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## **Remission of type 2 diabetes follows decrease in liver and pancreas fat content but is determined by capacity for beta cell recovery**

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## Summary

New understanding of the physiologic mechanisms underlying type 2 diabetes has depended upon studies of small groups over short periods. Detailed metabolic studies were performed on 64 people with type 2 diabetes over 12 months. Liver fat content decreased ( $16.0\pm 1.3\%$  to  $3.1\pm 0.5\%$ ,  $p<0.0001$ ) immediately after weight loss. Similarly, plasma triglyceride and pancreas fat content decreased in all, whether or not glucose control normalised. Only those who returned to non-diabetic glucose control recovered first phase insulin response ( $0.04[-0.05-0.32]$  to  $0.11 [0.0005-0.51]$  nmol/min/m<sup>2</sup>,  $p<0.0001$ ) and this was durable at 12 months ( $0.11 [0.005-0.81]$  nmol/min/m<sup>2</sup>,  $p=0.0001$ ). Responders were similar to non-responders at baseline but had shorter diabetes duration ( $2.7\pm 0.3$  vs.  $3.8\pm 0.4$  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 and progressive loss of beta cell function in type 2 diabetes.

## Keywords

Type 2 diabetes; beta cell dedifferentiation; type 2 diabetes remission; liver fat; very low density lipoprotein; pancreas fat, insulin secretion; low calorie diet; 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; 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 ( $148.9 \pm 6.8$  vs.  $167.8 \pm 11.9$  mg/dl,  $p=0.18$ ; Figure 3B), and HbA1c was  $7.4 \pm 0.2$  vs.  $7.9 \pm 0.2\%$  respectively ( $p=0.04$ ; Figure 3C). At baseline, liver fat in the whole intervention group was  $16.0 \pm 1.3\%$  and was not significantly different in responders and non-responders ( $16.7 \pm 1.5$  vs.  $14.5 \pm 2.6\%$  respectively;  $p=0.47$ ). There was no significant difference at baseline between responders and non-responders in VLDL1-TG production ( $560.7 \pm 30.9$  vs.  $581.1 \pm 52.1$  mg/kg/day,  $p=0.70$ ; Figure 4C) or total plasma triglyceride ( $162 \pm 11$  vs.  $168 \pm 22$  mg/dl,  $p=0.08$ ). The non-responders had a longer duration of diabetes ( $2.7 \pm 0.3$  vs.  $3.8 \pm 0.4$  years;  $p=0.02$ ), lower fasting plasma insulin ( $108.3 \pm 10.0$  vs.  $77.2 \pm 8.5$  pmol/l;  $p=0.02$ ) and lower plasma ALT ( $34.1 \pm 2.8$  vs.  $26.3 \pm 2.6$  pmol/l;  $p<0.05$ ). Data on the whole intervention group compared with the conventionally treated control group are shown in Table 3.

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 26% 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 ( $100.6 \pm 2.6$  to  $84.4 \pm 2.1$  kg;  $p < 0.0001$ ;  $n = 40$ ) and in non-responders ( $102.1 \pm 4.4$  to  $88.7 \pm 4.4$  kg;  $p < 0.0001$ ;  $n = 18$ ; Figure 3A). The change during the weight loss phase did not differ significantly between groups ( $-16.2 \pm 1.2$  vs.  $-13.4 \pm 1.4$  kg;  $p = 0.14$ ; 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  $3.3 \pm 0.8$  kg to  $86.2 \pm 3.0$  kg ( $p < 0.0001$ ). Over the same period weight in non-responders increased by  $4.9 \pm 0.8$  kg to  $92.5 \pm 4.6$  kg but stayed lower than baseline ( $p < 0.0001$ ). By 12 months the overall change was greater in the responders ( $-14.1 \pm 1.5$  vs.  $-9.4 \pm 1.3$  kg;  $p < 0.02$ ).

## Glucose control

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

HbA1c decreased in responders (to  $5.9 \pm 0.1\%$ ;  $p < 0.0001$ ), but not in non-responders ( $8.0 \pm 0.4\%$ ,  $p = 0.67$ ; Figure 3C). At 12 months, HbA1c did not change further in either group so that differences were maintained ( $5.8 \pm 0.1$  vs.  $7.6 \pm 0.2\%$ ,  $p < 0.0001$ ; Table 2). 69.0% of the Intervention group (40/58) achieved non-diabetic blood glucose control after weight loss, and 64.4% at 12 months (29/45).

## Liver fat

Liver fat content decreased after weight loss in both groups (responders: to  $3.3 \pm 0.6\%$ ;  $p < 0.0001$ ; non-responders: to  $2.6 \pm 0.5\%$ ;  $p < 0.0001$ ; Figure 4A). The change in liver fat was similar ( $-13.4 \pm 1.4$  vs.  $-11.9 \pm 2.4\%$ ,  $p = 0.60$ ; Table 2). At 12 months, liver fat in responders was  $3.0 \pm 0.6\%$  and in non-responders  $6.1 \pm 1.9\%$  ( $p = 0.11$  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 ( $3.2 \pm 1.1$  vs.  $3.2 \pm 1.0\%$ ;  $p = 0.95$ ;  $n = 16$ ). In contrast, in those who gained more than 3.3kg, there was a resultant increase in liver fat ( $1.5 \pm 0.3$  vs.  $2.8 \pm 0.5\%$ ;  $p = 0.03$ ;  $n = 15$ ). In those randomised to the conventionally treated control group, there was no significant change in liver fat throughout the study (Figure 4A; Table 3).

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



Plasma VLDL1-TG concentration decreased in responders after weight loss ( $0.69\pm 0.07$  to  $0.44\pm 0.06$  mmol/l,  $p=0.001$ , Figure 4E), followed by a small increase during weight maintenance (to  $0.49\pm 0.08$  mmol/l,  $p=0.04$ ; Figure 4E). There was no significant change in non-responders after weight loss ( $0.73\pm 0.11$  to  $0.55\pm 0.12$  mmol/l,  $p=0.12$ ) nor during the weight maintenance phase (to  $0.64\pm 0.12$  mmol/l,  $p=0.12$ ).

Total plasma triglyceride (largely chylomicrons plus VLDL-TG) fell similarly in responders and non-responders after weight loss ( $1.84\pm 0.13$  to  $1.30\pm 0.13$  mmol/l,  $p<0.0001$  and  $1.91\pm 0.25$  to  $1.24\pm 0.14$  mmol/l respectively,  $p=0.002$ , Figure 4B). This remained stable at 12 months (responders: to  $1.24\pm 0.12$  mmol/l,  $p=0.43$ ; non-responders: to  $1.39\pm 0.21$  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 3).

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 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 VLDL-1 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 ( $8.7\pm 0.4$  vs.  $7.9\pm 0.6\%$ ;  $p=0.25$ , Figure 4D). Weight loss produced a similar fall in intrapancreatic fat in both groups (responders: to  $7.8\pm 0.4\%$ ;  $p<0.0001$  and non-responders: to  $7.1\pm 0.4\%$ ;  $p=0.004$ ). There was no significant difference in extent of change between the two groups ( $-0.90\pm 0.17$  vs.  $-0.78\pm 0.23$ ;  $p=0.67$ ; Table 2). During the weight maintenance phase, it remained stable in both groups ( $7.9\pm 0.4\%$  and  $6.8\pm 0.4\%$  in responders and non-responders, respectively). There was no change in pancreas fat in the control group (Table 3).

The magnetic resonance method of quantifying fat content 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 intra-pancreatic fat content, and these parameters change in step (Lee et al., 2010). *In vivo* measurement of intra-pancreatic 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 intra-pancreatic 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. Intra-pancreatic 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<sup>2</sup> ( $p < 0.0001$ ). 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<sup>2</sup>;  $p = 0.59$ ; Figure 5A; Table 2). In the responders, increased first phase insulin secretion was maintained during the weight maintenance phase, with improvement in some individuals which did not reach significance (to 0.11[0.005-0.81] nmol/min/m<sup>2</sup>;  $p = 0.34$ ). However, 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<sup>2</sup>;  $p = 0.04$  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 3).

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 were 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 and 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  $1.27 \pm 0.12$  at baseline to  $1.44 \pm 0.14$  and to  $2.00 \pm 0.19$  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 and controls ( $0.75 \pm 0.18$  vs.  $-0.11 \pm 0.23$ ;  $p=0.006$ ; Table 2). Conversely, lipid oxidation rates decreased in responders from  $0.96 \pm 0.05$  to  $0.87 \pm 0.06$  ( $p<0.28$ ) and to  $0.64 \pm 0.09$  mg/kg/min; ( $p<0.01$ ) with a greater decrease than in non-responders over the same period ( $-0.32 \pm 0.08$  vs.  $-0.05 \pm 0.09$  mg/kg/min;  $p<0.04$ ). There were no significant changes in substrate oxidation rates in the non-responders (Table 1).

### **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 intra-pancreatic 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, JM, NS, MEJL; Visualisation AAM, RT; Data curation and formal analysis – AAM, RT; Funding Acquisition, RT, MEJL, NS, JM; Resources, BSA, ACCB; Supervision, RT, NS, JM and MEJL.

### **Declaration of Interests**

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## References

- Adiels, M., Taskinen, M.R., Packard, C., Caslake, M.J., Soro-Paavonen, A., Westerbacka, J., Vehkavaara, S., Hakkinen, A., Olofsson, S.O., Yki-Jarvinen, H., et al. (2006). Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia* *49*, 755-765.
- Al-Mrabeh, A., Hollingsworth, K.G., Steven, S., Tiniakos, D., and Taylor, R. (2017). Quantification of intrapancreatic fat in type 2 diabetes. *Plos One* *12*, e0174660.
- Al-Shayji, I.A., Gill, J.M., Cooney, J., Siddiqui, S., and Caslake, M.J. (2007). Development of a novel method to determine very low density lipoprotein kinetics. *J Lipid Res* *48*, 2086-2095.
- Bensellam, M., Jonas, J.C., and Laybutt, D.R. (2018). Mechanisms of beta-cell dedifferentiation in diabetes: recent findings and future research directions. *The Journal of endocrinology* *236*, R109-R143.
- Bensellam, M., Laybutt, D.R., and Jonas, J.C. (2012). The molecular mechanisms of pancreatic beta-cell glucotoxicity: recent findings and future research directions. *Mol Cell Endocrinol* *364*, 1-27.
- Brereton, M.F., Iberl, M., Shimomura, K., Zhang, Q., Adriaenssens, A.E., Proks, P., Spiliotis, I.I., Dace, W., Mattis, K.K., Ramracheya, R., et al. (2014). Reversible changes in pancreatic islet structure and function produced by elevated blood glucose. *Nat Commun* *5*.
- Bril, F., and Cusi, K. (2017). Management of Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes: A Call to Action. *Diabetes Care* *40*, 419-430.
- Cinti, F., Bouchi, R., Kim-Muller, J.Y., Ohmura, Y., Sandoval, P.R., Masini, M., Marselli, L., Suleiman, M., Ratner, L.E., Marchetti, P., et al. (2016). Evidence of beta-Cell Dedifferentiation in Human Type 2 Diabetes. *The Journal of clinical endocrinology and metabolism* *101*, 1044-1054.
- Donnelly, K.L., Smith, C.I., Schwarzenberg, S.J., Jessurun, J., Boldt, M.D., and Parks, E.J. (2005). Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* *115*, 1343-1351.
- Frayn, K.N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol Respir Environ Exerc Physiol* *55*, 628-634.
- Harrison, L.B., Adams-Huet, B., Raskin, P., and Lingvay, I. (2012). Beta-cell function preservation after 3.5 years of intensive diabetes therapy. *Diabetes Care* *35*, 1406-1412.
- Henry, R.R., Wallace, P., and Olefsky, J.M. (1986). Effects of weight loss on mechanisms of hyperglycaemia in obese non-insulin dependent diabetes mellitus. *Diabetes* *35*, 990-998.
- Hiukka, A., Fruchart-Najib, J., Leinonen, E., Hilden, H., Fruchart, J.C., and Taskinen, M.R. (2005). Alterations of lipids and apolipoprotein CIII in very low density lipoprotein subspecies in type 2 diabetes. *Diabetologia* *48*, 1207-1215.
- Huang, C.J., Lin, C.Y., Haataja, L., Gurlo, T., Butler, A.E., Rizza, R.A., and Butler, P.C. (2007). High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress mediated beta-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes. *Diabetes* *56*, 2016-2027.
- Kumashiro, N., Erion, D.M., Zhang, D., Kahn, M., Beddow, S.A., Chu, X., Still, C.D., Gerhard, G.S., Han, X., Dziura, J., et al. (2011). Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. *Proc Natl Acad Sci U S A* *108*, 16381-16385.



Lambert, J.E., Ramos-Roman, M.A., Browning, J.D., and Parks, E.J. (2014). Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology* *146*, 726-735.

Laybutt, D.R., Preston, A.M., Akerfeldt, M.C., Kench, J.G., Busch, A.K., Biankin, A.V., and Biden, T.J. (2007). Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. *Diabetologia* *50*, 752-763.

Lean, M.E., Leslie, W.S., Barnes, A.C., Brosnahan, N., Thom, G., McCombie, L., Peters, C., Zhyzhneuskaya, S., Al-Mrabeh, A., Hollingsworth, K.G., et al. (2017). Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *Lancet* *391*, 541-551.

Lee, Y., Hirose, H., Ohneda, M., Johnson, J.H., McGarry, J.D., and Unger, R.H. (1994). B-Cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-B-Cell relationships. *Proceedings of the National Academy of Science of U.S.A.* *91*, 10878-10882.

Lee, Y., Lingvay, I., Szczepaniak, L.S., Ravazzola, M., Orci, L., and Unger, R.H. (2010). Pancreatic steatosis: harbinger of type 2 diabetes in obese rodents. *Int J Obes (Lond)* *34*, 396-400.

Leslie, W.S., Ford, I., Sattar, N., Hollingsworth, K.G., Adamson, A., Sniehotta, F.F., McCombie, L., Brosnahan, N., Ross, H., Mathers, J.C., et al. (2016). The Diabetes Remission Clinical Trial (DiRECT): protocol for a cluster randomised trial. *BMC Fam Pract* *17*, 20.

Lim, E.L., Hollingsworth, K.G., Aribisala, B.S., Chen, M.J., Mathers, J.C., and Taylor, R. (2011). Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia* *54*, 2506-2514.

Magkos, F., Su, X., Bradley, D., Fabbrini, E., Conte, C., Eagon, J.C., Varela, J.E., Brunt, E.M., Patterson, B.W., and Klein, S. (2012). Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. *Gastroenterology* *142*, 1444-1446 e1442.

McGarry, J.D. (2002). Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* *51*, 7-18.

Menke, A., Casagrande, S., Geiss, L., and Cowie, C.C. (2015). Prevalence of and Trends in Diabetes Among Adults in the United States, 1988-2012. *JAMA : the journal of the American Medical Association* *314*, 1021-1029.

Ohneda, M., Inman, L.R., and Unger, R.H. (1995). Caloric restriction in obese pre-diabetic rats prevents beta-cell depletion, loss of beta-cell GLUT 2 and glucose incompetence. *Diabetologia* *38*, 173-179.

Perry, R.J., Peng, L., Cline, G.W., Wang, Y., Rabin-Court, A., Song, J.D., Zhang, D., Zhang, X.M., Nozaki, Y., Dufour, S., et al. (2018). Mechanisms by which a Very-Low-Calorie Diet Reverses Hyperglycemia in a Rat Model of Type 2 Diabetes. *Cell Metab* *27*, 210-217 e213.

Petersen, K.F., Dufour, S., Befroy, D., Lehrke, M., Hendler, R.E., and Shulman, G.I. (2005). Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* *54*, 603-608.

Pinnick, K., Neville, M., Clark, A., and Fielding, B. (2010). Reversibility of metabolic and morphological changes associated with chronic exposure of pancreatic islet beta-cells to fatty acids. *Journal of cellular biochemistry* *109*, 683-692.

Pinnick, K.E., Collins, S.C., Londos, C., Gauguier, D., Clark, A., and Fielding, B.A. (2008). Pancreatic ectopic fat is characterized by adipocyte infiltration and altered lipid composition. *Obesity* *16*, 522-530.

Poitout, V., Amyot, J., Semache, M., Zarrouki, B., Hagman, D., and Fontés, G. (2010). Glucolipototoxicity of the pancreatic beta cell. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* *1801*, 289-298.

Ravikumar, B., Gerrard, J., Dalla Man, C., Firbank, M.J., Lane, A., English, P.T., Cobelli, C., and Taylor, R. (2008). Pioglitazone decreases fasting and postprandial endogenous glucose production in proportion to decrease in hepatic triglyceride content. *Diabetes* *57*, 2288-2295.

Samuel, V.T., Liu, Z.X., Qu, X., Elder, B.D., Bilz, S., Befroy, D., Romanelli, A.J., and Shulman, G.I. (2004). Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *The Journal of biological chemistry* *279*, 32345-32353.

Samuel, V.T., Petersen, K.F., and Shulman, G.I. (2010). Lipid-induced insulin resistance: unravelling the mechanism. *Lancet* *375*, 2267-2277.

Seppala-Lindroos, A., Vehkavaara, S., Hakkinen, A.M., Goto, T., Westerbacka, J., Sovijarvi, A., Halavaara, J., and Yki-Jarvinen, H. (2002). Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *The Journal of clinical endocrinology and metabolism* *87*, 3023-3028.

Shibata, M., Kihara, Y., Taguchi, M., Tashiro, M., and Otsuki, M. (2007). Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men. *Diabetes Care* *30*, 2940-2944.

Spijker, H.S., Song, H., Ellenbroek, J.H., Roefs, M.M., Engelse, M.A., Bos, E., Koster, A.J., Rabelink, T.J., Hansen, B.C., Clark, A., et al. (2015). Loss of  $\beta$ -Cell Identity Occurs in Type 2 Diabetes and Is Associated With Islet Amyloid Deposits. *Diabetes* *64*, 2928-2938.

Steven, S., Hollingsworth, K.G., Al-Mrabeh, A., Avery, L., Aribisala, B.S., Caslake, M., and Taylor, R. (2016a). Very Low Calorie Diet and 6 Months of Weight Stability in Type 2 Diabetes: Pathophysiological Changes in Responders and Nonresponders. *Diabetes Care* *39*, 158-165.

Steven, S., Hollingsworth, K.G., Small, P., Woodcock, S., Pucci, A., Aribisala, B.S., Al-Mrabeh, A., Daly, A.K., Batterham, R.L., and Taylor, R. (2016b). Weight loss decreases excess pancreatic triacylglycerol specifically in type 2 diabetes. *Diabetes Care* *39*, 158-165.

Talchai, C., Xuan, S., Lin, H.V., Sussel, L., and Accili, D. (2012). Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. *Cell* *150*, 1223-1234.

Taylor, R. (2008). Pathogenesis of Type 2 diabetes: Tracing the reverse route from cure to cause. *Diabetologia* *51*, 1781-1789.

Taylor, R. (2013). Type 2 diabetes: etiology and reversibility. *Diabetes Care* *36*, 1047-1055.

Taylor, R., Leslie, W.S., Barnes, A.C., Brosnahan, N., Thom, G., McCombie, L., Sattar, N., Welsh, P., Peters, C., Zhyzhneuskaya, S., et al. (2017). Clinical and metabolic features of the randomised controlled Diabetes Remission Clinical Trial (DiRECT) cohort. *Diabetologia* *61*, 589-598.

Timlin, M.T., Barrows, B.R., and Parks, E.J. (2005). Increased dietary substrate delivery alters hepatic fatty acid recycling in healthy men. *Diabetes* *54*, 2694-2701.

Toschi, E., Camastra, S., Sironi, A.M., Masoni, A., Gastaldelli, A., Mari, A., Ferrannini, E., and Natali, A. (2002). Effect of acute hyperglycemia on insulin secretion in humans. *Diabetes* *51 Suppl 1*, S130-133.

Turner, R.C., Cull, C.A., Frighi, V., and Holman, R.R. (1999). Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive

requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. *Journal of the American Medical Association* 281, 2005-2012.

Unger, R.H. (1995). Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* 44, 863-870.

Wang, Z., York, Nathaniel W., Nichols, Colin G., and Remedi, Maria S. (2014). Pancreatic  $\beta$  Cell Dedifferentiation in Diabetes and Redifferentiation following Insulin Therapy. *Cell Metabolism* 19, 872-882.

Weir, G.C., Aguayo-Mazzucato, C., and Bonner-Weir, S. (2013). beta-cell dedifferentiation in diabetes is important, but what is it? *Islets* 5, 233-237.

White, M.G., Marshall, H.L., Rigby, R., Huang, G.C., Amer, A., Booth, T., White, S., and Shaw, J.A.M. (2013). Expression of Mesenchymal and  $\alpha$ -Cell Phenotypic Markers in Islet  $\beta$ -Cells in Recently Diagnosed Diabetes. *Diabetes care* 36, 3818-3820.

White, M.G., Shaw, J.A.M., and Taylor, R. (2016). Type 2 diabetes: The pathologic basis of reversible beta-cell dysfunction. *Diabetes Care* 39, 2080-2088.

WHO (2016). Global Report on Diabetes. Last update 17.06.2017.  
<http://www.who.int/diabetes/global-report/en/>.

Wing, R.R., Koeske, R., Epstein, L.H., Nowalk, M.P., Gooding, W., and Becker, D. (1987). Long-term effects of modest weight loss in type II diabetic patients. *Arch Intern Med* 147, 1749-1753.

Zhou, Y.P., and Grill, V. (1995). Long term exposure to fatty acids and ketones inhibits B-cell functions in human pancreatic islets of Langerhans. *The Journal of clinical endocrinology and metabolism* 80, 1584-1590.

## 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; 4 weeks) 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. 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 VLDL-TG (E), and fasting plasma insulin (F) between baseline, post weight loss (5 months), and 12 months. 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 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)

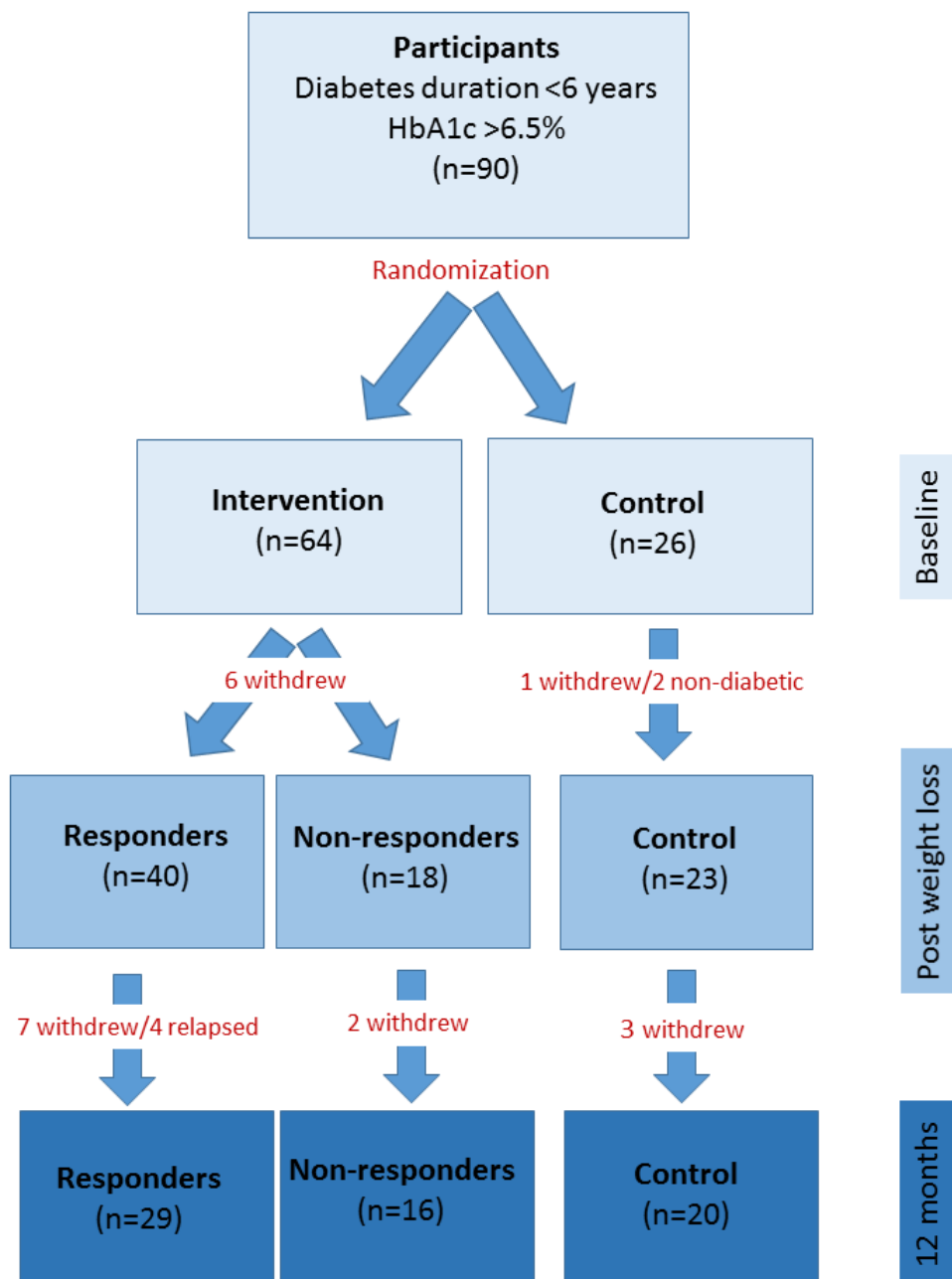
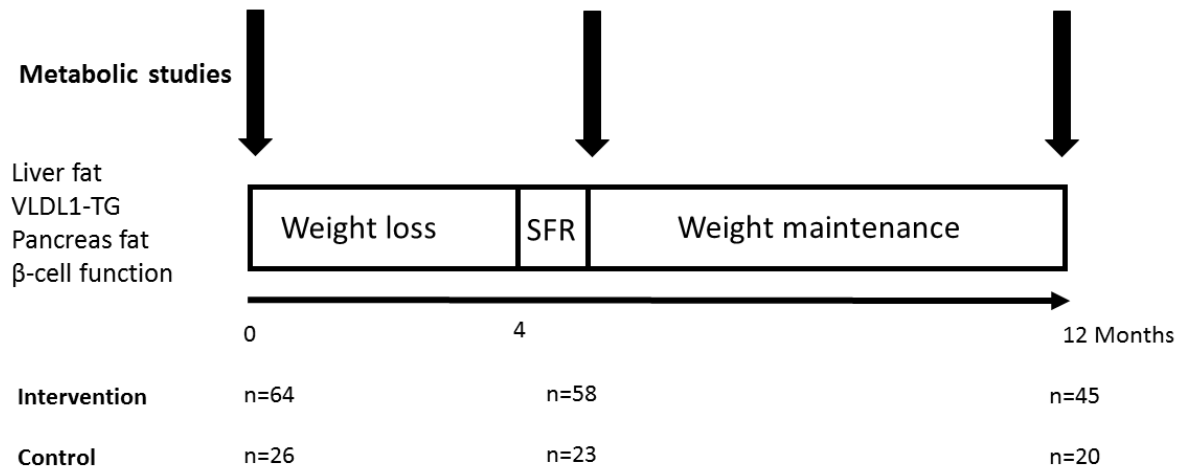


Fig 1



**Fig 2**

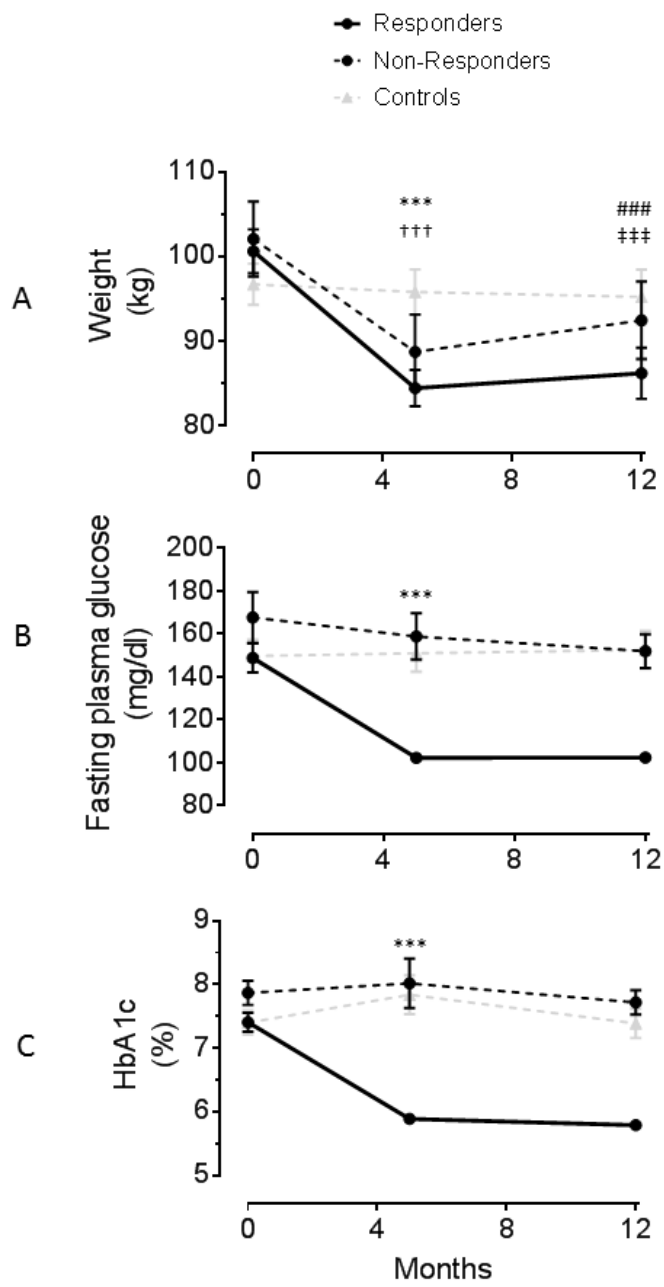


Fig 3

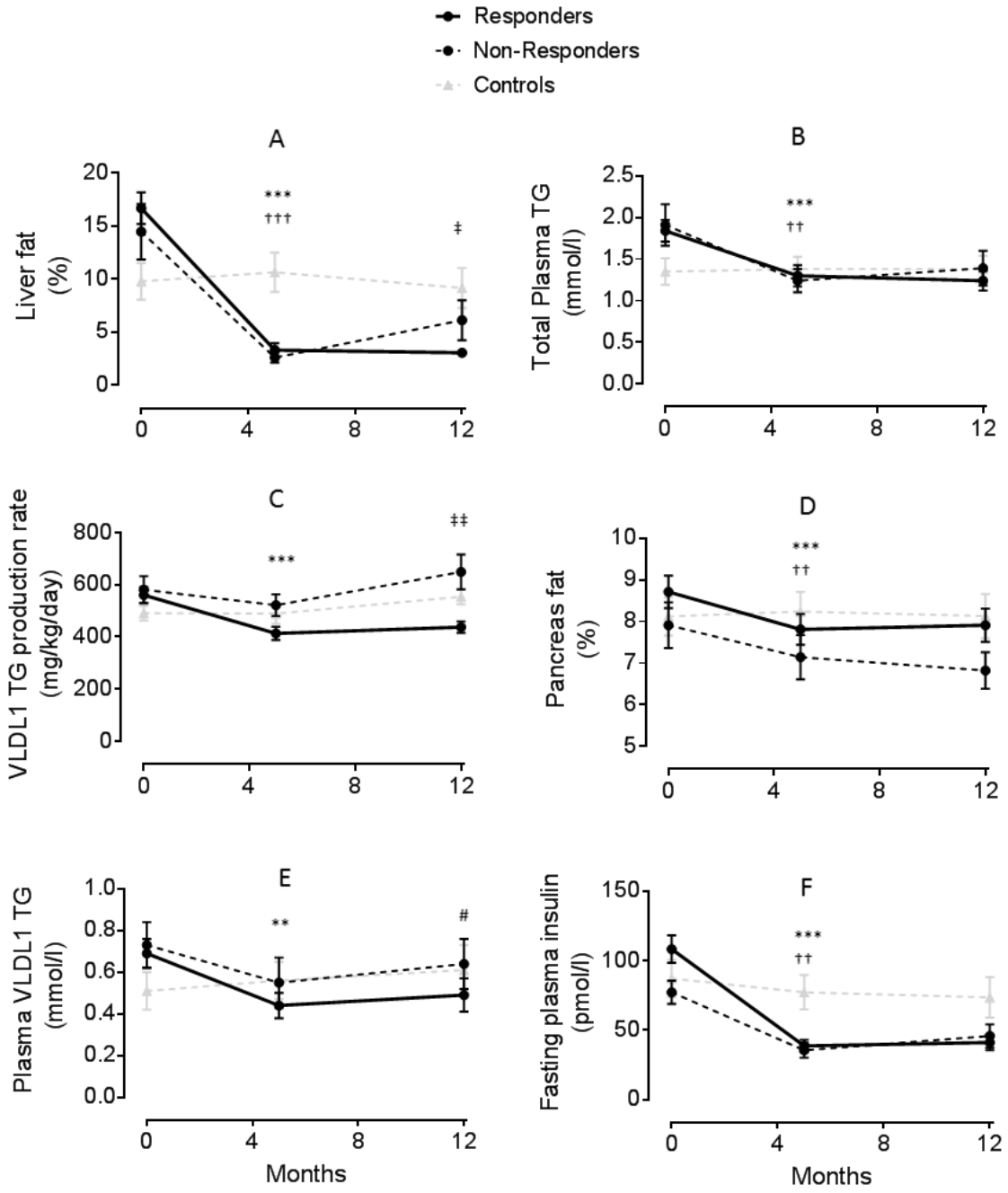


Fig4







**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)
<b>BMI (kg/m<sup>2</sup>)</b>	34.9±0.7	29.4±0.6***	29.6±0.8***###	35.7±1.2	31.1±1.3***	32.4±1.4***###
<b>Age (Year)</b>	53.0±1.2	-	-	53.3±1.9	-	-
<b>Sex (F/M)</b>	17/23	-	-	9/9	-	-
<b>Diabetes duration (years)</b>	2.7±0.3	-	-	3.8±0.4†	-	-
<b>VLDL1-TG pool(mg)</b>	2445.9±267	1258.4±168***	1461.7±240**##	2775.4±505	1866.4±432*	2234.1±570
<b>Fasting NEFA (mmol/l)</b>	0.56±0.03	0.55±0.03	0.51±0.03	0.66±0.04	0.59±0.05	0.61±0.04
<b>Alanine Aminotransferase (ALT) (U/l)</b>	34.1±2.8	-	17.1±1.0***	26.3±2.6†	-	18.3±2.0**
<b>Cholesterol (mmol/l)</b>	4.3±0.2	-	4.3±0.2	4.1±0.3	-	4.0±0.2
<b>HDL (mmol/l)</b>	1.09±0.05	-	1.23±0.08**	0.99±0.05	-	1.11±0.06*
<b>Ketone (mmol/L)</b>	0.19±0.02	0.29±0.04**	0.26±0.03*	0.18±0.02	0.20±0.02	0.24±0.04
<b>Lipid oxidation (mg/kg/min)</b>	0.96±0.05	0.87±0.06	0.64±0.09**##	0.84±0.08	0.89±0.11	0.83±0.07
<b>Glucose oxidation (mg/kg/min)</b>	1.27±0.12	1.44±0.14	2.0±0.19***###	1.48±0.17	1.19±0.21	1.31±0.20†
<b>Resting Energy Expenditure (kcal/day)</b>	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**

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

**Table 3: Comparison of metabolic parameters between Intervention and Control groups**

	Intervention			Control		
	Baseline (n=58)	5 months (n=58)	12 months (n=45)	Baseline (n=23)	5 months (n=23)	12 months (n=20)
<b>Weight (kg)</b>	101.1±2.2	85.8±2.0***	88.4±2.5***###	96.7±2.5	95.8±2.7	95.2±3.2
<b>HbA1c (%)</b>	7.6± 0.1	6.5± 0.2***	6.4± 0.2***	7.4± 0.2	7.8±0.3*	7.2±0.2
<b>Fasting plasma glucose (mg/dl)</b>	154.7±6.0	119.8±5.0***	120.0±4.8***	149.7±7.6	151.0±8.6	152.6±9.0
<b>Liver fat (%)</b>	16.0 ±1.3	3.1 ±0.5***	4.1±0.8***#	9.8±1.7 †	10.6±1.8	9.2±1.9
<b>VLDL1-TG PR (mg/kg/day)</b>	567.3±26.6	448.4±22.9***	511.5±31.2**##	491.1±27.6	489.6±40.1	554.3±28.9
<b>Plasma VLDL-TG (mmol/l)</b>	0.71±0.06	0.47±0.05***	0.54±0.07*#	0.51±0.09	0.56±0.09	0.61±0.12
<b>Pancreas fat (%)</b>	8.5±0.3	7.6±0.3***	7.5±0.3***	8.1±0.5	8.2±0.5	8.1±0.5
<b>Fasting plasma insulin (pmol/l)</b>	98.5±7.5	37.7±3.4***	42.8±4.6***##	87.1 ±13.4	77.3±12.4	73.6±14.6
<b>First phase ins. (nmol/min/m<sup>2</sup>)</b>	0.03[-0.07-0.32]	0.06[-0.04-0.51]***	0.06[-0.03-0.81]**	0.02[-0.05-0.29]	0.03[-0.06-0.25]	0.03[-0.05-0.38]
<b>Maximal insulin (nmol/min/m<sup>2</sup>)</b>	0.53[0.13-2.35]	0.63[0.01-3.21]	0.63[0.25-2.69]	0.54[0.13-1.76]	0.57[0.26-1.38]	0.56[0.25-1.45]

\*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 Intervention vs. Control at baseline.

Paired data were presented (baseline to post-weight loss or baseline/ post weight loss to 12 months).



## STAR Methods

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Instruments and software</b>		
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
<b>Low Calorie Diet</b>		
Liquid formula diet (825– 853 kcal/day)	Cambridge Weight Plan Ltd., UK	N/A
<b>Laboratory reagents for lipoprotein separation</b>		
Sodium Chloride	Sigma-Aldrich, UK	Cat No: S9888
Sodium Bromide 97%	Alfa Aesar, USA	Cat No: 14037
Na <sub>2</sub> 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
<b>Clinical reagents for metabolic and calorimetric studies</b>		
0.9% Sodium Chloride	Fresenius Kabi Ltd, UK	Freeflex®
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%)
<b>Plasma assays</b>		
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)
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## CONTACT FOR REAGENT AND RESOURCE SHARING

All queries should be directed to the lead author, Roy Taylor (roy.taylor@ncl.ac.uk)..  
The dataset of this study is available in Mendeleev Data (\_\_\_\_\_).

## 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<sup>2</sup>, 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<sup>2</sup>. 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 and this was withdrawn after the baseline studies. 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 as previously described (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) as described by Al-Shayji et al. (2007). Triglyceride concentration of this fraction was quantified using the standard method (Roche Diagnostics, U.K.), 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 as previously described (Lim et al., 2011).

### **Indirect calorimetry**

Indirect calorimetry was carried out in the fasting state after 30 minutes 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 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 each of the two phases, weight loss and weight maintenance. Data are presented as mean $\pm$ SEM for normally distributed data and median (range) for skewed data. 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., State College, PA) and a P value <0.05 was considered as significant. Paired data were presented in all tables, and n represents the number of subjects in each group. 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 69.0% remission of diabetes after

weight loss and 64.4% at 12 months the above assumption for statistical analysis was satisfied.