Reversal of Type 2 diabetes Upon Normalisation of Energy intake in non-obese people (ReTUNE)

Synopsis of Protocol in non-technical language

People who have been diagnosed with type 2 diabetes but who are not obese will be studied. Although only half of all newly diagnosed people with type 2 diabetes are obese, it is widely believed by doctors that obesity itself causes the condition. This leads to non-obese people with type 2 diabetes being told they have a different form of the disease and that they must not lose weight.

We have published a discussion of this, with reasons why some people can develop type 2 diabetes at a body mass index within the population range of 'normal'. Clinical observation has shown that non-obese people can reverse their diabetes after weight loss just as well as obese people. Indeed the first patient of RT's to do this had a BMI of 24. By dropping to just under 20 (which had been the person's BMI in their 20's), diabetes went away, and the person still does not have diabetes almost 13 years later. It appears that everyone has a personal threshold beyond which they can no longer store fat safely under the skin – where it is safely stored without metabolic problems. This project aims to obtain experimental evidence of the phenomenon.

Rare forms of diabetes will be excluded. To allow identification of the weight at which each individual experiences return of normal sugar control, body weight will be decreased in three stages. A very low calorie liquid diet will be used, as this is well accepted and surprisingly easy to live with, according to our volunteers. After each short weight loss period, weight will be kept constant on normal foodstuffs as it takes 8 weeks for the full effect of any weight loss to become apparent. So for a person of 70kg, approximately 3.5kg (5%) would be lost, then weight kept steady until week 8 when the tests would be done. This would be repeated 3 times. However, if diabetes has gone away after any one stage, no further weight loss will be undertaken.

Tests would be carried out before starting the weight loss, and at 8, 16, 24 and 52 weeks. The tests are: a) The special magnetic resonance measurement of the amount of fat in the pancreas and liver; b) A standard breakfast with blood sampling to show how well the body is coping with food in terms of control of blood sugar and also insulin response; c) blood tests to detect overfilling of the subcutaneous fat stores.

Scientific Synopsis

Type 2 diabetes in non-obese individuals is frequently regarded as having a different aetiology from that affecting obese people. However, experimental evidence does not support this, and the concept of a personal level of tolerance of total body fat allows potential understanding of BMI-independent occurrence of the disease. It is proposed to test the Personal Fat Threshold hypothesis and to identify plasma markers which may be of assistance in the management of individuals. People classified by BMI as normal or marginally overweight (BMI<27kg/m^2) will be asked to undergo stepwise weight loss with quantification of insulin secretion, liver and pancreas fat and markers of adipose tissue over-expansion. The primary aim is to determine whether type 2 diabetes in non-obese individuals can be reversed by weight loss, with potential profound consequences for clinical management of this group. The secondary aims are to identify markers of adipose tissue overexpansion as potential guides to defining the Personal Fat Threshold, to examine the relationship between the threshold and pancreas fat content, and to evaluate and refine a Diet Decision Aid to facilitate long term individual normalisation of energy intake.

Scientific Background

Type 2 diabetes

Although type 2 diabetes is commonly assumed to be caused by obesity, only ~50% of people at diagnosis have a BMI over 30kg/m^2 [1]. It is widely believed that non-obese individuals with type 2 diabetes have a different diabetes subtype with greater beta cell defect and less insulin resistance than those who are overweight or obese [2-5]. This view is reinforced by the belief that type 2 diabetes is not one condition but a composite of many different aetiological entities [6]. However, close examination of data leads to different conclusions: A) The relative difference in insulin resistance between groups with type 2 diabetes or normal glucose tolerance is similar whether groups are obese or non-obese [7; 8]. B) The apparent enigma of the higher fasting plasma insulin levels seen in obese compared with non-obese individuals with Type 2 diabetes, is explained when the obesity itself is taken into account [9; 10]. C) Test meals elicit similar increases in plasma C-peptide in non-obese and obese people with type 2 diabetes (2.5 and 1.8-fold respectively) [11]. Overall, there is a lack of evidence for difference in pathophysiology between obese and non-obese people with Type 2 diabetes.

In populations, the incidence and prevalence of type 2 diabetes rises or falls simply as a function of the state of the food supply - as documented in Cuba in 1990-96 and in Britain during the first and second world wars [12; 13]. The relationship between type 2 diabetes and obesity has not always been obvious.

In the 1970s and 80s, when the average weight of the UK population was considerably less than at present and only 7% were obese, there was no detectable effect of obesity on the development of type 2 diabetes [14-16]. The Nurses' Health Study established that the risk of type 2 diabetes increases steadily as weight increases throughout the BMI range, with a four-fold increase in type 2 diabetes prevalence for women of BMI 23-25 compared with those of BMI less than 22kg/m^2 [17]. This critically important finding has not been widely recognized. As a result, studies of the effect of weight loss in type 2 diabetes typically exclude those with BMI less than 25kg/m^2 [18; 19], and current guidelines do not recommend weight loss for people with type 2 diabetes who have a 'normal' BMI.

UK Prospective Diabetes Study (UKPDS)

Knowledge about the natural history of type 2 diabetes largely derives from the UKPDS, which enrolled 5,102 people with newly diagnosed type 2 diabetes between 1977 and 1991. It is not widely appreciated that 36% of this cohort had a BMI less than 25 kg/m^2 at entry into the study. The BMI frequency distribution was unimodal with no suggestion of a distinct, non-obese sub-population [20]. Notably, the distribution was merely right-shifted from that of a contemporaneous adult UK population, in which 64% had a BMI less than 25kg/m^2 [21; 22]. From today's perspective it may appear remarkable that so many people with newly-diagnosed type 2 diabetes had normal BMI's. Nevertheless, given that the risk of type 2 diabetes rises steeply at higher BMI's and that such higher BMI's are now more prevalent, it is not surprising that the association between obesity and type 2 diabetes is more evident today. The most recent information suggests that 11.3% (~350,000 people in the UK) now have a BMI <25kg/m^2 at the time of diagnosis of type 2 diabetes [23].

In the initial 3 month UKPDS dietary run-in phase, weight loss was the main aim. Overall, 16% of the cohort achieved fasting plasma glucose of <6.0mmol/l during this period. There was no relationship between initial body weight and achievement of normal fasting glycaemia. Indeed, normoglycaemia was observed with a lesser degree of weight loss (13%) if initial body weight was normal, compared with the whole cohort (21% weight loss) [24].

Mechanism of reversal

Recent studies have shown that the reversal of type 2 diabetes is primarily associated with a reduction in pancreatic fat levels [25-27]. Rodent islets exposed to excess fatty acids decrease insulin secretion, and removal reverses this [28]. Human islets also take up fatty acids avidly with substantial increase in islet triglyceride content and impaired insulin secretion [29]. Importantly, acute weight loss only brings about an early decrease in pancreatic fat in individuals with type 2 diabetes and not those without diabetes [27]. It is important to note that optimized magnetic resonance imaging technology is required for determination of the relatively small absolute differences in pancreatic fat levels and low precision methods cannot achieve this [30]. The excess fat in the environment of the pancreatic islet and decreased insulin secretion appear linked. Such exposure can reduce expression or activity of key beta-cell transcription factors [31-33], and loss of the differentiated phenotype including

insulin production [28; 34]. This is known to be associated with markers of endoplasmic reticulum stress, typically elevated in human beta-cells from individuals with type 2 diabetes [35-37]. The metabolic stress of chronic nutrient oversupply can lead to loss of the critical end-differentiated genes including insulin [38]. Removal of the nutrient excess leads to redifferentiation, with consequent recovery of insulin gene expression [28; 39]. Hence, the reversal of type 2 diabetes is dependent upon changing beta cell fat exposure rather than any aspect of initial BMI *per se*.

In 2008, the aetiology and potential reversibility of type 2 diabetes were described hypothetically [40]. In 2011, the mechanisms were confirmed by the Counterpoint study. In 2016 the Counterbalance study confirmed the long term maintenance of normal metabolic health after the weight loss phase [25; 26]. Currently, the Diabetes Remission Controlled Trial (DiRECT) is underway entirely in Primary Care, with the primary aim of establishing what proportion of all people with type 2 diabetes can reverse to long term (2 year) metabolic normality after up to 6 years disease duration [41]. As the lower limit of BMI for recruitment into DiRECT is 27kg/m^2, the proposed study will include individuals with a BMI up to this level. DiRECT has been fully recruited on schedule, and the 1 year data will be available in autumn 2017. The emerging 1 and 2 year follow up data are very encouraging for practical NHS implementation (see Section 12).

Personal Fat Threshold (PFT)

The PFT hypothesis [22] potentially explains commonly occurring type 2 diabetes as a single pathophysiological disease entity. It postulates that type 2 diabetes occurs when the capacity of subcutaneous tissue fat stores has been exceeded and excess fat has to be stored within liver and pancreas. Some individuals have considerable capacity to store subcutaneous fat without metabolic problems - 72% of people with a BMI over 40kg/m^2 do not have diabetes [42]. Others appear to have lesser subcutaneous fat capacity and excess fat appears in liver and pancreas. The state of lipoatrophy displays these mechanism at an extreme level [43]. Although there must be individual differences in the susceptibility of the pancreas to take up the excess fat, the PFT hypothesis would suggest that everyone with type 2 diabetes has proven susceptibility. It is likely that secretion of inflammatory mediators from adipose tissue, a typical finding in type 2 diabetes, will be particularly related to overfull fat stores [44; 45]. These include plasminogen activator inhibitor-1, TNFα and interleukin 6. There are also typical abnormalities in adipose tissue hormones leptin and adiponectin [46]. Although the evidence appears overwhelming that type 2 diabetes is a reversible state of nutrient excess [25; 26; 39; 47; 48], beliefs about non-obese type 2 diabetes are particularly resistant to change. It is therefore important to examine the potential reversibility and mechanistic basis of type 2 diabetes in non-obese individuals.

Some basic predictions of the PFT hypothesis have already been shown to be correct. There is no relationship between initial BMI and reversibility of type 2 diabetes [25; 26]. Moreover, in the Counterbalance study, those with a high BMI at diagnosis of type 2 diabetes who were still technically obese after losing 15% of body weight (a change of approximately 4kg/m^2) remained normoglycaemic over 6 months, with normal liver and pancreas fat content [26].

Emerging observations from DiRECT extend this to 2 years of metabolically normal follow up (see Section 12 – report on DiRECT). The post-diabetes state is thus characterized by low liver fat content, low VLDL-TG production and hence low pancreatic fat content. It differs profoundly from the state of pre-diabetes by absence of abnormal liver fat content, by absence of an atherogenic plasma lipid profile and by lower rates of hypertension. Nonetheless, with the exception of UKPDS, only anecdotal evidence suggests that people with a normal BMI can achieve reversal of their type 2 diabetes by weight loss alone [47-49]. Definitive evidence is required to inform management guidelines.

Long term weight control

Long term maintenance of stable body weight after weight loss has been reported to be challenging. In the LookAhead study, maximum weight loss of 8.5% decreased to 4.7% despite intensive support [50]. After the Counterpoint study [25], which was a solely an acute intervention to test the Twin Cycle hypothesis, participants reported uncertainty and even feelings of panic when having to decide what to eat and what the portion size should be. This led to the design of the stepped food re-introduction used in Counterbalance [26], with directive advice on what to eat and what portion size to take. There was a blank slate upon which new dietary habits could be written. This was successful in that there was no weight gain during the planned 6 month follow up period with only monthly contact with the research team. Decision aids have been developed to help people with type 2 diabetes weigh up the advantages and disadvantages of different medication options [51], but to our knowledge no similar decision aid has been developed for choices of different dietary approaches. Yet evidence-based nutrition guidelines highlight that there is not one 'best diet' for diabetes prevention or management [52]. The Diet Decision Aid will offer a choice between three evidence-based approaches to avoiding weight regain: a low carbohydrate diet [53; 54]. A Mediterranean diet, replacing saturated fats with monounsaturated, and based on poultry, fish, vegetables, fruit and pulses [55; 56]. Intermittent calorie restriction (500-650kcal) for one day per week [57]. The overall aim is to establish optimal methodology for truly long term weight maintenance. The practical aim of the present study is to retune appetite and eating habits to achieve long term normalisation of energy intake.

Lipids and the pancreas

Type 2 diabetes is strongly linked to lipoprotein abnormalities [58; 59] and particularly of the VLDL fraction [60]. This fraction is largely responsible for delivery of fatty acids to all tissues, including the pancreas. Fatty acids derived continuously from this source will contribute to the fatty acid and acylglycerol intracellular pools which affect beta cell function in addition to those derived from local triglyceride stores. There are differential effects of a high fat diet on VLDL composition in type 2 diabetes [61]. Size and concentration of VLDL is known to be decreased after fish oil supplementation or by diets enriched with olive oil, but fatty acid composition has rarely been investigated [62; 63]. In particular full quantitation of fatty acid composition during and after reversal of type 2 diabetes is lacking. During separation of the very low density lipoprotein fraction in the DiRECT study, we have observed considerable individual variation in the physical properties of the VLDL fraction during harvesting which are

independent of total triglyceride concentration. This may reflect differences in fatty acid and structural lipid (especially phospholipid) composition, with possible influence upon tissue uptake of fatty acids. This observation requires further evaluation.

In obese people with Type 2 diabetes, we have reported that the pancreas has a markedly irregular border and is ~30% smaller in volume than in weight matched non-diabetic controls [64]. Subsequent work has shown that reversal of diabetes for 6 months brings about improvement in the irregularity of the border but no change in volume [65]. At least in the obese range, body size does not confound, as correction for surface area or BMI leaves the conclusions unchanged. The gross morphology of the pancreas appears important in type 2 diabetes and further study is required. The aims of defining the architecture of the pancreas underlying the irregularity of pancreas border will be simplified by study of non-obese individuals with smaller volume of surrounding adipose tissue. The morphology of the pancreas in the non-obese range of BMI will be able to be defined, with assessment of change after 12 months of reversal of diabetes.

Summary

The proposed study will define the relationships between type 2 diabetes, pancreatic fat and personal fat thresholds, but will also provide unique pathophysiological insight, uncomplicated by obesity, into mechanisms likely to be operating in all Type 2 diabetes. The concept of every individual having a personal threshold of fat accumulation above which they are at risk of developing type 2 diabetes has direct practical utility. Both people with type 2 diabetes and GPs receive the PFT explanation with enthusiasm. It is simple to grasp the idea that anyone with true type 2 diabetes has acquired more fat than their body can cope with. It is also a spur to action with clear motivational potential. But before the PFT concept can be rolled out in clinical practice it is essential to demonstrate formally that reversal of type 2 diabetes by weight loss can be brought about in non-obese people.

Scientific Objectives

Primary Aim:

To test the hypothesis that type 2 diabetes can be reversed by a very low calorie diet in people classified by BMI as normal or marginally overweight (BMI<27kg/m^2).

Secondary Aims:

a) To identify markers of adipose tissue overexpansion as potential guides to defining a Personal Fat Threshold;
b) To examine the relationship between the Personal Fat Threshold and pancreatic fat content and morphology, by comparison with a normal glucose tolerant group and with individual baseline levels;
c) To quantitate the fatty acid composition of very low density lipoprotein during and after restriction of food energy;

d) To evaluate and refine a Diet Decision Aid to facilitate long term individual normalisation of energy intake.

Inclusion/Exclusion criteria

Inclusion
Documented type 2 diabetes of duration less than 6 years
All ethnicities
BMI of 21-27kg/m2 (Caucasian) or 19-27 (non-Caucasian) due to documented differing metabolic risks per BMI unit
Age 20-70 years
Exclusion
Current insulin use
HbA1c ≥12%
Substance abuse
Known cancer
Myocardial infarction within previous 6 months
Learning difficulties
Diagnosed eating disorder or purging
Pregnant or considering pregnancy
Previous hospitalisation for depression
Presence of metal implants preventing magnetic resonance scanning
Claustrophobia likely to cause discomfort in the MR scanner

Interventions

Protocol:
A schematic outline is shown in Appendix 1.

Individuals will be invited to participate via newspaper advertisement (n=21). The ethnic composition of the North East of England is expected to lead to an almost exclusively Caucasian group, with ~5% South Asians. It would be impractical to advertise for ethnicity-based BMI characteristics despite differing distributions of BMI. GAD, IA2 antibodies and zinc transporter will be measured to identify and exclude individuals with slow-onset type 1 diabetes. Genetic screening for all common types of maturity onset diabetes of youth (MODY) will be undertaken [66]. Baseline magnetic resonance and metabolic tests will be performed as detailed below.

All oral hypoglycaemic agents will be stopped immediately prior to the very low calorie diet. As this diet achieves a major and rapid effect upon blood pressure (mean 25mm Hg systolic decrease [26], antihypertensive agents will also be stopped to avoid postural hypotension. Experience from DiRECT shows this is well accepted and is associated with the expected fall in blood pressure. A very low calorie diet (600 kcal/day from liquid formula product, plus 240g of non-starchy vegetables per day [25; 67] will then be used to achieve a 5%

decrease in body weight over approximately 2 weeks. This intervention will be supervised by the research dietitian. Participants will monitor fasting and post-prandial blood glucose levels twice weekly, and this information will be downloaded electronically from their glucose meters. A stepped return to an isocaloric diet of normal foodstuffs over 2 weeks will be then be undertaken. The energy content will initially be calculated from body weight and adjusted in accordance with observed weight change as we have previously described [26]. After a total of 8 weeks since the start of the weight loss, the magnetic resonance and metabolic tests will be repeated. As illustrated in Figure 1, the cycle of weight loss, weight maintenance and tests will be repeated twice. If an individual achieves normalization of both fasting plasma glucose and HbA1c after the second cycle, then these tests will constitute the final end point for the individual.

After each transition to normal eating, the Diet Decision Aid, based on simple illustrated resources, will outline each approach during face to face consultation. This will take place in the last week of each weight loss stage, exploring the suitability of each approach for the individual's food preferences and lifestyle. Success of the decision process will be assessed by number of changes of choice after each weight loss phase. Adherence will be measured by blinded assessment of web-based dietary assessment (INTAKE24) (Rowland et al 2016). The overall success will be measured by observed weight change at 12 months within each choice.

Participants will be seen monthly by the research dietitian from months 3-6 and every 2 months thereafter. A questionnaire (used in DiRECT) about motivation and experiences of taking part will be administered at 6 and 12 months. Anthropometric, metabolic and magnetic resonance measurements will be repeated at 12 months.

Non-diabetic control individuals with no family history of type 2 diabetes and matched for BMI achieved after weight loss will be studied on a single occasion (n= 20). Age, sex and ethnicity will be matched with the type 2 diabetes group. Oral glucose tolerance testing will be performed to ensure normal glucose tolerance.

Anthropometric tests
Weight, height, waist and hip circumferences and body fat by impedance will be recorded.

Metabolic tests
Fasting blood samples will be taken at each test point for HbA1c, glucose, insulin, ALT, non-esterified fatty acid, total cholesterol, HDL-cholesterol, total triglyceride and VLDL1-triglyceride. GC-MS will be used to identify and quantify fatty acids and phospholipids within VLDL fraction at baseline, and comparing it with the lipid profile for matched healthy controls and in relation to the metabolic response to weight loss in the participants with Type 2 diabetes. Chylomicrons and VLDL1 fraction will be separated from plasma by sequential flotation using density gradient ultracentrifugation [68]. Identification of the

fatty acids profile in these fractions will be carried out by GC-MS using the fatty acid methyl esters method [69] following extraction from the VLDL1 fraction [70]. Internal standards will be added before lipid extraction to permit fatty acid quantification. Phospholipids will be identified [69] after optimization of GC-MS methodology [71].

Markers of adipose tissue inflammation will be measured (plasminogen activator inhibitor-1, C-reactive protein, TNFα, interleukin 6) plus adipose tissue derived hormones (adiponectin and leptin). Plasma glucose, insulin and C-peptide will be measured at -15, 0, 10, 20, 30, 45, 60, 90, 120, 150 and 180 minutes during a standard meal test. The standard meal will be consumed over the well tolerated time course previously described with approximately 50% eaten within 5 minutes and all within 10 minutes. The primary outcomes for overall control of the meal response will be 180 minute incremental area under the glucose curve. Both early and total insulin secretory response will be quantified by modelling collaboration with Professor Cobelli [72]. Achievement of non-diabetic glucose control will be defined using the published criteria [73].

Magnetic resonance assessments
Magnetic resonance data will be acquired using a 3.0 Tesla Philips scanner using a 28-channel torso array for signal detection and dStream fibre optic architecture to achieve the maximum possible signal to noise ratio (Philips Best, The Netherlands). The protocols run will include a 3 point Dixon for fat fraction measurements together with an anatomical scan as previously described [74]. In 10 participants from each group the newly developed methodology for compressed sensing [75], with optimal balance between decreased duration of breath-hold and precision of the fat quantification, will be run in parallel in order to compare data derived by each technique. Additional high resolution 3D anatomical scans will be acquired. Pancreas volume will be measured by surface rendering and border irregularity by fractal analysis [65]. Liver fat content will be quantified as previously reported [25; 26]. Visceral and subcutaneous fat volumes will also be quantified. The time in the scanner to acquire all MR data will be approximately 30 minutes.

Definition of End of Study

Completion of all participant visits, blood sample analysis and data analysis.

Relevant statistics

Statistical considerations including sample size:
Primary outcome measure: non-diabetic HbA1c reversal rate.
Secondary measures: i) change in HbA1c from entry to study and post-diet; ii) change in levels of pancreas and liver fat pre- and post-diet; iii) safety; iv) serum markers and relationship with levels of fat; v) maintenance of reversal at 12-months; vi) feasibility measures including reasons for non-participation.
The primary outcome of the study is achievement of non-diabetic HbA1c levels, post-diet termed reversal reported as a % reversal (95%CI). The relationship between reversal and HbA1c will be estimated from logistic regression analysis,

unadjusted and adjusted for BMI. The clinically relevant reversal rate is 50%, as judged in previous studies (n=11)[25]. Twenty one patients will allow a confidence interval of width 0.4 to be calculated should the actual reversal rate be 50% i.e. 50% (95% CI: 30% to 70%).
Secondary outcome is the actual change in HbA1c from entry/ pre-diet to post-diet, and will reported as a mean change (95%CI) over the group of participants. The relationship between post-diet HbA1c and pre-diet levels will be estimated from linear regression analysis, unadjusted and adjusted for BMI. No data exist on a non-obese group, and data from the short duration group of the Counterbalance study (mean BMI 34) have been used (anticipated and relevant mean change in HbA1c pre- and post diet =0.6; SD=0.8 (ref)). To observe this large but relevant difference, with this assumed level of variability and with low type 1 error (alpha=5%) and high power (1-beta=90%) requires 21 matched pairs (patients) to be recruited. Data will be transformed if underlying assumptions of normality are questionable.
An important secondary outcome measure is the demonstration of a decrease in pancreas fat content from baseline to after completion of weight loss. This analysis will be descriptive since any statistical comparison is likely to be underpowered due to larger variance in this parameter requiring larger numbers to demonstrate a statistically significant difference. The association between levels of pancreas/ liver fat and serum markers will be investigated descriptively using linear regression, transforming non-normally distributed outcome and non-linear covariates where necessary.
A group size of 21 providing baseline and post-diet data, is the target recruitment. We estimate a 10% non-completion and hence this target is inflated to 24 (early drop outs will remain in the baseline analysis will not contribute to the comparative analysis of outcome).
The non-diabetic control group (n=20) will be used to provide a normative distribution to allow comparison with all parameters after weight loss.
Longitudinal data over 12months will be summarized graphically and descriptively.
Statistics input by Professor Deborah Stocken

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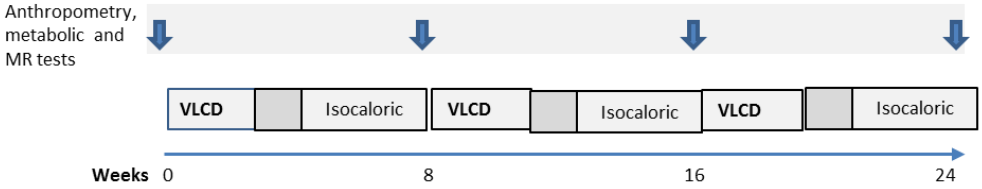

Figure 1: Schematic representation of weight loss phase. Each very low calorie diet (VLCD) phase will achieve 5% weight loss, and after stepped return to normal foodstuffs a weight maintaining (isocaloric) diet of normal foods will be followed for the remainder of each 8 week cycle.

ReTUNE: Gantt Chart of Activity by Time