Understanding the reversibility of Type 2 Diabetes

Roy Taylor¹ Ahmad Al-Mrabehe¹ & Naveed Sattar²

Magnetic Resonance Centre, Institute of Cellular Medicine¹, Newcastle University
& Institute of Cardiovascular and Medical Sciences, Glasgow University²

Correspondence:
Roy Taylor,
Professor of Medicine and Metabolism,
Newcastle Magnetic Resonance Centre,
Campus for Ageing and Vitality,
Newcastle upon Tyne NE4 5PL
United Kingdom
Telephone: +44 (0)191 222 1172
Fax: +44 (0)191 222 1151
Email: Roy.Taylor@ncl.ac.uk
Abstract

Recent clinical and pathophysiological studies have shown type 2 diabetes to be mainly a condition caused by excess, yet reversible, fat accumulation in liver and pancreas. Within the liver, excess fat worsens hepatic responsiveness to insulin leading to increased glucose production. Within the pancreas, the beta cell appears to enter a survival mode and fails in its specialised function due to the fat-induced metabolic stress. Removal of excess fat from these organs via substantial weight loss can normalise hepatic insulin responsiveness and, in the early years post-diagnosis, is associated with beta cell recovery of acute insulin secretion in many, possibly by re-differentiation. Collectively, these changes can normalise blood glucose levels. The Diabetes Remission Clinical Trial demonstrated in Primary Care that 46% of people with type 2 diabetes could achieve remission at 12 months mediated by weight loss. This major change in our understanding of the underlying mechanisms of disease permits a reassessment of advice for people with type 2 diabetes.

Search Strategy and selection criteria

We did a systematic search in PubMed for English language articles published between Jan 1, 1960, and October 15, 2018. Our search terms included “type 2 diabetes,” “pathogenesis” and “remission.” We searched articles by title and abstract to identify relevant studies. Studies were also sought within reference lists of eligible studies. We considered studies and trials that evaluated the reversibility or remission of type 2 diabetes and explored relevant mechanisms.
**Introduction**

The lifelong nature of both type 1 and type 2 diabetes has always appeared self-evident. In type 2 diabetes, observational studies have documented the gradual worsening of blood glucose control with need for increasing numbers of anti-diabetic agents. By 10 years after diagnosis, up to 50% of people require insulin therapy to achieve reasonable control. As a consequence of these findings, it is common practice to inform individuals at the time of diagnosis that they have a lifelong condition, which must be managed as well as possible. The importance of accepting this inevitability has been emphasised. The overall result is a learned helplessness on the part of both patients and health care professionals.

Just over a decade ago the importance of liver fat accumulation in determining hepatic insulin resistance became clear and the link with type 2 diabetes was recognised. As the degree of sensitivity to insulin determines effectiveness of regulation of liver glucose production and hence fasting plasma glucose, liver fat content appeared to be a central factor. Observation of the very rapid return to normal fasting plasma glucose in people with type 2 diabetes experiencing sudden food restriction after bariatric surgery led to formulation of the Twin Cycle Hypothesis to explain the potential nutritionally-dependent reversal of type 2 diabetes (Figure 1). This postulated that modest calorie balance over many years would lead to accumulation of fat in the liver and hence liver insulin resistance. As the liver continuously makes glucose, only restrained by insulin action, the fat-induced liver insulin resistance would cause a slight rise in fasting plasma glucose and triggering an increase in insulin production. Because higher insulin levels stimulate conversion of carbohydrate to fat within the liver, a vicious cycle would be initiated. But additionally, the excess liver fat would cause increased export of fat to the whole body and this would be taken up by many tissues including the pancreatic beta cells. Fat is known to decrease acute insulin production, and eventually this could lead to greater post-meal glucose levels. In turn, this would result in higher insulin levels and greater tendency to store excess carbohydrate as liver fat. The twin vicious cycles would continue until a point at which the beta cells became unable to produce enough insulin to compensate for the resistance to
insulin, and diabetes would result. Importantly, the hypothesis indicates that the twin vicious cycles could be made to reverse in direction if the excess fat load was removed.

Although it was initial assumed that bariatric surgery associated increase in post-prandial GLP-1 explained the rapid restoration of normal fasting plasma glucose, this has since been shown to be incorrect\(^9,10\). The series of studies and trials to test the Twin Cycle Hypothesis have brought about change in UK management of type 2 diabetes\(^11\) and the American Diabetes Association recently recognised remission as an appropriate aim of management\(^12\). There has been very clear and positive feedback about the new concept of practical reversal from people with the condition\(^13,14\). It is important that the mechanisms underlying the restoration of non-diabetic blood glucose control are understood by doctors and scientists. This review summarises the studies relevant to the understanding of type 2 diabetes as a reversible clinical state.

**Conventional view of type 2 diabetes**

The pathophysiology of type 2 diabetes has been regarded as having two distinct components: insulin resistance (i.e. tissues losing their ability to respond normally to insulin, leading to hyperinsulinemia), and a beta-cell defect. The insulin clamp has become regarded as the gold standard method for assessing insulin resistance although it mainly assesses muscle with a variable contribution from liver. Muscle insulin resistance is the earliest detectable feature indicating increased risk of developing type 2 diabetes\(^15\). The predominant focus of research in the treatment of type 2 diabetes has therefore been to identify better ways of improving muscle insulin resistance by drugs (e.g., glitazones) or by diet and exercise.

The beta cell dysfunction has, to date, proved less amenable to therapy. Data from the highly impactful United Kingdom Prospective Diabetes Study suggested that at the time of diagnosis, beta-cell function, albeit estimated by surrogate indices, had already declined to around 50% of normal\(^16\), and continued to decline in a linear fashion irrespective of pharmacological treatments\(^17\). Histological studies furthermore suggest an approximate 50% decrease in beta cell number and continued death of beta cells, possibly due to
apoptosis\textsuperscript{18,19}. Based on such evidence it has hitherto been widely accepted that ongoing death or apoptosis of beta cells plays a major role in the onset and progression of human type 2 diabetes even though direct evidence is lacking.

**Recent observations on the liver**

Over a decade ago, plasma levels of the liver enzyme ALT and triglyceride were reported by us to increase steadily over the 18 months prior to diagnosis of type 2 diabetes\textsuperscript{20}. This rang a chord with clinicians who had puzzled over elevated ALT and gamma GT levels in many with type 2 diabetes. As hepatic steatosis is now the commonest cause of moderately raised ALT levels the question of liver fat levels in type 2 diabetes became important. Several studies have highlighted the association between hepatic sensitivity to insulin and extent of fat storage in the liver\textsuperscript{5,21,22}.

The continuous production of glucose is one of the most important roles of the liver and this is regulated by insulin. The effect of low calorie diet on liver fat, hepatic glucose production and hence fasting plasma glucose, has been recognised for some time (Figure 2)\textsuperscript{23,24}. The extent of liver fat accumulation has a major effect upon control of plasma glucose concentrations via change in insulin sensitivity\textsuperscript{5,25,26}. This is particularly notable in the fasted state (Figure 1). Overall, it appeared that the rising ALT prior to the diagnosis of type 2 diabetes\textsuperscript{20} could indicate increasing hepatic fat accumulation and gradually increasing hepatic insulin resistance.

Around about the same time as the recognition of the rise in ALT prior to the onset of hyperglycaemia, the remarkable rapidity of normalisation of fasting plasma glucose after bariatric surgery became appreciated\textsuperscript{7}. Even though it had been recognised for over 50 years that bariatric surgery could eventually result in remission of type 2 diabetes the time course of change had not been recognised. As the women in this 2006 bariatric surgery study had an average weight of 152kg, it could be calculated that they would require to eat around 2,700 calories per day merely to meet their basal energy requirements. But on the evening before bariatric surgery a sudden severe restriction of calorie intake was instigated which was then maintained post-surgery. This restriction is relevant since a year earlier,
Petersen and colleagues had demonstrated rapidly decreasing liver fat levels and complete normalisation of hepatic insulin sensitivity following calorie restriction in type 2 diabetes. This could potentially explain the mechanism of rapid fall in fasting plasma glucose after bariatric surgery. Indeed, this rapid glycaemic normalisation by diet alone had been directly observed by the father of bariatric surgery, Walter Pories, many years earlier following an abandoned bariatric surgery procedure but application of the usual post-operative food restriction. The 2006 bariatric surgery paper postulated instead that this beneficial glycaemic effect was mediated by increased post-meal GLP-1 secretion. However, given that GLP-1 has negligible glucose effects in the fasting state and no enteral feeding had preceded the observation of normalised fasting plasma glucose, the concept that a post-meal spike in GLP-1 level explains the post-bariatric surgery improvement is physiologically improbable. Others have since shown GLP-1 changes post-surgery to be an association not a cause of early glycaemia changes. The role of oxidative capacity of the liver in enhancing liver fat accumulation and the roles of diacylglycerol and ceramide in the fat induced hepatic insulin resistance (at least in rodent studies) has now been demonstrated.

**Recent observations on the pancreas**

Whilst this new understanding of the role of liver fat in control of hepatic glucose production permitted better understanding of observed changes in fasting plasma glucose after calorie restriction, the inadequate pancreatic insulin secretion, characteristic of type 2 diabetes, required to be explained. Data from animal studies offered a potential solution to this problem as chronic in vitro exposure of beta-cells to triglyceride or fatty acids decreases ability to respond to an acute increase in glucose levels. The concept that fat could impair beta cell function was far from new. Early studies by Anne Clark demonstrated the ultrastructural intracellular damage brought about in rodent beta cells and insulin secreting cell lines by relatively low concentrations of saturated fatty acids, and this endoplasmic reticulum stress has since been recognised in in vivo studies of type 2 diabetes. Once hyperglycaemia is added, rodent studies suggest that this is likely to compound the fat-initiated metabolic insult.
If beta-cell dysfunction and hepatic insulin resistance shared the common cause of long term excess fat exposure, then the known pieces of this jigsaw puzzle could begin to fit together (Figure 1). This twin cycle hypothesis was an attempt to crystalise these concepts into a testable form. It postulated that long-standing calorie excess would initiate a self-reinforcing accumulation of liver fat and would increase VLDL-triglyceride output from the liver (leading to excess “ectopic” blood fat levels – i.e. accumulation in a site not able to provide safe storage of fat). The latter would in turn contribute to accumulation of ectopic fat including in the pancreas and so impaired beta cell function, resulting eventually in loss of plasma glucose control and type 2 diabetes. Individuals clearly differ in susceptibility to deleterious effects of increased intra-organ fat. Importantly, the hypothesis predicted that if the prime driver of the cycles could be reversed, via negative calorie balance, then type 2 diabetes could resolve in some, perhaps many, individuals.

Fat within the pancreas is contained within scattered adipocytes, giving a high background level (not relevant to local metabolic function) and within the cytoplasm of both exocrine and endocrine cells. It is vital that quantification excludes the fibro-fatty tissue between lobules, but precise quantification using advanced magnetic resonance methods is possible. Pancreas fat content varies widely between individuals and sequential measurement is required to examine metabolic effect. It is increased in type 2 diabetes and importantly does not decrease with weight loss over 8 weeks unless type 2 diabetes is present. Current data suggest that there is a small but metabolically significant pool of excess triglyceride within both endocrine and exocrine cells of the pancreas in type 2 diabetes.

**Testing the new hypothesis**

The hypothesis could be tested most simply by observing what happened in response to sudden calorie restriction in people with type 2 diabetes. If the hypothesis was correct, the body would have to call upon its supplies of stored energy, and there would be two main results. Firstly, fat stored within the liver would be utilised and both hepatic insulin resistance and fasting plasma glucose would fall. Secondly, it would be expected that pancreas fat content would fall and glucose induced insulin secretion would normalise. In
order to put this to the test, a precise method of measuring pancreas fat \textit{in vivo} was developed and a robust, practicable means of decreasing calorie intake for the purposes of was devised as part of a clinical mechanistic study \textsuperscript{44}. The Counterpoint (COUNTERacting Pancreatic inhibitiOn of INsulin secretion by Triglyceride) study was successful in achieving a 15.3kg weight loss over the planned eight weeks period in a typical group with type 2 diabetes \textsuperscript{44}.

Just as observed during the calorie restriction of bariatric surgery, fasting plasma glucose normalised within seven days of instituting sudden negative calorie balance even though metformin had been withdrawn on day 1 of the diet. Liver fat levels and hepatic insulin sensitivity also normalised over the same time course, returning, remarkably, to levels seen in weight matched non-diabetic controls within the seven days (Figure 3).

During the study, pancreas fat content declined and the first phase insulin response gradually increased, the latter becoming normal by week eight. These findings were paradigm-shifting, as hitherto it had been believed that restoration of normal first phase insulin secretion could not be achieved in type 2 diabetes (Figure 3e). The predictions of the twin cycle hypothesis had thus been confirmed (Table 1). [refs for Table to ensure correct order: \textsuperscript{33,44,50,51,20,44,45,47,52,44,47,52,53,10,44,46,54,55,56}]

Because of the documented effect of commencement of exercise programs to induce compensatory eating \textsuperscript{55-57} and limiting weight loss, a purely dietary approach was taken to achieve the necessary 15kg average weight loss in Counterpoint and subsequent studies. However, a sustained increase in daily physical exercise can be a very important component of long term avoidance of weight regain \textsuperscript{58}.

\textbf{Is reversal of type 2 diabetes durable after return to normal eating?}

Critically, in order to demonstrate durability (and so wider clinical applicability) of the return to non-diabetic plasma glucose levels, a nutritional and behavioural approach to achieving long term isocaloric eating after the acute weight loss phase was required. The
Counterbalance (COUNTERacting BetA cell failure by Long term Action to Normalize Calorie intake) study involved six months of follow up by specialist staff in a research centre. A liquid diet replacement was used to induce weight loss followed by stepped reintroduction of normal foods then supportive follow up with emphasis on portion size with regular check of body weight. No significant weight regain occurred leaving the whole group approximately 14kg lighter than before and with maintenance of post-weight loss normalisation of blood glucose control. The overall remission rate (defined as HbA1c < 6.5% [48mmol/mol] on two occasions at least 2 months apart) remained constant over the 6 months of follow up.

These finding were extended in DiRECT (Diabetes Remission Clinical Trial), but importantly this was conducted entirely in primary care, the intervention being training of primary care nurses or dietitians. It was much larger (n=149 in both intervention and control groups) and involved people with up to 6 years duration of type 2 diabetes. The mean weight loss in the intervention group at 12 months was 10kg, analysed on an intention to treat basis. The remission rate at 12 months was 46%, off all anti-diabetic agents. In both Counterpoint and DiRECT, people who had reversed their type 2 diabetes after weight loss remained free of diabetes if they avoided subsequent weight regain. The study characteristics and results of Counterpoint, Counterbalance and DiRECT are summarised in Table 2.

Some longer-term data are available from the LookAhead study in which mean weight loss was greater in the intensive lifestyle group compared to the comparator by 3.9% after 4 years of follow up, in turn associated with 5.3% more people undergoing remission of type 2 diabetes. In this study, the focus not only on diet but with emphasis on intensive exercise programmes from the outset, which are not easily sustained, may have led to greater weight regain. Notably, the LookAhead population was different to DiRECT in important ways in that around one fifth of patients were on insulin at the start and the median duration was longer (5 years), with many having had diabetes for more than a decade. The ethnic mix was also quite different with only around 60% being white in LookAhead, whereas this figure was close to 100% in DiRECT. Hence, these two trials are not easily comparable.

Many people continue to have BMI over 30 kg/m² after remission of type 2 diabetes has been achieved and the implications of this require to be considered. Following the acute
weight loss, the mean BMI was 28.6 and 31.5 kg/m² in Counterbalance and DiRECT respectively, and hence approximately half of each group remained obese by definition. Would the remaining excess fat redistribute into liver and pancreas when isocaloric eating resumed? Baseline mean liver fat content was 12.8% and 16.0% respectively in the two studies. In Counterbalance, the weight loss was associated with fall in liver fat to 2% (low normal) in those who reversed their diabetes and this level was unchanged at 6 months. In DiRECT responders, liver fat content fell to 3.3% then 3.0% at 12 months (Figure 4). It appears that if total body fat burden falls below an individual threshold, and this fall is sustained, then ectopic fat does not reaccumulate. This is consonant with the personal fat threshold concept 62, now supported by genetic data linking limited capacity for subcutaneous fat storage with cardiometabolic disease 63, and indicates that BMI is only a very general guide to a healthy metabolic weight for an individual. A parallel pattern of change was observed in the pancreas fat. Self-reported observations, open to bias, indicate that individuals who lost significant weight by dietary change remained free of diabetes over several years provided weight regain is avoided 6465.

**Can type 2 diabetes of any duration be reversed?**

The Counterpoint study involved people with type 2 diabetes up to 4 years after diagnosis. It was necessary to determine the effect of longer duration upon reversibility, and the subsequent Counterbalance study involved people with 0.5-23 years duration of type 2 diabetes 45. All 29 participants in the latter showed an improvement in fasting plasma glucose by the end of the first week 45. Almost 90% of people with less than 4 years duration diabetes achieved non-diabetic levels (<7mM). In this short duration group, achieved fasting plasma glucose after 6 months was 5.8 ± 0.2 mmol/l; for diabetes duration 8–12 years it was 6.2 ± 0.7 mmol/l; and for diabetes duration ≥ 12 years, it was 10.6 ±1.7 mmol/l. In those with diabetes duration of greater than 11 years none returned to non-diabetic fasting plasma glucose levels. In a subgroup of DiRECT studied in detail, the minority who lost weight without achieving reversal of their diabetes had longer duration of type 2 diabetes 4760. This was so even though only people with less than 6 years of diagnosed type 2 diabetes had been included in this study. Loss of the specialised beta cell phenotype (de-
differentiation) is a likely critical mechanism underlying the conversion to type 2 diabetes in susceptible individuals \(^{66-70}\). Markers of de-differentiation are expressed in beta cells from human type 2 diabetic pancreases \(^{71,72}\). There is not yet universal agreement that de-differentiation is the sole process underlying beta cell dysfunction, but if this state persists too long in an individual it appears that irreversible loss of endocrine function results. It is now possible to tie in the histological and cell biological processes with observations during clinical reversal of the disease process \(^{73}\). The histological observation of markedly lower beta cell numbers appears likely to relate to the insulin immunostain, so that beta cells which had stopped producing insulin were simply not identified, and incorrectly considered to be non-viable rather than non-functional.

**Differences between pre-diabetes and post-diabetes**

Although many people are returned to a state of glucose metabolism that is clearly normal, others achieve an HbA1c which is non-diabetic (<48mmol/mol), but not normal. Even so, the pattern of parallel blood pressure and lipids improvements indicates that these individuals have substantially improved their overall likelihood of long term good health. The Counterbalance study group were supervised to avoid weight regain for 6 months, and mean blood pressure and lipids improved substantially \(^{45}\). The ten year cardiovascular risk assessed by the QRISK score \(^{74}\) fell on average from 23% to 7% for those achieving remission in this closely supervised study. In this group with average chronological age of 55 years, calculated heart age fell from 71 to 56 years. As yet there are no outcome data following diet-induced weight loss in type 2 diabetes, although following bariatric surgery-induced weight loss, there was a 32% lower risk for macrovascular complications compared to the control group at 15 years \(^{75}\). These implied major health gains are dependent upon avoidance of return of weight to its previous level.

A term is now required to describe people who have achieved stable weight with normalised intra-hepatic and intra-pancreatic fat and reversed their type 2 diabetes. Approximately two thirds of the responders in DiRECT achieved normal fasting plasma glucose and HbA1c. For the one third who remained below the diabetic range but not normal it would be inappropriate to describe as having pre-diabetes, as this state is associated with cardiovascular risk (related mainly to the associated dyslipidaemia). It has
been proposed that their metabolic state would most appropriately be termed ‘post-diabetes’, retaining the implication that they remain susceptible to diabetes if weight regain occurs. Return to previous weight appears to be uniformly associated with return of diabetes by informal observation on former research participants but firm data upon this are expected from longer term follow up of DiRECT participants. People in the post-diabetes state do not have diabetes, and this is important not only for insurance purposes but also as a motivating factor to avoid weight regain. Sufficient weight regain will allow diabetes to return and hence post-diabetes ongoing support is essential. In UK Primary Care, they would appropriately be assigned Reid code C10P, indicating resolution of diabetes but need for ongoing annual checks.

Microvascular complications are highly unlikely to occur or advance in the post-diabetes state, as observed after pancreas transplantation in type 1 diabetes or after bariatric surgery-induced remission of type 2 diabetes. However, if moderately severe retinopathy is present then there is a risk of worsening to treatable maculopathy or proliferative retinopathy following the sudden fall in plasma glucose levels. Retinal imaging within 4-6 months is required for individuals with more than minimal retinopathy.

**Future questions**

Calorie restriction to obtain substantial weight loss and remission of type 2 diabetes has previously been observed with observation of 7.2kg weight loss at 2 years, raising the central question of how best to maintain the initial advantage provided by a low calorie liquid diet. Recent national dietary guidelines have emphasised the need to provide individualised advice, rather than any one dietary prescription, and high quality studies in Primary Care are required to test both the dietary advice and the nature of the behavioural support provided to maintain remission of type 2 diabetes.

Genetic traits appear likely to underlie the varying susceptibility of individuals to fat-induced beta cell de-differentiation, given that 72% of people with BMI over 40kg/m² do not have diabetes. These are likely to operate via both capacity for safe, subcutaneous storage of fat and beta cell susceptibility. All of the studies on reversing type 2 diabetes have involved
people with BMI greater than 27 kg/m², and the important question arises whether the large numbers of people of ‘normal’ BMI (approximately 10% at the time of diagnosis in the UK) can also expect to reverse type 2 diabetes. In this group it is likely that there will be a greater proportion of people who have been mis-labelled, actually having MODY or slow onset type 1 diabetes. There may be yet unrecognised diagnostic categories although the concept of heterogeneity of cause of type 2 diabetes itself appears unlikely.

The beta cell factors which prevent return to normal plasma glucose control require to be elucidated, as weight loss of >15kg does not reverse type 2 diabetes in 14% of individuals with duration up to 6 years. Weight loss of >25kg after bariatric surgery did not achieved remission of diabetes in 39% in whom the average duration of diabetes was 10 (2-19) years. In DiRECT, 14% of those losing 15kg or more did not achieve remission compared with 66% of those only achieving 5-10kg weight loss. Duration of diabetes appears to be the major determinant factor and weight loss above 20kg is not progressively associated with higher rates of remission.

Many of those who develop type 2 diabetes at younger ages (i.e. less than 30-35 years of age) are much heavier than those diagnosed much later in life. Whether such individuals need to lose more than 10-15kg to reverse their type 2 diabetes is also an important and testable question.

The studies on mechanisms of reversal of type 2 diabetes have so far involved individuals almost entirely of white European extraction and further work is now urgently needed in other ethnic groups (South Asians, Chinese, Black AfircanCaribbean), which are more susceptible to diabetes at lower average BMIs. Whether such individuals need to lose less weight than whites to reverse their diabetes is equally testable. Such work would be very appropriate given rapidly rising levels of diabetes worldwide, particularly in pockets like South Asia and other low and middle income countries where the consequent burden on individuals and the economics of healthcare are profound. Such work could be particularly helpful in areas where current access to diabetes drugs is scarce.

**Summary**

13
Type 2 diabetes is a condition of having eaten more than required over a long period. More fat than the individual’s body can safely store has accumulated, leading to excess liver and pancreatic fat and subsequent loss of plasma glucose control. Susceptibility to fat excess appears to vary considerably between individuals. In the early years after diabetes onset, removal of the excess fat in these organs via intensive but achievable weight loss allows for normalisation of hepatic glucose production and possible beta cell re-differentiation, and the condition can be reversed to normal. As physicians, we must grasp this paradigm shift in our understanding of type 2 diabetes for the benefit of our patients.

Acknowledgements

We are owe a great debt of gratitude to our research participants and to many colleagues, in particular Ee Lin Lim, Sarah Steven, Carl Peters, Sviatlana Zhyzhneuskaya, Kieren Hollingsworth, Michael Lean and the DiRECT team.

Contribution Statement

RT conceived the initial hypothesis, led the series of research studies concerned with pathophysiology of reversal of type 2 diabetes and drafted the manuscript. All authors contributed to the analysis of published data and interpretation of findings. NS also contributed earlier studies and concepts relevant to diabetes pathogenesis. All authors reviewed and revised the manuscript and have read and approved the final version.

Conflicts of interest

Dr. Taylor reports grants from Diabetes UK, grants from European Fund for the Study of Diabetes, grants from NovoNordisk Research Foundation, grants from National Institute of Health Research, Newcastle Biomedical Research Centre Grant, non-financial support from Cambridge Weight Plan, non-financial support from Nestle Ltd, during the conduct of the study; other from Wilmington Health Care, personal fees from Novartis, other from Lilly, personal fees from Janssen, outside the submitted work; and I am a member of the Low Carbohydrate Working Group, subgroup of the Scientific Advisory Committee on Nutrition but all opinions expressed are personal and not representative of the Working Group. Dr. Al-Mrabeh reports a grant from Diabetes UK to conduct the Re-TUNE study. Dr. Sattar reports grants from Diabetes UK, during the conduct of the study; personal fees from Astrazeneca, grants and personal fees from Boehringer Ingelheim, personal fees from Eli-Lilly, personal fees from Napp Pharmaceuticals, personal fees from Novo Nordisk, personal fees from Sanofi, personal fees from Amgen, outside the submitted work.
**Table 1:** Key observational and treatment-induced observations supporting the Twin (liver and pancreas) Cycle hypothesis for type 2 diabetes

<table>
<thead>
<tr>
<th></th>
<th><strong>Evidence for liver and pancreatic fat as key drivers of diabetes</strong></th>
<th><strong>Evidence for reversibility of liver and pancreatic fat and diabetes remission with intensive weight loss</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight</strong></td>
<td>Weight gain by far strongest modifiable risk factor for T2DM [33]</td>
<td>Intensive weight loss by any methods can reverse T2DM including also some people on insulin [44,50,51]</td>
</tr>
<tr>
<td><strong>Liver enzymes</strong></td>
<td>Higher ALT and GGT levels, as modest proxies for excess liver fat, are common prior to diabetes development; ALT levels also rise during short term conversion to type 2 diabetes [20]</td>
<td>ALT and GGT levels decline in parallel with weight loss and glucose reduction [44,47,52]</td>
</tr>
<tr>
<td><strong>Liver fat</strong></td>
<td>Hepatic fat levels excessive in at least 70% T2DM patients [53]</td>
<td>Fatty liver fat levels normalise rapidly with intense weight loss in diabetes [10,44,46]</td>
</tr>
<tr>
<td><strong>Liver insulin sensitivity</strong></td>
<td>Higher liver fat correlated with impaired hepatic insulin sensitivity [22,25,26]</td>
<td>Hepatic insulin sensitivity normalises rapidly with intensive weight loss via calorie restriction [44,45,47,52]</td>
</tr>
<tr>
<td><strong>Relationship between liver fat and liver insulin sensitivity</strong></td>
<td>High fatty acids levels in hepatocytes interfere with insulin-mediated suppression of hepatic glucose production [54]</td>
<td>Rapid return to normal hepatic insulin sensitivity during negative calorie balance [5,44,45]</td>
</tr>
<tr>
<td><strong>Pancreas fat</strong></td>
<td>Pancreatic fat levels elevated in beta cell dysfunction and T2DM [44,49,85]</td>
<td>Pancreatic fat levels decline gradually with intensive weight loss in diabetes only [46]</td>
</tr>
<tr>
<td><strong>Beta cell function</strong></td>
<td>First phase insulin response impaired in T2DM</td>
<td>First phase insulin response returns following substantial weight loss [44,45,47]</td>
</tr>
<tr>
<td><strong>Relationship between pancreas fat and beta cell function</strong></td>
<td>Excess fatty acids known to bring about beta cell de-differentiation and inhibit insulin release [35,36,86]</td>
<td>Evidence for beta cell recovery with weight loss and return of insulin production capacity [44,45,47]</td>
</tr>
<tr>
<td>Change in body weight</td>
<td>Weight regain post intensive weight loss linked to rapid re-emergence of excess liver fat and glucose rises $^{47}$</td>
<td>Sustained weight loss associated with sustained liver and pancreatic fat level reductions and sustained glycaemia benefits$^{45,47}$</td>
</tr>
</tbody>
</table>
Table 2: Comparative data from pathophysiological studies of type 2 diabetes reversal

Data summarised from Counterpoint \(^\text{44}\), Counterbalance \(^\text{45}\) and the pathophysiological substudy of DiRECT \(^\text{47}\).

<table>
<thead>
<tr>
<th>Description</th>
<th>Counterpoint</th>
<th>Counterbalance</th>
<th>DiRECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversal in short duration T2DM</td>
<td>6 months sustained reversal in duration up to 23 years T2DM</td>
<td>12 months sustained reversal by Primary Care staff in duration up to 6 years T2DM</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>29</td>
<td>64</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>0-4</td>
<td>0-23</td>
<td>0-6</td>
</tr>
<tr>
<td>Prior treatment</td>
<td>Diet ± metformin</td>
<td>Any oral agents except thiazolidinediones</td>
<td>Any agents except insulin</td>
</tr>
<tr>
<td>Weight loss (kg)</td>
<td>15.3±1.2 (all responders)</td>
<td>Responders – 15.8 ± 0.5</td>
<td>Responders – 16.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-responders – 13.6 ± 0.7</td>
<td>Non-responders – 13.4 ± 1.4</td>
</tr>
<tr>
<td>Timing of observations after weight loss</td>
<td>Immediately post weight loss</td>
<td>2 weeks then 6 months post-weight loss</td>
<td>2-4 weeks then 7-8 months post-weight loss</td>
</tr>
<tr>
<td>HbA1c (% and mmol/mol)</td>
<td>Baseline: 7.4±0.3% (57±3 mmol/mol) Post: 6.0±0.2 (42±2 mmol/mol)</td>
<td>Responders – Baseline: 7.1 ± 0.3% [55 ± 4 mmol/mol] Post: 5.9 ± 0.2% [41 ± 2 mmol/mol] Non-responders – Baseline: 8.4 ± 0.3% [68 ± 3 mmol/mol] Post: 7.8 ± 60.3% [62 ± 3 mmol/mol]</td>
<td>Responders – Baseline: 7.4% ± 0.2% [57 ± 2 mmol/mol] Post: 5.8% ± 0.1% [40 ± 1 mmol/mol] Non-responders – Baseline: 7.9% ± 0.2% [63 ± 2 mmol/mol] Post: 7.6% ± 0.2% 90 ± 2 mmol/mol</td>
</tr>
<tr>
<td>Number (%) achieving non-diabetic state</td>
<td>11/11 (100%)</td>
<td>13/30 (43%)</td>
<td>29/45 (64%)</td>
</tr>
</tbody>
</table>
**Figure 1: The Twin Cycle Hypothesis**

During chronic positive calorie balance, there is only one pathway to handle excess carbohydrate - *de novo* lipogenesis - which particularly promotes fat accumulation in the liver. As the process is stimulated by insulin, individuals with a degree of insulin resistance (determined by genetic or lifestyle factors) will accumulate liver fat more readily than others due to the higher plasma insulin levels. The increased liver fat in turn will cause relative resistance to insulin-suppression of hepatic glucose production. Over many years a small increase in fasting plasma glucose level will bring about increased basal insulin secretion rates to maintain euglycaemia. The consequent hyperinsulinaemia will increase further the conversion of excess calories into liver fat. A vicious cycle of hyperinsulinaemia and blunted suppression of hepatic glucose production becomes established. Fatty liver leads to increased export into the circulation of VLDL triglyceride (so excess ectopic fat in the blood) which will increase fat delivery to all tissues including the islets. This process is further stimulated by elevated plasma glucose levels. Excess fatty acid availability in the pancreatic islet would be expected to impair the acute insulin secretion in response to ingested food, and at a certain level of fatty acid exposure, post-prandial hyperglycaemia will supervene. The hyperglycaemia will further increase insulin secretion rates, with consequent increase of hepatic lipogenesis, spinning the liver cycle faster and driving on the pancreas cycle. Eventually the fatty acid and glucose inhibitory effects on the islets reach a trigger level leading to beta cell failure and a relatively sudden onset of clinical diabetes. Figure adapted from 8 and reproduced with permission from 88.

**Figure 2: MRI scans of liver colour coded for liver fat, showing regulation of liver glucose output in health and in type 2 diabetes**

*Upper panel:* In the presence of low liver fat content, insulin sensitivity of the liver is normal and the low plasma insulin concentration typical of the overnight fasting state is able to regulate liver glucose output and so keep circulating levels normal [Data from 89].

*Lower panel:* In type 2 diabetes, liver fat content is typically high 84,87, with consequent resistance to insulin action. Even though fasting plasma insulin levels are raised, these levels are unable to restrain liver glucose output adequately and circulating levels may rise into the diabetes range [Data from 90].

**Figure 3: Results of the Counterpoint study**

Sequential changes over 8 weeks after withdrawal of metformin therapy and initiation of a very low energy diet (600 kcal)/day in people with type 2 diabetes (solid line) and matched non-diabetic controls studied at a single time point: a) Fasting plasma glucose; b) hepatic
triglyceride content; c) hepatic insulin sensitivity index; d) pancreas triglyceride content; and e) first phase insulin response to a 3mmol/l rise in plasma glucose.

Figure replotted with permission 44.

**Figure 4: Results of the DiRECT mechanistic studies**

Sequential changes over 12 months after withdrawal of antidiabetic drugs and initiation of a low energy diet (825-853kcal/day) in people with type 2 diabetes: a) fasting plasma glucose; b) fasting plasma insulin; c) hepatic triglyceride content; d) VLDL1-plasma TG; e) total plasma TG; f) pancreas triglyceride content; g) first phase insulin response to intravenous glucose stimulation and h) maximal insulin secretion in response to arginine. Data are mean± SEM for panels a, b, c, d, e, and f and median with interquartile range for panels g and h. Figure replotted with permission from 47.
References


74. The QRISK*2-2016 risk calculator. 2016. [https://www.heart.org/lifetime/](https://www.heart.org/lifetime/).


Fig 1
Normal

% liver fat

Insulin
24 pmol/l

Glucose
9.6 g/hour

Type 2 diabetes

% liver fat

Insulin
67 pmol/l

Glucose
14.4 g/hour
Fig 3