DR, Johnson PA, et al. Apoptosis and proliferation of acinar and islet cells in chronic pancreatitis: evidence for differential cell loss mediating preservation of islet function. Gut. 2002;50(4):542-8.
21. Mossner J, Logsdon CD, Williams JA, Goldfine ID. Insulin, via its own receptor, regulates growth and amylase synthesis in pancreatic acinar AR42J cells. Diabetes. 1985;34(9):891-7.
22. Bonal C, Thorel F, Ait-Lounis A, Reith W, Trumpp A, Herrera PL. Pancreatic inactivation of c-Myc decreases acinar mass and transdifferentiates acinar cells into adipocytes in mice. Gastroenterology. 2009;136(1):309-19.e9.
23. Steven S, Hollingsworth KG, Al-Mrabeh A, Avery L, Aribisala B, Caslake M, 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(5):808-15.
24. Porta M, Curletto G, Cipullo D, Rigault de la Longrais R, Trento M, Passera P, et al. Estimating the delay between onset and diagnosis of type 2 diabetes from the time course of retinopathy prevalence. Diabetes Care. 2014;37(6):1668-74.
25. Friedrich N, Thuesen B, Jorgensen T, Juul A, Spielhagen C, Wallaschofksi H, et al. The association between IGF-I and insulin resistance: a general population study in Danish adults. Diabetes Care. 2012;35(4):768-73.
26. Saisho Y, Butler AE, Meier JJ, Monchamp T, Allen-Auerbach M, Rizza RA, et al. Pancreas volumes in humans from birth to age one hundred taking into account sex, obesity, and presence of type-2 diabetes. Clin Anat. 2007;20(8):933-42.
27. Patel S, Alvarez-Guaita A, Melvin A, Rimmington D, Dattilo A, Miedzybrodzka EL, et al. GDF15 Provides an Endocrine Signal of Nutritional Stress in Mice and Humans. Cell Metab. 2019;29(3):707-18.e8.
28. Fisher FM, Maratos-Flier E. Understanding the Physiology of FGF21. Annu Rev Physiol. 2016;78:223-41.
29. Singhal G, Fisher FM, Chee MJ, Tan TG, El Ouaamari A, Adams AC, et al. Fibroblast Growth Factor 21 (FGF21) Protects against High Fat Diet Induced Inflammation and Islet Hyperplasia in Pancreas. PLoS One. 2016;11(2):e0148252.
30. Xie T, So WY, Li XY, Leung PS. Fibroblast growth factor 21 protects against lipotoxicity-induced pancreatic beta-cell dysfunction via regulation of AMPK signaling and lipid metabolism. Clin Sci (Lond). 2019;133(19):2029-44.
31. Murtaugh LC, Keefe MD. Regeneration and repair of the exocrine pancreas. Annu Rev Physiol. 2015;77:229-49.
32. Zhou Q, Melton DA. Pancreas regeneration. Nature. 2018;557(7705):351-8.
33. Szczepaniak LS, Victor RG, Mathur R, Nelson MD, Szczepaniak EW, Tyer N, et al. Pancreatic steatosis and its relationship to beta-cell dysfunction in humans: racial and ethnic variations. Diabetes Care. 2012;35(11):2377-83.
34. Bynoe K, Unwin N, Taylor C, Murphy MM, Bartholomew L, Greenidge A, et al. Inducing remission of Type 2 diabetes in the Caribbean: findings from a mixed methods feasibility study of a low-calorie liquid diet-based intervention in Barbados. Diabetic medicine : a journal of the British Diabetic Association. 2019.

	Responders				Non-responders				NDC
	0 M	**5M**	**12M**	**24M**	**0 M**	**5M**	**12M**	**24M**	**0 M**
Age (year)	53.2(7.3)	-	-	-	52.8(8.2)	-	-	-	55.8(6.0)
Sex (M/F)	22/16	-	-	-	8/8	-	-	-	13/12
D. Duration (year)	2.7(1.6)#	-	-	-	3.8(1.7)	-	-	-	-
Weight (kg)	100.7(16.6)	84.5(13.7)***	86.8(15.7)***	91.7(17.3)***	99.8(17.6)	86.2(16.7)***	89.3(15.9)***	90.3(14.6)***	86.6(14.9)
BMI (kg/m^2)	34.8(4.4)	29.3(4.1)***	30.1(4.6)***	31.8±(4.9)***	34.7(4.6)	30.0(4.8)***	31.1(4.7)***	31.7(4.5)***	29.7(3.8)
HbA1c (mmol/mol)	57.5(10.6)#	40.9(4.4)***	42.499.5)***	50. 5(14.6)**	62.6(9.2)	65.3(18.8)	61.5 (9.1)	65.3(14.4)	35.2(3.4)§§§
Fasting insulin (pmol/l)	85.0[55.5-143.5]#	31.0[19.5-52.3]***	34.3[19.1-58.4]***	46.2.[18.5-82.3]**	65.5[48.2-86.9]	29.0[19.6-38.8]***	28.0[23.2-47.7]**	34.4[16.5-49.5]**	16.4[10-37.2]§§
Fasting glucose (mmol/l)	8.(2.4)	5.7(0.8)***	5.8(0.9)***	6.6(1.9)**	9.4(2.9)	8.9(2.7)	8.4(1.9)	9.3(4.0)	5.1(0.4)§§§
Fasting glucagon (pg/ml)	22.0[19-23.8]	14.5[11.3-20.8]***	16.0[13.0-25.5]**	20.0[13.0-29.0]*	24.5[18.5-31.8]	12.5[7.0-21]**	17.5[15.3-25.8]*	19.5[14.0-27.3]	14.0 [8.0-21.0]§
HOMA2-IR(%)	1.86[1.25-3.03] #	0.60 [0.38-1.03] ***	0.68[0.37-1.14] ***	0.89[0.37-1.57] ***	1.33[1.10-2.00]	0.61[0.43-0.82]**	0.58[0.48-0.98]**	0.79[0.34-1.19]**	0.31[0.18-0.69] §§
Total plasma TG (mmol/l)	1.72[1.15-2.29]	1.05[0.70-1.63] ***	1.15[0.79-1.65] **	1.23[0.89-1.73]**	1.55[1.21-1.96]	1.00[0.88-1.25]***	1.13[0.87-1.30]**	1.46[0.95-1.56]	1.10[0.80-1.50]
Pancreas fat (%)	8.7(2.4)	7.8(2.3)***	7.9(2.0)***	8.0(2.5)***	7.8(2.3)	7.1(2.3)**	6.7(1.7)*	7.0(1.2)	6.2(2.1) §§
IGF-1 (ng/ml)	70.7±(29.9)	104.0(38.6)***	96.1(37.3)**	80.2(36.2)	76.2(33.0)	97.9(42.1)*	92.6±(54.3)	81.3(38.5)	77.0(24.8)
GDF-15 (ng/ml)	0.92[0.71-1.48]	0.75[0.57-0.96]**	0.72[0.5-0.91]*	0.77[0.59-0.90]*	0.99[0.73-1.16]	0.75[0.70-1.00]	0.75[0.68-0.97]	1.09([0.79-1.42]	0.67[0.51-0.76]

FGF-21 (ng/ml)	0.61[0.45-0.89]	0.36[0.23-0.54]***	0.33[0.17-0.75]**	0.33[0.26-0.56]**	0.57[0.37-0.73]	0.40[0.29-0.50]**	0.25[0.13-0.58]	0.48[0.23-1.03]	0.57[0.19-1.16]
SAT(cm^2)	320.2(129.3)	233.3(120.7)***	248.9(115.5)***	277.4(116.7)**	305.1(105.8)	244.5(120.8)***	303.6(145.1)**	307.5(126.1)*	264.3(95.1)
VAT(cm^2)	281.9(79.1)	159.5(63.4)****	179.4(74.2)***	222.9(85.4)***	246.5(74.5)	141.5(56.0)***	171.1(66.8)***	194.6(67.3)*	193.9±(117.3)

Table 1: Clinical, anthropometric and metabolic change in responders, non-responders, and non-diabetic controls (NDC)

Data are presented as Mean(SD) or Median(IQ) based on distribution. Baseline data of responders and non-responders were presented as paired with 5 months (n=39, n=16), 12 months (n=32 , n=14), and 24 months (n=32, n=13).

The non-diabetic controls (NDC/n=25) were matched for with the Intervention group after weight loss.

Comparison with baseline data of responders and non-responders (*p<0.05, **p<0.01, ***p<0.001)

Comparison of responders vs. non-responders ((#p<0.05).

Comparison of NDC with responders at 24 months (§p<0.05, §§ p<0.01, §§§ p<0.001)

			Responders	Non-Responders	Diabetic Controls
Pancreas Volume	Baseline	N	39	16	21
		Mean (SD)	62.8 (16.8)	58.9 (13.9)	62.9 (15.0)
	5 Months	N	32	13	16
		Mean (SD)	62.3 (4.0)	62.1 (4.0)	61.7 (4.1)
	12 Months	N	32	13	16
		Mean (SD)	67.6 (5.6)	64.5 (5.7)	63.8(5.8)
	24 Months	N	32	13	16
		Mean (SD)	72.2 (5.4)	65.4 (5.5)	65.6 (5.6)
Change from Baseline	5 Months	Estimate (95% CI) p-value	-0.5 (-4.3, 3.5) p=0.84	3.0 (-1.6, 7.7) p=0.39	-1.3 (-5.6, 3.2) p=0.62
	12 Months	Estimate (95% CI) p-value	4.8 (1.5, 8.1) p=0.13	5.5 (1.8, 9.3) p=0.13	0.8 (-2.8, 4.4) p=0.71
	24 Months	Estimate (95% CI) p-value	9.4 (6.1, 12.8) p=0.02	6.4 (2.5, 10.3) p=0.10	2.6 (-1.0, 6.4) p=0.35
Difference vs. Diabetic Controls	5 Months	Estimate (95% CI) p-value	0.64 (-2.4, 3.7) p=1.00	0.37 (-3.4, 2.9) p=1.00	-
	12 Months	Estimate (95% CI) p-value	3.8 (-0.5, 8.1) p=0.10	0.76 (-4.5, 6.0) p=1.00	-
	24 Months	Estimate (95% CI) p-value	6.6 (2.4, 10.8) p=0.001	0.26 (-4.9, 5.4) p=1.00	-
Difference vs. Non-Responders	5 Months	Estimate (95% CI) p-value	0.27 (-2.9, 3.4) p=1.00	-	-
	12 Months	Estimate (95% CI) p-value	3.1 (-1.4, 7.5 p=0.29	-	-
	24 Months	Estimate (95% CI) p-value	6.8 (2.5, 11.2) p=0.001	-	-

Table 2: Mean and standard deviation (SD) of pancreas volume at each time point within each study group. Estimates (with 95% CIs and p-values) of within-group changes over time, and between-group differences at each time point, based on a mixed effects regression model

(repeated measures ANOVA) adjusting for baseline pancreas volume, weight, age, sex, and diabetes duration

Legends to figures

Figure 1: Flow diagram of study protocol and participants details

90 people with type 2 diabetes (<6 years duration) were randomized to intervention or control and studied at baseline and studied at 5, 12, and 24 months. 25 non-diabetic controls (NDC) were studied on one single occasion.

The intervention group were randomized to receive liquid formula diet (825-853 kcal/day) for 3-4 months followed by stepped food reintroduction (SFR) and weight maintenance up to 24 months. Controls were randomized to continue with their routine diabetes management based on current clinical guidelines. There were 64 participants within the intervention group at the baseline of the study. By the end of weight loss stage, 6 people had left the study for personal reasons, and 3 had no MR data for technical reasons. At 12 months, 9 other people had left the study for personal reasons, and by 24 months, an additional participant had left the study for personal reasons.

Of the 26 participants randomized to diabetic control group at baseline, 4 participants withdrew at 5 months (2 for personal reasons, 1 without MR data, and 2 who had lost more than 5kg of weight and therefore were excluded from the study). There were 3 subjects who withdrew at 12 months for personal reasons. By 24 months, 2 participants withdrew (1 for personal reason and 1 without MR data).

Figure 2: Change in pancreas volume over 2 years

Changes in pancreas volume from baseline to 5, 12, and 24 months in responders, non-responders, and diabetic controls. Data for each time point are shown mean (95% CI). P=0.001 reflects the difference in change of pancreas volume between responders and non-responders/diabetic controls at 24 months. All data and statistics were derived after adjusting for age, sex, diabetes duration, baseline body weight, and baseline pancreas volume in the ANOVA model.

Figure 3: Extent of return to normal over 24 months of pancreas morphology and β-cell capacity

Surface-rendered image of pancreas morphology in a representative participant (A), pancreas volume (B), fractal dimension (C), and maximal insulin secretion (D) in responders compared with non-diabetic controls (NDC) at baseline, 5 months, 12 months, and 24 months. Data paired with baseline at each time point were presented as mean(SD) in B and C or Median (interquartile range) for insulin secretion in panel D. Statistics were derived from the ANOVA model after adjusting for age, sex, diabetes duration, baseline body weight, and baseline pancreas volume.

** p<0.01 vs. responders baseline, *** p<0.001 responders vs. baseline

# p<0.05 responders vs. NDC, ## p<0.01 responders vs. NDC, ### p<0.001 responders vs. NDC

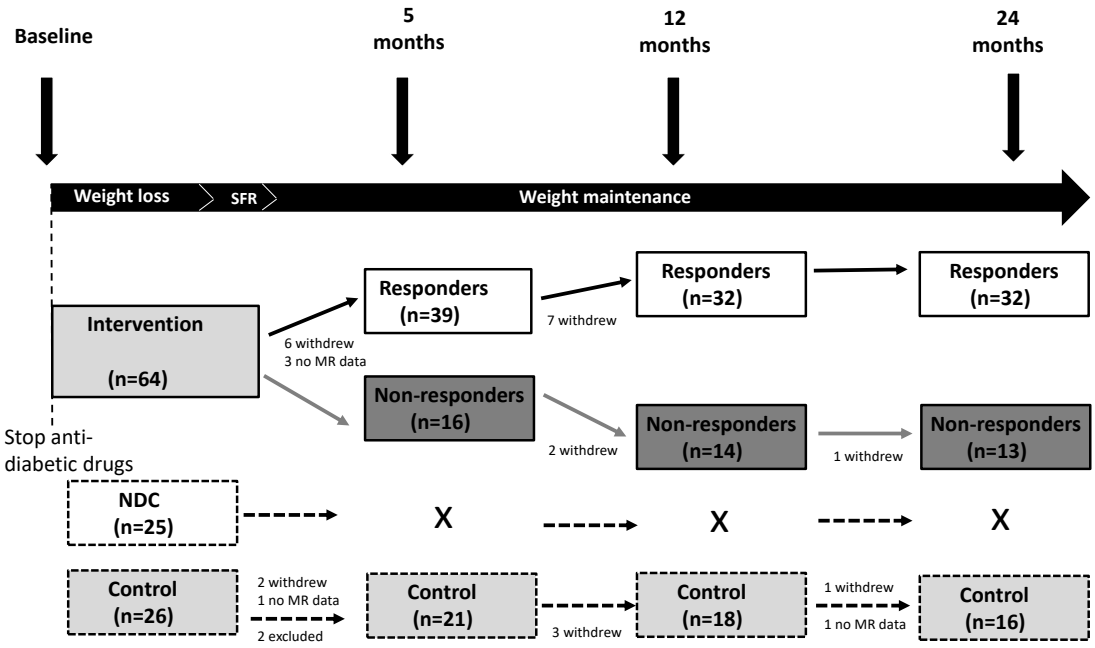

Baseline
5 months
12 months
24 months
Weight loss
SFR
Weight maintenance
Intervention (n=64)
Stop anti-diabetic drugs
6 withdrew
3 no MR data
Responders (n=39)
7 withdrew
Responders (n=32)
Responders (n=32)
Non-responders (n=16)
2 withdrew
Non-responders (n=14)
1 withdrew
Non-responders (n=13)
NDC (n=25)
X
X
X
Control (n=26)
2 withdrew
1 no MR data
2 excluded
Control (n=21)
3 withdrew
Control (n=18)
1 withdrew
1 no MR data
Control (n=16)

Figure 1

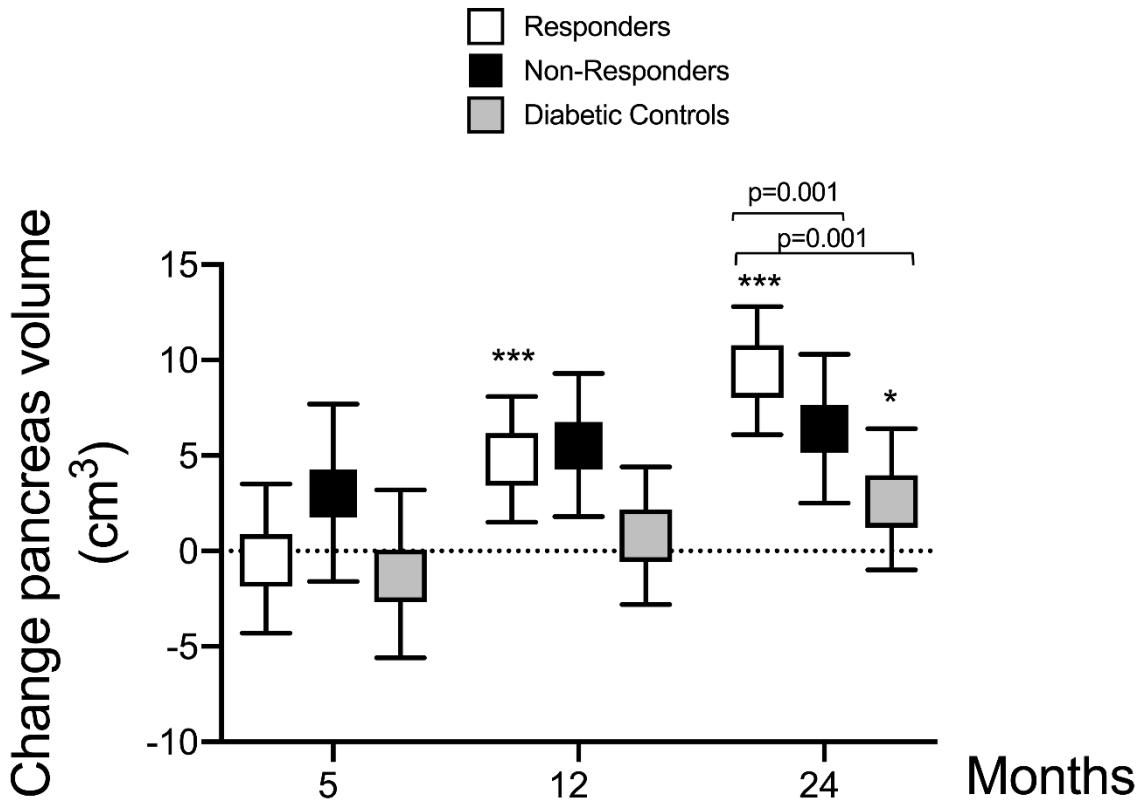


Figure 2: Change in pancreas volume over 2 years

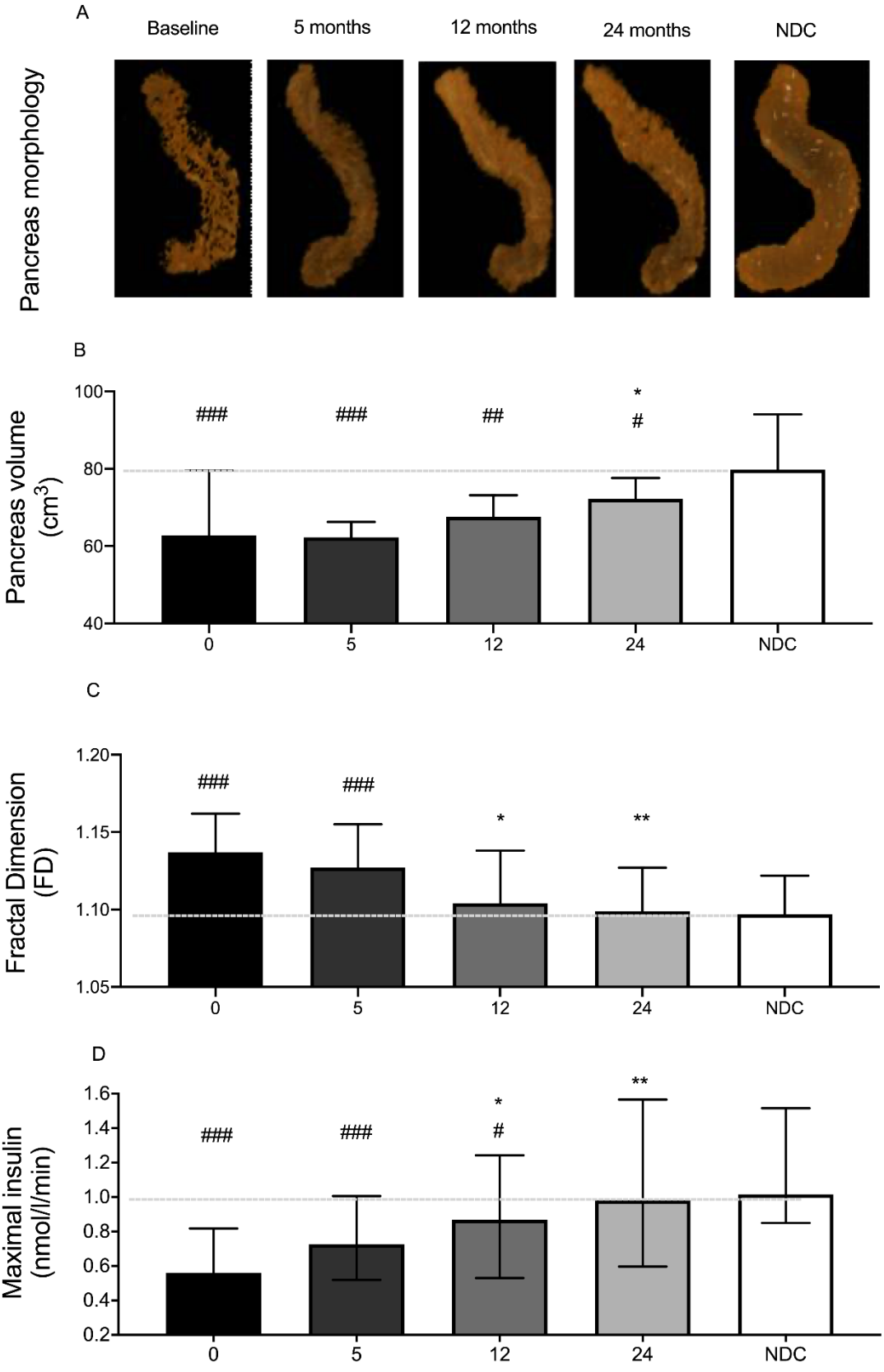

A
Baseline
5 months
12 months
24 months
NDC
Pancreas morphology
B
Pancreas volume (cm3)
100
80
60
40
###
###
##
*
#
0
5
12
24
NDC
C
Fractal Dimension (FD)
1.20
1.15
1.10
1.05
###
###
*
**
0
5
12
24
NDC
D
Maximal insulin (nmol/l/min)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
###
###
*
#
**
0
5
12
24
NDC

Figure 3

Supplementary material

	Baseline	**5 months**	**12 months**	**24 months**
Intervention	N=55	N=55	N=46	N=45
Control	N=21	N=21	N=18	N=16
Weight (kg)				
Intervention	100.4(16.7)	85.0(14.5)***	87.6(15.6)***	91.3(16.4)***
Control	94.5(9.6)	93.6(10.9)	92.9(13.1)	92.9(13.1)
BMI (kg/m^2)				
Intervention	34.8(4.4)	29.5(4.3)***	30.4(4.6)***	31.8(4.8)***
Control	33.3(3.6)	32.9(3.7)	32.5±4.3	32.4±4.5
HbA1c (mmol/mol)				
Intervention	59.0(10.4)	48.0(15.4)***	48.2(12.8)***	54.8(15.9)**
Control	56.2(11.5)	62.1(15.9)	55.6(11.5)	55.9(11.2)
Fasting insulin (pmol/l)				
Intervention	81.6[53.6-135.0]	31.0[19.5-45.2]***	30.1[20.2-54.2]***	44.1[18-59.9]***
Control	54.4[43.6-121.0]	56.7[35.5-79.7]	65.5[37.6-75.4]	44.5[28.5-63.4]
F. Glucose (mmol/l)				
Intervention	8.6(2.6)	6.6(2.2)***	6.6(1.7)***	7.4(2.9)***
Control	8.2(2.1)	8.3(2.3)5	8.3(2.3)	7.9(2.0)
HOMA2-IR (%)				
Intervention	1.65[1.14-2.83]	0.60[0.38-0.93]***	0.61[0.42-1.08]***	0.89[0.36-1.27]***
Control	1.11[0.91-2.53]	1.15[0.76-1.61]	1.33[0.76-1.57]	0.87[0.64-1.26]*
Total plasma TG (mmol/l)				
Intervention	1.66[1.18-2.24]	1.05[0.78-1.43]***	1.15[0.81-1.60]***	1.28[0.90-1.71]***
Control	1.00[0.80-1.50]##	1.20[1.00-1.50]	1.16[0.92-1.44]	1.12[0.90-1.32]
Pancreas fat (%)				
Intervention	8.5(2.4)	7.6(2.3)***	7.5(2.0)***	7.7(2.3)***
Control	8.0(2.2)	8.1(2.2)	8.0(2.2)	8.1(2.1)
IGF-1(ng/ml)				
Intervention	72.3(30.7)	102.3(39.3)***	95.1(42.4)***	80.5(36.4)
Control	93.4(34.6)#	84.6(31.3)	91.8(40.4)	97.1(54.9)
GDF-15 (ng/ml)				
Intervention	0.93[0.72-1.34]	0.93[0.72-1.34]	0.73[0.60-0.96]**	0.83[0.59-1.09]
Control	0.93[0.71-1.05]	0.99[0.65-1.33]	1.05[0.93-1.39]	1.12[0.84-1.28]
FGF-21(ng/ml)				
Intervention	0.58[0.42-0.85]	0.37[0.24-0.55]***	0.33[0.16-0.64]**	0.34[0.25-0.60]*
Control	0.41 [0.27-0.70]#	0.37 [0.29-0.92]	0.41 [0.25-0.76]	0.39 [0.29-0.65]
CRP (mg/l)				
Intervention	2.7[1.0-4.6]	-	1.1[0.5-1.8]***	1.3[0.4-3.4]*
Control	1.2[0.4-2.2] #		1.2[0.8-3.0]	1.5[0.8-2.6]
Glucagon (pg/ml)				
Intervention	22.0[19-23.8]	14.0[11.0-20.8]***	16.0[13.0-26.0]**	20.0[13.5-29.0]**
Control	29.0[19-50.0]	24.0[12-39.0]	22.0[15.0-34.0]	20.0[16.0-32.0]
SAT(cm^2)				
Intervention	316.0(122.4)	236.3(119.7)***	264.7(125.6)***	286.1(118.8)***
Control	298.4(104.9)	301.3(114.7)	295.6(123.2)	276.3(110.9)
VAT(cm^2)				
Intervention	272.1(78.8)	154.7(61.5)***	177.0(71.5)***	214.7(80.9)***
Control	234.7(87.2)	239.3(91.4)	223.8(87.8)	207.3(79.4)

Supplementary Table 1: Characteristics of Intervention and Control groups

Data on the whole intervention group and control group from DiRECT. Data presented as Mean (SD) or Median (IQ) based on distribution. Baseline data were presented as paired with 5 months and the number of subjects at baseline reflects this comparison.

Comparison with baseline data (*p<0.05, **p<0.01, ***p<0.001)
Comparison of Intervention vs. Control groups at baseline (#p<0.05, ##p<0.01, ###p<0.001).

			Responders	Non-Responders	Diabetic Controls
Fractal Dimension	Baseline	N	39	16	21
		Mean (SD)	1.137 (0.025)	1.138 (0.030)	1.126 (0.032)
	5 Months	N	32	13	16
		Mean (SD)	1.127 (0.028)	1.123 (0.029)	1.130 (0.028)
	12 Months	N	32	13	16
		Mean (SD)	1.104 (0.034)	1.112 (0.032)	1.122 (0.036)
	24 Months	N	32	13	16
		Mean (SD)	1.099 (0.028)	1.121 (0.025)	1.125 (0.028)
Change from Baseline	5 Months	Estimate (95% CI) p-value	-0.010 (-0.012, -0.008) p=0.173	-0.015 (-0.015, -0.014) p=0.234	0.004 (0.002, 0.004) p=0.659
	12 Months	Estimate (95% CI) p-value	-0.033 (-0.037, -0.029) p=0.021	-0.026 (-0.030, -0.022) p=0.142	-0.004 (-0.009, 0.000) p=0.732
	24 Months	Estimate (95% CI) p-value	-0.038 (-0.039, -0.036) p=0.0036	-0.017 (-0.017, -0.018) p=0.186	-0.001 (-0.002, -0.001) p=0.905
Difference vs. Diabetic Controls	5 Months	Estimate (95% CI) p-value	-0.003 (-0.025, 0.020) p=1.000	-0.006 (-0.033, 0.020) p=1.000	-
	12 Months	Estimate (95% CI) p-value	-0.018 (-0.045, 0.009) p=0.304	-0.010 (-0.043, 0.022) p=1.000	-
	24 Months	Estimate (95% CI) p-value	-0.026 (-0.047, -0.004) p=0.013	-0.005 (-0.030, 0.021) p=1.000	-
Difference vs. Non-Responders	5 Months	Estimate (95% CI) p-value	0.004 (-0.018, 0.026) p=1.000	-	-
	12 Months	Estimate (95% CI) p-value	-0.008 (-0.019, 0.035) p=1.000	-	-
	24 Months	Estimate (95% CI) p-value	-0.021 (-0.043, 0.000) p=0.056	-	-

Supplementary Table 2: Mean and standard deviation (SD) of the Fractal Dimension (FD) of pancreas borders at each time point within each study group

FD for the non-diabetic comparator group was 1.097 (SD 0.025). Estimates (with 95% CIs and p-values) of within-group changes over time, and between-group differences at each time point, based on a mixed effects

regression model (repeated measures ANOVA) adjusting for baseline FD, baseline weight, age, sex, and diabetes duration

All participants with diabetes (Intervention+Controls)		
	Pancreas Volume (cm^3)	Fractal Dimension
Baseline	-	-
Number assessed	76	76
Mean value	62·0 (15·6)	1.134 (0.028)
5 months	-	-
Number assessed	61	61
Mean value	62·0 (4.1)	1.127 (0.031)
12 months	-	-
Number assessed	61	61
Mean value	65.3 (5.8)	1.113 (0.039)
24 months	-	-
Number assessed	61	61
Mean value	67.7 (5.6)	1.115 (0.031)
Change from baseline	-	-
5 months	0.03 [-2.4 to 2.5] p=0.989	-0.007 [-0.009 to -0.007] p=0.220
12 months	3.3 [1.3 to 5.3] p=0.046	-0.021 [-0.024 to -0.019] p=0.038
24 months	5.7 [3.7 to 7.7] p=0.004	-0.019 [-0.020 to -0.019] p=0.022

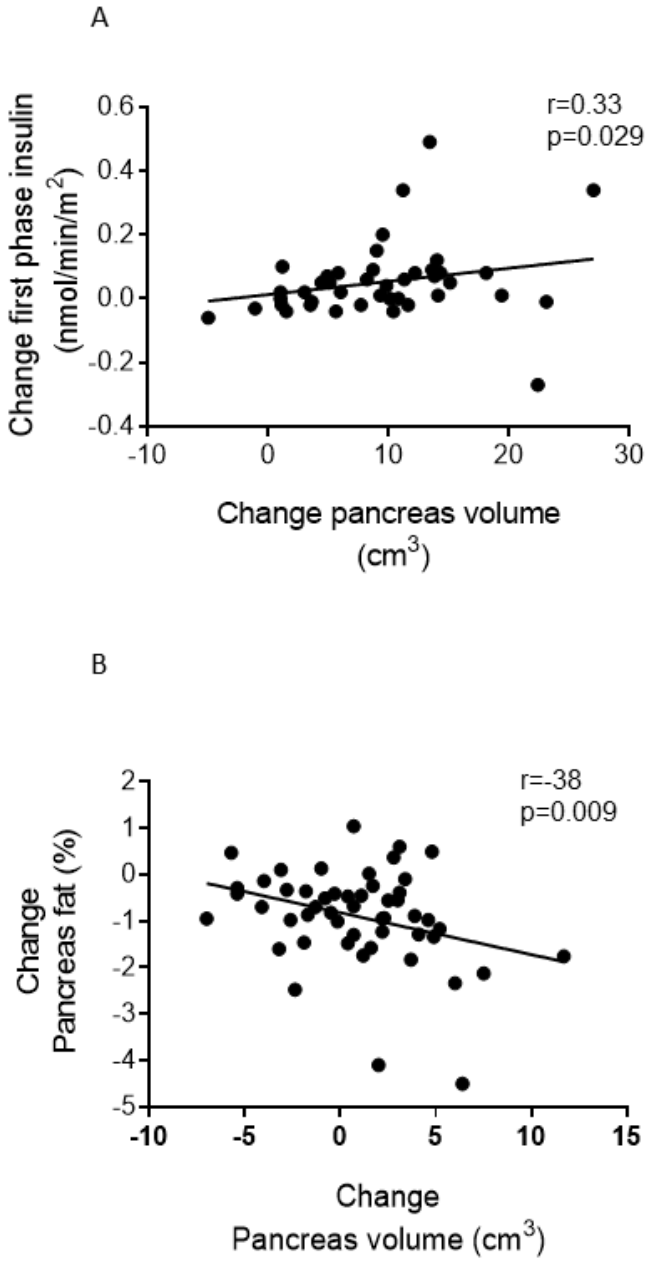
Supplementary Table 3: Pancreas volume and Fractal Dimension (FD) at each time point within all type 2 diabetes participants

Pancreas volume and fractal dimension for the non-diabetic comparator group were 79.8 cm^3 (SD 14.3), and 1.097 (SD 0.025), respectively. Data are n, mean (SD), or estimate (95% CI); p value. Estimates of within-group changes over time based on a mixed-effects regression model adjusting for baseline weight, age, sex, diabetes duration and either baseline pancreas volume or baseline fractal dimension.

HbA1c (mmol/mol)	**Baseline** N=13	**5 months** N=13	**Delta** N=13
Tertile 1 (N=7)	67.1 (7.1)	57.9(10.9)	-9.3[-17.9 to -0.7]
Tertile 2 (N=6)	58.7(10.0)	63.8(16.9)	5.2[-2.2 to 12.6]*
Fractal Dimension	**Baseline** N=13	**24 months** N=13	**Delta** N=13
Tertile 1 (N=7)	1.154(0.028)	1.120(0.024)	-0.035[-0.046 to -0.024]
Tertile 2 (N=6)	1.115(0.018)*	1.124(0.029)	0.008[-0.016 to 0.033]**

Supplementary Table 4: Fractal Dimension (FD) of pancreas borders and glucose control in non-responders

Data presented as Mean (SD) or Mean (95%CI).*p<0.05, **p<0.001 vs. Tertile 1.



Supplementary Figure 1: Relationship of pancreas volume to insulin secretion and intra-pancreatic fat

Panel A: Correlation between change in pancreas volume and change in first phase insulin secretion in the intervention group at 24 months (n=43).

Panel B: Correlation between change in pancreas volume and change in intra-pancreatic fat in the intervention group at 12 months (n=46).

Spearman ρ (panel A) or Pearson (panel B) correlations were applied.

	Baseline	**5 months**	**12 months**
Responders	N=39	N=38	N=31
Non-responders	N=16	N=16	N=14
Relapsers	N=13	N=12	N=12
Control	N=21	N=21	N=18
1st phase insulin(nmol/min/m^2)			
Responders	0.041[0.004-0.067]	0.106[0.058-0.155]***	0.093[0.053-0.198]***
Non-responders	0.018[0.002-0.033]	0.014[0.006-0.032]	0.022[-0.005-0.037]
Relapsers	0.026[0.013-0.055]	0.096[0.054-0.122]*	0.069[0.032-0.122]
Control	0.025[-0.007-0.061]	0.028[0.005-0.043]	0.030[-0.004-0.081]
Maximal insulin (nmol/min/m^2)			
Responders	0.561[0.474-0.811]	0.725[0.536-0.975]	0.869[0.538-1.241]*
Non-responders	0.451[0.310-0.660]	0.490[0.383-0.638]	0.485[0.406-0.607]
Relapsers	0.532[0.380-0.764]	0.709[0.548-0.768]	0.936[0.522-1.240]
Control	0.517[0.448-0.842]	0.547[0.462-0.719]	0.559[0.448-0.804]

Supplementary Table 5: First phase and maximal insulin secretion in responders, non-responders, relapsers, and diabetic controls

Data presented as Median (IQ).
*P<0.05 vs. baseline, **p<0.001 vs. baseline, ***p<0.0001 vs baseline.
# P<0.05 vs. 5 months