Remission of human type 2 diabetes requires decrease in liver and pancreas fat content but is dependent upon capacity for beta cell recovery

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Summary

The Diabetes Remission Clinical Trial reported return and persistence of non-diabetic blood glucose control in 46% of people with type 2 diabetes of up to 6 years duration. Detailed metabolic studies were performed on a subgroup (Intervention n=64; Control n=26). In the intervention group, liver fat content decreased (16.0±1.3 to 3.1±0.5%, p<0.0001) immediately after weight loss. Similarly, plasma triglyceride and pancreas fat content decreased whether or not glucose control normalised. Recovery of first phase insulin response (0.04[-0.05-0.32] to 0.11 [0.0005-0.51] nmol/min/m^2, p<0.0001) defined those who returned to non-diabetic glucose control and this was durable at 12 months (0.11 [0.005-0.81] nmol/min/m^2, p=0.0001). Responders were similar to non-responders at baseline but had shorter diabetes duration (2.7±0.3 vs. 3.8±0.4 years; p=0.02). This study demonstrates that beta cell ability to recover long term function persists after diagnosis, changing the previous paradigm of irreversible loss of beta cell function in type 2 diabetes.

Keywords

Type 2 diabetes; low calorie diet; type 2 diabetes remission; beta cell dedifferentiation; liver fat; very low density lipoprotein; pancreas fat, insulin secretion; weight loss; human.

Introduction

Type 2 diabetes now affects at least one in ten US adults, and 422 million worldwide (Menke et al., 2015; WHO, 2016). It has long been regarded as an inevitably progressive, lifelong condition. However, the Diabetes Remission Clinical Trial (DiRECT) has demonstrated that nearly half of those with early (< 6 years) type 2 diabetes can be returned to long term non-diabetic glucose control using an effective method to achieve and maintain substantial weight loss (Lean et al., 2017). The initial period of weight loss was followed by weight maintenance and the data were reported at 12 months. This population-based study built upon the results of earlier small studies which revealed the detailed physiological basis of the transition from type 2 diabetes to normal (Lim et al., 2011; Petersen et al., 2005; Steven et al., 2016a; Steven et al., 2016b). However, whether these mechanisms operate in all individuals with type 2 diabetes and the critical factor(s) that determine the capacity to return to non-diabetic glucose metabolism remain uncertain.

During a very low calorie diet in type 2 diabetes an initial study showed that liver fat content rapidly decreased, with normalisation of hepatic insulin sensitivity within 7 days (Lim et al., 2011). Over an 8 week period, pancreas fat content decreased more slowly, as first phase insulin response gradually returned. A follow up study demonstrated that as duration of type 2 diabetes increased beyond 10 years, the possibility of restoring beta cell function decreased (Steven et al., 2016a). These observations led to a simplified view of the aetiology of type 2 diabetes (Taylor, 2013), consistent with earlier observations (Henry et al., 1986; Wing et al., 1987), in that linked but distinct mechanisms in liver and pancreas appeared to explain the condition. Recently, the molecular basis of the liver abnormalities in type 2 diabetes has been clarified (Perry et al., 2018). In addition, a major decline in beta cell function is necessary before type 2 diabetes develops. In the last few years, metabolic stress-induced beta cell de-differentiation and subsequent re-differentiation with significant weight loss have been demonstrated, potentially

explaining any return from type 2 diabetes to normal glucose tolerance (Cinti et al., 2016; Pinnick et al., 2010; Talchai et al., 2012; White et al., 2016). A larger study was required to determine the extent to which this explains common type 2 diabetes.

Detailed pathophysiologic studies were carried out in a geographically pre-defined sub-group of DiRECT participants. These were designed to test the hypothesis that there would be differences between those who did or did not return to non-diabetic glucose control in some or all of the factors previously identified as underlying type 2 diabetes. We have examined liver fat content, liver export of triglyceride, pancreas fat content and beta cell function in type 2 diabetes during conventional therapy, after weight loss and after 12 months of weight maintenance.

Results and Discussion

Baseline characteristics

Responders, who returned to non-diabetic glucose control after weight loss, and non-responders, who did not, were similar in age, weight and sex (Table 1; Figure 3A). Responders had a non-significantly lower fasting plasma glucose than non-responders (148.9±6.8 vs. 167.8±11.9 mg/dl, p=0.18; Figure 3B), and HbA1c was 7.4±0.2 vs. 7.9±0.2% respectively (p=0.04; Figure 3C). At baseline, liver fat in the whole intervention group was 16.0±1.3% and was not significantly different in responders and non-responders (16.7±1.5 vs. 14.5±2.6% respectively; p=0.47). There was no significant difference at baseline between responders and non-responders in VLDL1-TG production (560.7±30.9 vs. 581.1±52.1 mg/kg/day, p=0.74; Figure 4C) or total plasma triglyceride (1.84±0.13 vs. 1.91±0.25mmol/l; p=0.76). The non-responders had a longer duration of diabetes (2.7±0.3 vs. 3.8±0.4 years; p=0.02), lower fasting plasma insulin (108.3±10.0 vs. 77.2±8.5pmol/l; p=0.02) and lower plasma ALT (34.1±2.8 vs. 26.3±2.6 pmol/l; $p<0.05$). Data on the whole intervention group compared with the conventionally treated control group are shown in Table S1.

Lower fasting plasma insulin levels and lower plasma ALT in individuals with type 2 diabetes who cannot achieve non-diabetic plasma glucose levels despite adequate weight loss have previously been observed in a group with up to 23 years duration of diabetes (Steven et al., 2016a). In that study, longer duration was clearly associated with inability to achieve remission of diabetes. There was an evident failure at baseline to maintain fasting plasma insulin levels in the non-responders which was accompanied by lower hepatic fat levels. This is consistent with a slowing of insulin-driven de novo lipogenesis as the beta cell defect advances. Type 2 diabetes of more than 8 years duration was observed to be associated with considerably lower plasma ALT (Steven et al., 2016b). In contrast, the present study focussed upon the first 6 years of type 2 diabetes and within this shorter time span only a modest, non-significant difference in liver fat levels was observed between responders and non-responders. Nonetheless, plasma ALT levels were 23% lower in non-responders ($p<0.05$, Table 1), suggesting that the metabolic stress on the hepatocyte had diminished.

Weight

During the weight loss phase, weight decreased in responders (100.6±2.6 to 84.4±2.1kg; $p<0.0001$; n=40) and in non-responders (102.1±4.4 to 88.7±4.4kg; $p<0.0001$; n=18; Figure 3A). The change during the weight loss phase did not differ significantly between groups (-16.2 ±1.2 vs. -13.4 ±1.4kg; p=0.14; Table 2).

Between the end of weight loss and 12 months (weight maintenance phase), four responders failed to maintain remission, and nine participants dropped out of the total Intervention group. Paired data for the weight maintenance phase were thus available on 29 responders and 16 non-responders (Figure 1). No participants defined as non-responders after weight loss achieved remission during the weight maintenance phase. Weight in responders increased by 3.3±0.8kg to 86.2±3.0 kg ($p<0.0001$). Over the same period weight in non-responders increased by 4.9±0.8kg to 92.5±4.6 kg but stayed lower than baseline ($p<0.0001$). By 12 months the overall change was greater in the responders (-14.1±1.5 vs. -9.4±1.3kg; p=0.02).

Glucose control

Weight loss lowered fasting plasma glucose from 148.9±6.8 to 102.2±2.2 mg/dl ($p<0.0001$) in responders, and had no significant effect in non-responders (167.8 ±11.9 to 158.9 ±10.9 mg/dl; $p=0.47$; Figure 3B). By 12 months there was no significant further change in either group (responders: 102.4±2.3 mg/dl; non-responders: 152.0±7.9 mg/dl; Figure 3B).

HbA1c decreased in responders (to 5.9±0.1%; $p<0.0001$), but not in non-responders (8.0±0.4 %, $p=0.67$; Figure 3C). At 12 months, HbA1c did not change further in either group so that differences were maintained (5.8±0.1 vs. 7.6±0.2 %, $p<0.0001$; Table 2). 69% of the Intervention group (40/58) achieved non-diabetic blood glucose control after weight loss, and 64% at 12 months (29/45).

Liver fat

Liver fat content decreased after weight loss in both groups (responders: to 3.3±0.6%; $p<0.0001$; non-responders: to 2.6±0.5%; $p<0.0001$; Figure 4A). The change in liver fat was similar (-13.4±1.4 vs. -11.9±2.4%, $p=0.60$; Table 2). At 12 months, liver fat in responders was 3.0±0.6% and in non-responders 6.1±1.9% ($p=0.11$ and $p=0.04$ respectively compared with post-weight loss). The increase in liver fat in the weight maintenance phase was related to degree of weight gain. Those who gained less than the mean weight gain of the responder group (3.3kg) had no change in liver fat (3.2±1.1 vs. 3.2±1.0%; $p=0.95$; $n=16$). In contrast, in those who gained more than 3.3kg, there was a resultant increase in liver fat (1.5±0.3 vs. 2.8±0.5%; $p=0.03$; $n=12$). In those randomised to the conventionally treated control group, there was no significant change in liver fat throughout the study (Figure 4A; Table S1).

The importance of high levels of liver fat in the pathogenesis of type 2 diabetes is now recognised (Bril and Cusi, 2017; Petersen et al., 2005; Shibata et al., 2007; Steven et al., 2016b; Taylor, 2013). Raised liver fat levels are associated with hepatic insulin resistance, inadequate suppression of hepatic glucose production and hence

increased fasting plasma glucose (Petersen et al., 2005; Ravikumar et al., 2008; Seppala-Lindroos et al., 2002). Excess diacylglycerol has a profound effect in activating PKCε which inhibits the signalling pathway from the insulin receptor to IRS-1, the first post-receptor step in intracellular insulin action (Samuel et al., 2010). So, under circumstances of chronic energy excess, a raised level of intracellular diacylglycerol specifically prevents normal insulin action, and hepatic glucose production fails to be controlled. High-fat feeding of rodents brings about raised levels of diacylglycerol, PKCε activation and insulin resistance (Samuel et al., 2004), and these changes have recently been shown to reverse after 3 days on a very low calorie diet (Perry et al., 2018). The relationship between raised diacylglycerol, PKCε activation and hepatic insulin resistance leading to increased hepatic glucose output was clearly demonstrated in this recent study. In obese humans, intrahepatic diacylglycerol concentration has been shown to correlate with hepatic insulin sensitivity (Kumashiro et al., 2011; Magkos et al., 2012). It must be noted that raised diacylglycerol and PKCε activation are corrected early during calorie restriction and before major weight change (Lim et al., 2011; Perry et al., 2018). This should not be interpreted to indicate that the more gradually occurring weight loss is not necessary for long term normalisation. The mechanisms underlying hepatic insulin resistance are now established, and the present observations demonstrate the complete reversibility of the liver abnormalities of human type 2 diabetes.

Lipid metabolism

In responders, VLDL1-TG production decreased after weight loss (to 413.6±25.8 mg/kg/day, $p<0.0001$; Figure 4C). In non-responders, there was a non-significant fall (to 521.8 ±41.9 mg/kg/day; $p=0.28$). The change during weight loss was not significantly different between responders and non-responders (-147.2±33.8 vs. -59.2±52.7; $p=0.17$). VLDL1-TG production rate did not change during the weight maintenance phase in responders (at 12 months: 437.5±22.4 mg/kg/day, $p=0.12$) but increased in non-responders (to 649.6±67.0 mg/kg/day, $p=0.008$).

Plasma VLDL1-TG concentration decreased in responders after weight loss (0.69±0.07 to 0.44±0.06 mmol/l, $p=0.001$, Figure 4E), followed by a small increase

during weight maintenance (to 0.49±0.08 mmol/l, p=0.04; Figure 4E). There was no significant change in non-responders after weight loss (0.73±0.11 to 0.55±0.12 mmol/l, p=0.12) nor during the weight maintenance phase (to 0.64±0.12 mmol/l, p=0.12).

Total plasma triglyceride (largely chylomicrons plus VLDL-TG) fell similarly in responders and non-responders after weight loss (1.84±0.13 to 1.30 ±0.13 mmol/l, p<0.0001 and 1.91±0.25 to 1.24 ±0.14 mmol/l respectively, p=0.002, Figure 4B). This remained stable at 12 months (responders: to 1.24±0.12 mmol/l, p=0.43; non-responders: to 1.39±0.21 mmol/l, p=0.52). In those randomised to control, there was no significant change in any parameter of lipid metabolism during the study (Table S1).

The series of studies which led to DiRECT was initiated to test the twin cycle hypothesis (Taylor, 2008). This described vicious cycles within the liver and pancreas, respectively ratcheting up hepatic insulin resistance and beta cell dysfunction. Critically, it postulated that these cycles were linked by elevated insulin driving increased *de novo* lipogenesis and hepatic VLDL1-TG export. The increased exposure of beta cells to fat metabolites was postulated to lead ultimately to beta cell failure. VLDL1-TG is a major determinant of plasma triglyceride (Hiukka et al., 2005) and plasma concentration is proportional to liver fat content (Adiels et al., 2006). The dramatic and sustained normalisation of liver fat content in the present study was associated with a fall in both VLDL1-TG production rate and plasma levels. The fall was most pronounced in responders and with the continuing normoglycemia and low liver fat content, both production rate and plasma VLDL1-TG concentration remained significantly lower than baseline at 12 months. In non-responders, the modest weight gain during weight maintenance was followed by a significant rise in VLDL1-TG production rate even though the fasting plasma VLDL1-TG and total triglyceride concentrations remained lower than baseline. This suggests that non-responders may exhibit a different relationship between the two major components of plasma triglyceride - chylomicron and VLDL1-TG. This may possibly relate to different hepatic insulin sensitivity or plasma glucose levels in responders compared to non-responders. Although the major source of fatty acids supplying VLDL1-TG

export is from adipose derived fatty acids (around 60%), the contribution of *de novo* lipogenesis to VLDL1-TG is much greater when liver triglyceride levels are raised (Donnelly et al., 2005). In non-obese individuals this process accounts for only a small proportion of the fatty acids of VLDL1-TG, whereas in NAFLD it accounts for up to 26% (Lambert et al., 2014; Timlin et al., 2005). The present observations on change in VLDL1-TG and total plasma triglyceride are consistent with the predictions of the twin cycle hypothesis.

Pancreas fat

Pancreas fat did not differ significantly between responders and non-responders at baseline (8.7±0.4 vs. 7.9±0.6%; p=0.25, Figure 4D). Weight loss produced a similar fall in intrapancreatic fat in both groups (responders: to 7.8±0.4%; p<0.0001 and non-responders: to 7.1±0.5%; p=0.004). There was no significant difference in extent of change between the two groups (-0.90±0.17 vs. -0.78±0.23%; p=0.67; Table 2). During the weight maintenance phase, it remained stable in both groups (7.9±0.4% and 6.8±0.4% in responders and non-responders, respectively). There was no change in pancreas fat in the control group (Table S1).

The magnetic resonance method quantifies fat content of both exocrine and endocrine pancreas, and the relationship of this to islet fat content and exposure must be considered. In rodents, beta cell triglyceride content is directly related to total intrapancreatic fat content, and these parameters change in step (Lee et al., 2010). *In vivo* measurement of intrapancreatic fat content in human studies during an 8 week period of calorie restriction in type 2 diabetic humans is concordant with the rodent observations, and also matches the gradual return of first phase insulin secretion in that study (Lim et al., 2011). The change in total intrapancreatic fat is not seen during equivalent loss of weight in non-diabetic humans (Steven et al., 2016b), implying a specific excess of intracellular triglyceride within both exocrine and endocrine cells of the pancreas in type 2 diabetes. In addition to this endogenous pool, the ongoing exposure of beta cells to excess fatty acid delivered from plasma VLDL1-TG and chylomicrons will contribute to the metabolic load

experienced by the beta cell. Intrapancreatic triglyceride content and lipid supply were decreased in both responders and non-responders.

Beta cell function

Fasting plasma insulin decreased in both groups during weight loss (responders: 108.3±10.0 to 38.7±4.4 pmol/l; p<0.0001; non-responders 77.2±8.5 to 35.5±5.3 pmol/l; p=0.0002, Figure 4F). Because of the higher baseline level in responders, there was a greater decrease in this group (-69.7±9.3 vs. -41.7 ±5.8 pmol/l; p<0.01). At 12 months fasting plasma insulin remained steady in both groups (responders: 41.1±5.5 pmol/l, p=0.41) and non-responders: 45.8±8.4 pmol/l, p=0.40).

First phase insulin secretion increased in responders after weight loss from 0.04[-0.05-0.32] to 0.11 [0.0005-0.51] nmol/min/m^2 (p<0.0001). Whereas no change was observed in the non-responders (0.02[-0.07-0.13] to 0.01[-0.04-0.05] nmol/min/m^2; p=0.96; Figure 5A; Table 2). In the responders, increased first phase insulin secretion was maintained during the weight maintenance phase (to 0.11[0.005-0.81] nmol/min/m^2; p=0.97). Between baseline and 12 months the change was highly significant (p=0.0001).

There was a gradual increase in maximal insulin secretion in responders after weight loss that became significant at 12 months (0.62[0.13-1.95] to 0.94 [0.25-2.69] nmol/min/m^2; p<0.04 compared with baseline; Figure 5B). It remained unchanged in non-responders.

First phase and total insulin secretion rates remained unchanged in the control group throughout the study (Table S1).

It was established many years ago that chronic *in vitro* exposure of beta cells to triglyceride or fatty acids decreases ability to respond to an acute increase in glucose levels (Lee et al., 1994) and the concept that excess fat can impair beta cell function is not new (McGarry, 2002; Unger, 1995). In the ZDF rat, the onset of hyperglycemia is preceded by a rapid increase in pancreatic fat (Lee et al., 1994) and diabetes is completely preventable by restriction of food intake (Ohneda et al., 1995). Chronic exposure of human beta cells to lipid excess brings about decreased function (Zhou

and Grill, 1995). Early studies demonstrated the ultrastructural damage brought about even by relatively low concentrations of saturated fatty acids (Pinnick et al., 2010; Pinnick et al., 2008), and this endoplasmic reticulum stress has been identified in other *in vivo* studies of type 2 diabetes (Huang et al., 2007; Laybutt et al., 2007). Clearly, hyperglycemia cannot explain the initiation of beta cell stress in type 2 diabetes, but once the increased glucose levels are added to lipid-induced stress the increased glucose supply will compound and perpetuate the metabolic insult (Bensellam et al., 2012; Poitout et al., 2010; Weir et al., 2013). Loss of fully differentiated beta cell phenotype is now recognised as the most likely mechanism underlying type 2 diabetes (Bensellam et al., 2018; Brereton et al., 2014; Spijker et al., 2015; Talchai et al., 2012; Wang et al., 2014; White et al., 2013). Very recent work has identified markers of dedifferentiation in the islets from people with type 2 diabetes (Cinti et al., 2016). In the non-responders, lower baseline fasting plasma insulin levels, lower ALT levels and higher HbA1c are consistent with a more advanced, irreversible stage of beta cell dysfunction.

A potential alternative explanation could be that the non-responders have a different etiology of diabetes, in which non-lipid driven defects in beta cells are more important. However, the responders and non-responders were similar in anthropological characteristics at baseline, and exhibited the same abnormalities of grossly elevated liver fat content and all other metabolic abnormalities. Even though the DiRECT cohort was selected to have duration of type 2 diabetes of less than 6 years, the non-responders had a modestly longer recorded duration of disease.

It is established that the natural history of beta cell decline follows widely different time courses between individuals (Harrison et al., 2012; Turner et al., 1999), but this is the first time that a difference in disease duration has been shown to relate to the capacity for redifferentiation during the first 6 years of type 2 diabetes. This observation carries potentially important implications for the initial clinical approach to management. At present, the early management of type 2 diabetes tends to involve a period of adjusting to the diagnosis plus pharmacotherapy with lifestyle changes which in practice are modest. The present data suggest that substantial

weight loss at the time of diagnosis may be more appropriate to prevent ongoing loss of beta cell capacity.

Substrate oxidation

The return to non-diabetic glucose control was accompanied by an increase in basal glucose oxidation rates and a fall in basal lipid oxidation rates (Table 1). These changes were not fully developed immediately after weight loss, but maximal at 12 months. The glucose oxidation rates in responders increased from 1.27±0.12 at baseline to 1.44±0.14 and to 2.00±0.19 mg/kg/min after weight loss and weight maintenance respectively ($p<0.0001$ between baseline and 12 months). This was reflected in a greater increase in responders between baseline and 12 months compared to non-responders (0.75 ±0.18 vs. -0.11±0.23 mg/kg/min; $p=0.006$; Table 2). Conversely, lipid oxidation rates decreased in responders from 0.96±0.05 to 0.87±0.06 ($p=0.28$) and to 0.64±0.09 mg/kg/min; $p<0.01$) with a greater decrease than in non-responders over the same period (-0.32±0.08 vs. -0.05±0.09 mg/kg/min; $p<0.04$). There were no significant changes in substrate oxidation rates in the non-responders (Table 1).

Limitations of study

Reflecting the population of Tyneside, 98% of participants were white, and comparable studies in other ethnic groups are required. However, socio-economic deprivation was well represented, with 39% being from the lowest two quintiles for deprivation (Taylor et al., 2017). At baseline, participants were taking a range of anti-diabetic medications, reflective of current practice. Although people on insulin therapy were excluded from this primary care based study for practical reasons, prior insulin therapy is not a major determinant of diabetes remission following major weight loss (Panunzi et al., 2016). The study participants were not followed closely in a specialist centre but lived normal lives and reviewed by different primary care nurse, possibly introducing some heterogeneity. This could be interpreted to

indicate generalisability of the conclusions. Finally, the observations relate to only 12 months of observation, and 24 month follow up is underway (Taylor et al., 2017).

Conclusions

This study demonstrates the physiologic changes associated with the return to normal glucose homeostasis in type 2 diabetes. It also quantifies the responses to weight loss in those who returned to normal glucose control compared with those who did not and shows most marked differences between these two groups to be in their ability to recover first phase insulin response. An average decrease in body weight of 15% was achieved by a structured programme delivered by Primary Care staff. This brought about profound changes in lipid metabolism, irrespective of response in terms of glucose control. The greatest change was in liver fat content which fell from high levels to normal in the whole Intervention group at 12 months. Fall in plasma levels of VLDL1-TG was accompanied by fall in intrapancreatic fat content. All changes in lipid metabolism and intra-organ lipid remained steady over 12 months if weight loss was maintained, but, critically, only the responders demonstrated early and sustained improvement in beta cell function as measured by gold standard methodology, with difference at 12 months being striking. In summary, weight loss in early type 2 diabetes brings about similar correction of intra-organ fat content in all, but the defect in those who do not return to non-diabetic glucose control appears intrinsic to the beta cell.

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Author Contributions

Conceptualisation, RT; Methodology, AAM, KGH, BSA, CP; Investigation, SZ, CP, ACB, AAM; Writing – Original Draft, RT and AAM; Writing, Review and Editing, RT, SZ, AAM, CP, KGH, JCM, NS, MEJL; Visualisation AAM, RT; Data curation and formal analysis – AAM, RT, SZ; Funding Acquisition, RT, MEJL, NS, JCM; Resources, BSA, ACB; Supervision, RT, NS, JCM and MEJL.

Declaration of Interests

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Figure Legends

Figure 1: CONSORT diagram of study randomization and sample size

In the Intervention group 6 subjects withdrew during the weight loss phase and 7 during weight maintenance. To permit the paired analysis within each group, 4 subjects were excluded due to reversion to diabetic status.

In the Control group of type 2 diabetes followed over time, 1 subject withdrew and other 2 subjects were excluded due to dietary weight loss and remission of diabetes. During the weight maintenance phase, 3 subjects withdrew.

Figure 2: Study protocol

Participants were randomized to receive a low calorie diet (Intervention) or to continue with their normal medication (Control). This was lasted for an average of 4 months then followed by Stepped Food Reintroduction (SFR) and weight maintenance.

Figure 3: Changes in weight and glucose control

Change in weight (A), fasting plasma glucose (B), and HbA1c (C) within the study groups between baseline, post weight loss (5 months), and 12 months. Data are shown as mean ±SEM. Responders were presented as solid line, non-responders dotted black line, and control as grey dotted line.
***P<0.0001 vs. baseline (responders); †††P<0.0001 vs. baseline (non-responders)
###P<0.0001 vs. 5 months (responders); ‡‡‡P<0.0001 vs. 5 months (non-responders)

Figure 4: Induced changes in lipid metabolism and fasting plasma insulin

Change in liver fat (A), total plasma triglycerides (B), hepatic VLDL1-TG production (C), intrapancreatic fat (D), fasting plasma VLDL1-TG (E), and fasting plasma insulin (F) between baseline, post weight loss (5 months), and 12 months. Data are shown as mean ±SEM. Responders were presented as solid line, non-responders dotted black line, and control as grey dotted line.
**P<0.01 vs. baseline, ***P<0.0001 vs. baseline (responders)
††P<0.01 vs. baseline, †††P<0.0001 vs. baseline (non-responders)
#P<0.05 vs. 5 months (responders); ‡P<0.05 vs. 5 months (non-responders); ‡‡P<0.01 vs. 5 months (non-responders)

Figure 5: Induced changes in beta cell response to glucose stimulation

Median (range) first phase insulin response (A) and maximal insulin secretion (B) at baseline, post weight loss (5 months), and 12 months. Responders - a black box; non-responders - dark grey box; control - light grey box.
*P<0.05 vs. baseline (responders), ***P<0.0001 vs. baseline (responders)

Table 1: Anthropometric, clinical and metabolic features of responders and non-responders before and after intervention

	Responders			Non-responders		
	Baseline (n=40)	**Post-weight loss (n=40)**	**12 months (n=29)**	**Baseline (n=18)**	**Post-weight loss (n=18)**	**12 months (n=16)**
BMI (kg/m^2)	34.9±0.7	29.4±0.6***	29.6±0.8***###	35.7±1.2	31.1±1.3***	32.4±1.4***###
Age (Year)	53.0±1.2	-	-	53.3±1.9	-	-
Sex (F/M)	17/23	-	-	9/9	-	-
Diabetes duration (years)	2.7±0.3	-	-	3.8±0.4†	-	-
VLDL1-TG pool(mg)	2445.9±267	1258.4±168***	1461.7±240**##	2775.4±505	1866.4±432*	2234.1±570
Fasting NEFA (mmol/l)	0.56±0.03	0.55±0.03	0.51±0.03	0.66±0.04	0.59±0.05	0.61±0.04
Alanine Aminotransferase (ALT) (U/l)	34.1±2.8	-	17.1±1.0***	26.3±2.6†	-	18.3±2.0**
Cholesterol (mmol/l)	4.3±0.2	-	4.3±0.2	4.1±0.3	-	4.0±0.2
HDL (mmol/l)	1.09±0.05	-	1.23±0.08**	0.99±0.05	-	1.11±0.06*
Ketone (mmol/l)	0.19±0.02	0.29±0.04**	0.26±0.03*	0.18±0.02	0.20±0.02	0.24±0.04
Lipid oxidation (mg/kg/min)	0.96±0.05	0.87±0.06	0.64±0.09***##	0.84±0.08	0.89±0.11	0.83±0.07
Glucose oxidation (mg/kg/min)	1.27±0.12	1.44±0.14	2.0±0.19***##	1.48±0.17	1.19±0.21	1.31±0.20†
Resting Energy Expenditure (kcal/day)	1996.5±57.0	1647.1±48.1***	1696.4±67.3***#	1981.7±108.0	1641.1±89.5**	1733.2±109.5**

*P<0.05 vs. baseline, **P<0.01 vs. baseline, ***P<0.0001 vs. baseline, # P<0.05 vs. 5 months, ## P<0.01 vs. 5 months, ### P<0.0001 vs. 5 months, † P<0.05 responders vs. non-responders, †† P<0.01 responders vs. non-responders. Paired data were presented (baseline to post-weight loss or baseline /post weight loss to 12 months).

Table 2: Metabolic changes in responders and non-responders during weight loss, weight maintenance and from baseline to 12 months

	Baseline to post-weight loss		Post weight loss to 12 months		Baseline to 12 months	
Δ change	**Responders (n=40)**	**Non-responders (n=18)**	**Responders (n=29)**	**Non-responders (n=16)**	**Responders (n=29)**	**Non-responders (n=16)**
Weight (kg)	-16.2±1.2	-13.4±1.4	3.3±0.8	4.9±0.8	-14.1±1.5	-9.4±1.3*
Fasting plasma glucose (mg/dl)	-46.6 ±7.3	-8.9±12.0**	1.6±2.6	-3.6±11.2	-47.9±8.7	-21.3±7.8*
HbA1c (%)	-1.5±0.2	0.2±0.4***	-0.1±0.05	-0.4±0.35	-1.6±0.2	-0.4±0.2***
Liver fat (%)	-13.4±1.4	-11.9±2.4	0.6±0.3	3.6±1.6	-13.5±1.9	-9.7±2.1
VLDL1-TG production (mg/kg/day)	-147.2 ±33.8	-59.2±52.7	43.1 ±26.7	155.8±50.8	-119.2 ±39.0	72.2±73.6*
Plasma VLDL1-TG (mmol/l)	-0.26±0.07	-0.19±0.12	0.10±0.05	0.16±0.10	-0.21±0.09	-0.04±0.08
VLDL1-TG pool (mg)	-1187.5 ± 245.9	-909.0 ± 385.4	391.5 ± 149.0	659.1 ± 364.7	-993.7 ± 303.8	-313.5 ± 301.1
Total plasma TG (mmol/l)	-0.54±0.12	-0.67±0.19	0.08±0.10	0.13±0.19	-0.58±0.18	-0.57±0.13
Fasting plasma insulin (pmol/l)	-69.7±9.3	-41.7±5.8*	7.2±3.9	10.7±5.2	-65.3±12.1	-32.7±5.7*
Pancreas fat (%)	-0.90±0.17	-0.78±0.23	-0.14±0.28	0.17±0.23	-1.31±0.28	-0.74±0.27
First phase insulin (nmol/min/m^2)	0.08[-0.17-0.47]	-0.002[-0.11-0.06]***	0.02[-0.32-0.51]	-0.01-0.06—0.08]	0.08[-0.30-0.81]	-0.004[-0.17-0.11
Maximal insulin secretion (nmol/min/m^2)	0.08[-1.37-2.66]	-0.03[-1.80-0.66]	0.09[-2.17-1.81]	0.02[-0.44-0.61]	0.17[-1.24-2.05]	0.06[-1.72-0.86]*
Glucose oxidation rate (mg/kg/min)	0.17±0.19	-0.29±0.27	0.82±0.22	0.06±0.30*	0.75±0.18	-0.11±0.23**
Lipid oxidation rate (mg/kg/min)	-0.09±0.08	0.05±0.13	0.31±0.10	-0.02±0.15	-0.32±0.08	-0.05±0.09*

*P<0.05 responders vs. non-responders, **P<0.01 responders vs. non-responders, ***P<0.0001 responders vs. non-responders

Paired data were used to present changes between different phases of the study.

STAR Methods

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Instruments and software		
3T Philips Achieva scanner	Philips, Netherlands	SN: 17497
Six-channel cardiac array	Philips, Netherlands	PN: 453567009711
Three-point Dixon acquisition	Philips, Netherlands	mDixon
Balanced Turbo Field Echo acquisition	Philips, Netherlands	BTFE
Glucose analyzer	Yellow Springs Inc, USA	YSI 2300 STAT Plus
Ultracentrifuge	Beckman Coulter Inc, USA	Model L7-80
Low speed centrifuge	MSE Ltd., UK	Harrier 18/80R
Low speed centrifuge	Scientific Laboratory Supplies Ltd, UK.	Sigma 6K15
SW 40 Ti rotor	Beckman Coulter, Inc, USA	PN: 331302
Peristaltic pump	eBay, China	BT100M
Quark RMR	COSMED, Italy	PN:C09074-01-99
Infusion pump	Arcomedical Infusion Ltd, UK	Volumed® VP7000 PVC
MATLAB	MathWorks, UK	Version R2013a

ImageJ	National Institutes of Health (NIH), USA	Version 1.50
Minitab	Minitab Inc., USA	Version 17
MRIcro	University of South Carolina, USA	Version 1.40
Low Calorie Diet		
Liquid formula diet (825–853 kcal/day)	Cambridge Weight Plan Ltd., UK	N/A
Laboratory reagents for lipoprotein separation		
Sodium Chloride	Sigma-Aldrich, UK	Cat No: S9888
Sodium Bromide 97%	Alfa Aesar, USA	Cat No: 14037
Na_2 EDTA	VWR International Ltd, UK	Cat No: 100935V
Sodium Hydroxide	VWR International Ltd, UK	Cat No: 102525P
Ultracentrifuge tubes	SETON SCIENTIFIC, INC, USA	Cat No:7031W
Glass Pasteur Pipettes	VWR International Ltd, UK	Cat No: VWRI 1822
Clinical reagents for metabolic		
0.9% Sodium Chloride	Fresenius Kabi Ltd, UK	Free*flex*®
Intralipid 20%	Fresenius Kabi Ltd, UK	Intralipid® 20%
Intralipid 10%	Fresenius Kabi Ltd, UK	Intralipid® 10%
20% Dextrose	Fresenius Kabi Ltd, UK	20% Dextrose
Arginine	Martindale Pharmaceuticals, UK	L-Arginine hydrochloride (50%)
Plasma assays		
Triglyceride	Roche Diagnostics, U.K.	Cat No: 05171407 190

Insulin ELISA kit	Mercodia AB, Sweden	10-1128-01
C-peptide ELISA kit	Mercodia AB, Sweden	10-1136-01
HbA1c	Tosoh Bioscience, UK	HPLC-923G8
NEFA enzymatic calorimetric kit	BMG labtech, Germany	FLUOstar Omega microplate reader
Ketone meter	Abbott Diabetes Care Ltd, UK	Optium Xceed (XCF644-3826)
Deposited data		
Raw data	Mendeley dataset	doi:10.17632/9k9cgb8mwy.1

CONTACT FOR REAGENT AND RESOURCE SHARING

All queries should be directed to the lead author, Roy Taylor (roy.taylor@ncl.ac.uk).

DATA AVAILABILITY

The dataset of this study is available in Mendeley Data (doi:10.17632/9k9cgb8mwy.1).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The metabolic study was nested within the cluster-randomised controlled Diabetes Remission Clinical Trial (DiRECT; ISRCTN03267836) (Leslie et al., 2016). Ethical approval was obtained from the West of Scotland Ethics Committee, and written informed consent was obtained from all participants. The primary aim of DiRECT was to assess the effect of weight loss by low calorie diet on type 2 diabetes remission in a routine primary care setting. Individuals with type 2 diabetes living in the Tyneside region of England (n=90, 38F/52M, (mean± SD): age 52.8±7.9 years, weight 100.2±16.3kg, BMI 34.7±7.4 kg/m^2, diabetes duration 3.0±1.7 years, HbA1c 7.5±1.0 %) were recruited by their general practices (Figure 1). Inclusion criteria were diabetes duration of <6 years, age between 20-65 years, HbA1c $\geq$ 6.5 % ($\geq$6.1%

if taking anti-diabetes agents), and BMI of 27-45kg/m^2. Subjects were not recruited if pregnant, experienced weight loss of more than 5kg within the past 6 months or they have serious health problems. The majority of participants were white European with <2% of other ethnic minorities including Black African and South Asian. Most participants were on glucose lowering medication. Baseline characteristics of the whole direct cohort have been described (Taylor et al., 2017) and the baseline anthropometric, clinical and metabolic features for geographically defined subgroup who underwent detailed physiologic study are presented in Table 1.

GP practices were randomized to either Intervention or Control groups. Intervention group subjects stopped all anti-diabetic medication on day 1 of the Counterweight Plus weight management programme consisting of 825–853 kcal/d liquid formula diet (Cambridge Weight Plan Ltd., UK) continued for 12-20 weeks, followed by a 2-6 week food reintroduction phase, then ongoing support for weight maintenance. The Control group continued usual diabetes management by their GP practice according to current UK clinical guidelines.

The Tyneside cohort was designed to further study metabolic changes occurring during weight loss and remission of diabetes (Figure 2). Intervention group subjects were classified as responder or non-responder at the end of each phase. Responders were defined as those achieving non-diabetic levels of HbA1c (<6.5%) and blood glucose (<126mg/dl) off any anti-diabetes medication for at least 2 months. The purpose of the Control group was to examine sequential changes over the time course of the study in type 2 diabetic subjects, and participants (n=2) who lost >5kg weight and became non-diabetic were excluded from the analysis. All studies were performed after an overnight fast, and subjects drove or were transported to the MR Centre by taxi to minimise variability of physical activity and stress of travel.

METHOD DETAILS

Intraorgan fat quantification

All participants underwent Magnetic Resonance (MR) quantification of pancreatic and hepatic fat on three occasions: at baseline, following return to isocaloric eating after weight loss and at 12 months (Figure 2). MR data were acquired using a 3T Philips Achieva scanner 2 with six-channel cardiac array (Philips, Netherlands). Data were acquired by three-point Dixon method, with gradient-echo scans acquired during one breath hold (Al-Mrabeh et al., 2017). Hepatic fat content was measured by selecting homogenous regions of interest on five image slices of liver (Lim et al., 2011). Intrapancreatic fat content was quantified using the MR-opsy method optimized to exclude interlobular adipose tissue areas (Al-Mrabeh et al., 2017). Analysis of pancreas fat was carried out by a single observer (AAM) in a blinded manner.

Lipoprotein separation and VLDL1-TG Production

VLDL1-triglyceride levels were determined from fasting plasma samples taken at each time point. Briefly, the VLDL1-triglyceride production rate was measured by accumulation of plasma VLDL1-triglyceride during competitive blockade of lipoprotein lipase by excess Intralipid (Al-Shayji et al., 2007). To do so, 20% Intralipid (Fresenius Kabi Ltd, UK) was injected intravenously as a bolus (0.1 g/kg body mass) followed by continuous infusion of 10% Intralipid at 0.1 g/kg/h by infusion pump (Arcomed Infusion Ltd, UK). Plasma samples were collected at six points over 75 min. After two step centrifugation, to remove blood cells then chylomicrons plus Intralipid particles (Scientific Laboratory Supplies Ltd, UK), the VLDL1 fraction was separated by ultracentrifugation at 278,000g for 98 minutes using the SW 40 Ti swinging-bucket rotor (Beckman Coulter, Inc, USA). Triglyceride concentration of this fraction was quantified using the standard method (Roche Diagnostics, UK), and VLDL1-triglyceride production rates were calculated from the gradient of the linear increase in plasma concentration over time.

Beta cell function

A Stepped Insulin Secretion Test with Arginine stimulation (SISTA) was used to quantitate first phase insulin secretion and maximal rate of insulin secretion (Lim et

al., 2011; Toschi et al., 2002). Square wave hyperglycemia (50.4 then 100.8 mg/dl above baseline) was achieved by bolus of 20% Dextrose (Fresenius Kabi Ltd, UK) followed by variable 20% Dextrose infusion for each 30 minute step using an infusion pump (Arcomedical Infusion Ltd, UK). An arginine bolus of 5g L-Arginine hydrochloride 50% (Martindale Pharmaceuticals, UK) was diluted in 10 ml of 0.9% sodium chloride (Fresenius Kabi Ltd, UK), and injected during the second step of hyperglycemia to assess maximal insulin secretory capacity, followed by sampling every 2 min for 10 min. Blood samples for determination of C-peptide concentrations were obtained every 2 min for the first 10 min of each step, then every 5 min. Insulin secretion rates were calculated using a deconvolution method, modelling C-peptide kinetics (Lim et al., 2011).

Indirect calorimetry

Indirect calorimetry was carried out in the fasting state after 30 min of supine rest using a Quark ventilated hood calorimeter (COSMED, Italy). Substrate oxidation was calculated using standard equations (Frayn, 1983).

Analytical Procedures

Glucose was measured by the oxidase method (Yellow Springs Inc., USA). HbA1c was quantified using HPLC (Tosoh Bioscience, UK). Liver function tests were analysed by standard methods at the Institute of Cardiovascular and Medical Sciences, University of Glasgow. C-peptide, insulin, glucose, NEFA, VLDL1-triglyceride, ketones and other metabolites were analysed at Clinical Pathology Accreditation Laboratory (Newcastle upon Tyne Hospital NHS Foundation Trust, UK) using standard kits as described in the key resources table.

QUANTIFICATION AND STATISTICAL ANALYSIS

Analyses were conducted on all subjects with paired data both before and after weight loss and weight maintenance phases. Data are presented as mean±SEM for normally distributed data and median (range) for skewed data as stated in the Figure legends and main text. Student paired or two-sample t test was used as appropriate for parametric data and Mann Whitney U test for nonparametric data. All statistical

analyses including testing the normality of data distribution were performed using Minitab 17 (Minitab Inc., USA) and a P value <0.05 was considered as significant. Paired data were presented in all tables, and the number of subjects in each group is stated in the column headings of each Table. We excluded from the analysis 6 subjects (2 controls who lost weight and became non-diabetic, and 4 intervention participants who changed responder status between 5 and 12 months)

The study was designed to compare change in parameters between responders and non-responders, assuming a 60% rate of return to non-diabetic glucose control and a 25% loss to follow up. It was powered on the most stringent variable (change in pancreas fat) in responders compared with non-responders. The calculated sample size was achieved by randomising a greater proportion of general practices to Intervention in the Tyneside region. As there was 69% remission of diabetes after weight loss and 64% at 12 months, the above assumptions for statistical analysis were satisfied.