The Blood Sugar Diet

Dear Colleague,

We are pleased to write that your patient is taking steps to reduce their blood sugars and lose weight by implementing a low Carb Mediterranean style diet. They are doing this either through the 8 Week Blood Sugar Diet Book, with support from the online community, or by enrolling on an online Program (www.thebloodsugardiet.com.au) which has been developed and supported by GPs and other health professionals.

Your help in providing support and monitoring is very much appreciated whilst your patient makes the necessary dietary and lifestyle changes.

The diet is based on extensive research done by Professor Roy Taylor of Newcastle University, and then developed by Dr Michael Mosley in his bestselling book *The 8-week Blood Sugar Diet*. Dr Clare Bailey, who wrote the accompanying recipe book, is a GP in the UK and has helped many patients improve their blood sugars and reverse their diabetes. She is currently involved in academic research with Oxford University into this relatively novel approach.

Professor Taylor, who has shown in a number of studies that most well motivated Type 2 Diabetics can lose significant amounts of weight and return their blood glucose levels to the normal range, is also doing a large multicentre trial in the UK (see link on information sheet below)

The success of The Blood Sugar Diet (BSD) has inspired us to create an online program to support both patients and their health practitioners. It is a step-by-step guide, based on a low carb Mediterranean style diet combined with various options for calorie restriction ranging from the 800 calories daily approach, to 5:2 intermittent fasting or simply reducing portions. We have found that when it is tailored to the patient's needs they are more likely to implement and maintain the lifestyle changes required to achieve long-term success.

Our philosophy is to educate patients about food; provide practical support via weekly shopping lists and recipes; and – critically - engage with them in an online forum where medical professionals are available to offer support.

From experience, it is often necessary to reduce or stop insulin, SGLT-2 Inhibitors ('flozins') and sulphonylureas as well as anti-hypertensive medication early on. To provide pointers for medical professionals we have attached a summary below to help you support your patient.

Your help with arranging standard blood testing, such as monitoring HbA1c and the patient's home blood sugars, is very much appreciated (see more information in the summary below). In some areas, we are also able to arrange more detailed tests such as DEXA scans, which can be very useful in identifying the extent of unhealthy visceral fat. It is also highly motivating to be able to compare the pre and post diet scans.

In addition to developing a nurse run course for patients, we are planning to run training courses for medical professionals who wish to learn more about this approach so they can better support their patients to lose weight and improve their blood sugars. A professional's forum will also be available soon to answer queries either about the program or regarding changes to medication that can be expected over the course of the diet, as well as discussing amongst the member's, successes and issues that may arise. We hope you will find this helpful.

All the best,

Dr Clare Bailey, GP Buckinghamshire, UK Dr Patrick Garratt, GP Perth, Western Australia

Supporting Patients to improve their blood sugars

All options are based on a moderately low carb Mediterranean style diet. Options to choose a more intensive 800cal 'fasting' approach to intermittent fasting or simply portion control.

The BSD FAST 800: Fast, intensive and effective. Involves eating just over 800 calories a day. Requires motivation and commitment. This is the 'treatment phase'.

The 5:2 BSD with INTERMITTENT FASTING: More flexible, less intensive. Cut down to 800 calories, on some days, also known as 'fasting'. Usually means 5 days eating a Mediterranean style diet with some portion control and 2 days 'fasting' on about 800 calories. Not suitable for those on certain medications such as insulin, gliclazide or warfarin.

The BSD MED STYLE WAY OF LIFE: Slower & gentler. No fasting, just portion control, suitable for most people including; those who don't need to lose weight, are less motivated, the elderly, and with medical supervision.

MAINTENANCE: Once target is reached, continue to base food on the Mediterranean style diet. Many can relax a bit, no longer counting, just watching portions. Some prefer to continue intermittent fasting, perhaps doing a 6:1 version (800 cals 1 day/week) to maintain the benefits. Continue to avoid snacking if possible! However if you return to previous habits the diabetes is likely to return.

TIPS: On a low calorie day increase water intake by 1-1.5 litres (to about 2.5L depending on activity and circumstances), plan ahead, tell other people and try to avoid snacking (if you must, a small portion of nuts is best). As with all diets, we recommend doing it with the support of a health professional.

Considerations:

1. **Consider a different variant of diabetes or type 1;** If the patient is atypical or not responding as expected.
2. **May involve significant restriction of food intake (800 calories);** for up to 8-12w.
3. **Managing diabetic medication:** Aim to reduce medication that could cause hypos first. Otherwise on a last in, first out basis. Reduce evening hypoglycaemic medication first. Reassure that there may be a temporary increase in blood sugar, but if they stick to the diet it will continue to improve.

   Insulin; If making a significant change to a low carb diet & particularly if reducing to 800 calories, reduce insulin by half if on >20 Units (do this the previous night for long acting insulin) Advise re risk of hypos and management. Continue to reduce by half again, depending on fasting blood sugars (Can usually reduce or discontinue by 2 weeks if fasting blood sugars are around 8 or below). If insulin <20 Units stop it altogether. Ask patient to check FBS regularly during the day (about 4 times a day initially). Aim to run a bit high for a few weeks. Review at 1w or sooner as required.

   Sulphonylureas; Stop or reduce by half on commencement of the BSD Fast 800 diet.

   All other oral hypoglycaemic agents; Can be decreased or stopped according to degree of control achieved. Advise re hypo risk and management.

   SGLT-2 Inhibitors ('flozins'); usually stop (risk of Euglycaemic DKA)

   Antihypertensives: Unless poor control or on 2 or more medications, this can be halved or stopped on commencement of the BSD. BP likely to reduce within days as insulin resistance improves, so advise patient to watch out for feeling light headed and/or check BP at home – may require further reduction or discontinuation.

   Agree a plan for the patient to contact appropriate healthcare professional if blood glucose levels become very high (fasting >14mmol per litre) or they are getting hypos. Or if the BP is too high or too low. *More details in table on final page.*

4. **Tests - baseline bloods**;

   HbA1C; although advised to do only 3monthly, significant improvements usually seen within 6 wks.

Fasting glucose; may return to normal within a few weeks.
Lipid profile; usually improves alongside reduced blood sugars, despite increase in fat intake.
ALT/GGT; Improves as liver recovers.
Hb & Iron status; should be assessed prior to starting, especially for the elderly or vegetarians.
U&Es; TFTs;
Measurements: BP, weight, height, BMI, waist circumference (via umbilicus)

5. **Goal:** Depending on starting weight. Aim to lose 10-15% of body weight. If original BMI > 40, goal may need to be 15-20%. South Asians may need to aim for BMI closer to 22 or 23.

6. **Encourage patient to choose which approach to follow**. Check lifestyle, individual suitability, motivation & clinical needs. Consider the 5:2 BSD or the easier Mediterranean style way of life. Can move from one approach to another.

7. **Extra retinal screening required if moderate or more severe retinopathy** is present. Re-screen within six months of achieving a substantial improvement in blood glucose. Sudden normalisation in retinal blood flow can disadvantage damaged areas of the retina, resulting in deterioration in retinopathy.

8. **Side effects;** Commonest are probably headache, constipation and tiredness, usually due to dehydration. Normally settles with extra water (1-1.5L). Sometimes helped by a little extra salt in the diet. Consider vitamin supplementation on 800 calorie days.

9. **Although a low calorie Mediterranean style diet is suitable for most people, AVOID** reduced calorie diet if the patient is**;**
    - Underweight and/or has a history of an eating disorder
    - Under 18 years of age
    - Breastfeeding or pregnant (can do Mediterranean style diet with monitoring)
    - Diagnosed with a significant psychiatric disorder or substance abuse
    - Frail or recovering from surgery, uncontrolled BP, cardiac arrhythmia or other abnormalities.
    - Under active investigation or treatment or has a significant medical condition affecting ability to comply with diet, a history of intermittent porphyria
    - Unwell, has a fever, renal failure (stage 4 or 5), recent cardiac event, stroke or heart failure.
    - Some medications such as Warfarin and Lithium need adjusting and are not suitable for intermittent fasting due to dose fluctuations.
    - Careful monitoring for patients with history of seizure is also recommended.

10. **Review;** Review adherence, hypos, side effects, blood sugars, medication, BP, weight & waist at 2weeks, then monthly for 2-3m, then as required. Monitor HbA1C. Maintain routine diabetic reviews, even if blood sugar returns to normal.

11. **Resources;** Professional support at https://thebloodsugardiet.com/information-for-professionals/
Information of Prof Roy Taylor's research:
http://www.ncl.ac.uk/magres/research/diabetes/reversal/#publicinformation
Patient advice, useful resources, recipes and online community www.thebloodsugardiet.com.au and www.thebloodsugardiet.com . See **The 8 Week Blood Sugar Diet Recipe Book, by Dr Clare Bailey** for program and recipes. **The 8 Week Blood Sugar diet, by Michael Mosley** for scientific studies, stories and more information.

Type 2 Diabetes: Diabetic Medications on a Low Carbohydrate Diet - Summary & Suggestions

There are three main considerations with the use of diabetic medications in type 2 diabetes when on a low carbohydrate diet:

1. Is there a risk of hypoglycaemia?
2. What is the degree of carbohydrate restriction?
3. Does the medication provide benefit, and if so, do any potential side effects outweigh the benefit.

We include some general information about this here and on the website, but it is not a substitute for proper, individual medical advice.

Drug Group	Action	Hypo risk?	Suggested action (to continue/stop)
Sulfonylureas (e.g. Gliclazide)	Increase pancreatic insulin secretion	**YES**	**STOP** (or if gradual carbohydrate restriction then wean by e.g. halving dose successively)
Insulins*	Exogenous insulin	**YES**	**REDUCE/STOP** (Convert to all basal and wean appropriately, e.g. successive 30-50% reductions, towards elimination) *see below
Meglitinides (e.g. Repaglinide)	Increase pancreatic insulin secretion	**YES**	**STOP** (or if gradual carbohydrate restriction then wean by e.g. halving dose successively)
SGLT-2 inhibitors (e.g. Empagliflozin)	Increase renal glucose secretion	No	**STOP**. Risk of Euglycaemic DKA with normal/near normal sugars (especially if LADA that has been misdiagnosed as T2DM).
GLP-1 agonists (e.g. Liraglutide)	Slow gastric emptying. Glucose dependent pancreatic insulin secretion.	No	Optional, consider clinical pros/cons (expensive).
Biguanides (e.g. Metformin)	Reduces insulin resistance	No	Optional, consider clinical pros/cons.
Thiazolidinediones (e.g. Pioglitazone)	Reduce peripheral insulin resistance	No	Usually stop. Concern over risks usually outweigh benefits.
DPP-4 inhibitors (e.g. Sitagliptin)	Inhibit DPP-4 enzyme	No	Stop. No significant risk, but no benefit in most cases.
Alpha-glucosidase inhibitors (e.g. Acarbose)	Delay digestion of starch and sucrose	No	Stop. No benefit on a low carbohydrate diet.
Blood glucose testing strips	Provide feedback on blood glucose response to food	N/A	A period of measuring blood glucose helpful for informing them about the effect of various foods on blood glucose. Measurement may also be useful if HbA1c is not improving as expected.

*Insulin reduction suggestion -Tailor to individual. Usually requires close supervision with healthcare professional, and if in doubt seek expert input.

T2DM without 'beta cell failure': If using basal-bolus regime convert to long-acting insulin only, BD in equal doses (OD may suit some people). On commencing low carb diet reduce total insulin by 30-50%. Monitor QDS initially for hypoglycaemia (rescue glucose if required). Continue down-titration of insulin as insulin resistance improves (can take months).

***Caution:* Some T2DM may have significant 'beta cell failure'**; *or other forms of pancreatic insufficiency (e.g. LADA or T3c) misdiagnosed as T2DM. Consider this if rapidly increasing HbA1c, thirst, polydipsia, weight loss, low C-peptide. Insulin should not be eliminated in this cohort, although basal and bolus dose adjustment needed for carbohydrate restriction.*

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INFORMATION FOR DOCTORS

1. Diagnosis

The possibility of reversing type 2 diabetes relates specifically to this common form of diabetes. It is important to identify rare forms of diabetes, as they will not respond in the same way.

a) Pancreatic Diabetes. Most commonly caused by chronic pancreatitis and rarely by haemochromatosis. The associated clinical features are likely to make this diagnosis evident.
b) Monogenic diabetes. Onset of diabetes in teens or early adult life, usually but not exclusively in slim individuals and with a very strong family history of diabetes. Although if individuals are overweight, blood glucose control may be improved by weight loss, beta cell function will not normalise as the specific genetic change cannot be reversed.
c) Slow Onset type 1 diabetes. Typically individuals present with high blood glucose levels but appear to respond to diet. Despite adequate diet blood glucose levels rise relatively rapidly and insulin therapy is required within a few years. The presence of ketones+++ in the urine associated with hyperglycaemia may be a clue to diagnosis, but any recent hypocaloric dieting would also produce urinary ketones which merely reflect the healthy physiological mechanism.

2. Significant Restriction of Food Intake

Most individuals will be able to reduce food intake substantially with no short or medium term risks to health. However iron status should be assessed and vitamin supplementation considered when prolonged hypocaloric dieting is undertaken.

3. Medication

a) Sulphonylureas. These agents can be withdrawn with benefit as soon as hypocaloric dieting is commenced in order to ensure that hypoglycaemia cannot occur.
b) Insulin. At the time of commencement of decreasing food intake, insulin dose in type 2 diabetes may be substantially decreased, and advice to cut insulin dose by approximately 50% is appropriate. Monitoring of blood glucose must be done daily with a plan to contact appropriate healthcare professional if blood glucose levels become very high (fasting over 10mmol per litre) or very low. It may be anticipated that insulin may be withdrawn after approximately two weeks but this depends upon blood glucose response.
c) Other Medication. All other oral hypoglycaemic agents can be decreased or stopped in accordance with degree of control achieved.

4. Importance of setting a weight target

Everyone must know exactly what is to be achieved and a sustained effect is then essential. It is most important that body weight is decreased to target and then maintained steady. An individual target can be agreed on the basis of body weight and is usually 15kg lower than starting weight. Some individuals will have type 2 diabetes with a BMI only just above the normal range, and for them a low normal BMI is an appropriate target. For others, a BMI substantially less than that present, can be agreed with the patient as a target. Both motivation for the individual patient and support from both family and the diabetes care team will be important.

5. Diabetes complications

It is most important to consider the individual's microvascular complications before embarking upon major dietary change. If there is no retinopathy, or only early changes (scattered micro aneurysms with few blot haemorrhages) then no additional precaution is required other than an annual screening. However, if moderate or more severe retinopathy is present then arrangements should be made to re-screen the eyes within six months of achieving a substantial improvement in blood glucose control. The reason for this is that the sudden normalisation (reduction) in retinal blood flow associated with the return of normal blood glucose control can disadvantage areas of the retina in areas of marginal circulation with resulting deterioration in retinopathy. This effect is entirely restricted to individuals with pre-existing moderate or worse retinopathy. (Arun CS, Pandit R, Taylor R. *Diabetologia* 2004; 47:1380-84. PMID: 15309288).

For those individuals who achieve reversal of their type 2 diabetes, retinal screening should be continued for two years if there is no pre-existing retinopathy. If retinopathy is present, it should be continued until all changes remit.

All macrovascular complications will be improved by the dietary changes. It should be noted that blood pressure control will be substantially improved, with the possibility of decreasing number or dose of anti-hypertensive agents.

All of this general information upon diabetes and its management has to be interpreted in the light of the circumstances of each individual patient.

Professor Roy Taylor

Diabetologia (2011) 54:2506–2514
DOI 10.1007/s00125-011-2204-7

ARTICLE

Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol

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Received: 22 March 2011 / Accepted: 5 May 2011 / Published online: 9 June 2011

Abstract
Aims/hypothesis Type 2 diabetes is regarded as inevitably progressive, with irreversible beta cell failure. The hypothesis was tested that both beta cell failure and insulin resistance can be reversed by dietary restriction of energy intake.
Methods Eleven people with type 2 diabetes (49.5±2.5 years, BMI 33.6±1.2 kg/m^2, nine male and two female) were studied before and after 1, 4 and 8 weeks of a 2.5 MJ (600 kcal)/day diet. Basal hepatic glucose output, hepatic and peripheral insulin sensitivity and beta cell function were measured. Pancreas and liver triacylglycerol content was measured using three-point Dixon magnetic resonance imaging. An age-, sex- and weight-matched group of eight non-diabetic participants was studied.
Results After 1 week of restricted energy intake, fasting plasma glucose normalised in the diabetic group (from 9.2±0.4 to 5.9±0.4 mmol/l; p=0.003). Insulin suppression of hepatic glucose output improved from 43±4% to 74±5% (p=0.003 vs baseline; controls 68±5%). Hepatic triacylglycerol content fell from 12.8±2.4% in the diabetic group to 2.9±0.2% by week 8 (p=0.003). The first-phase insulin response increased during the study period (0.19±0.02 to 0.46±0.07 nmol min^{-1} m^{-2}; p<0.001) and approached control values (0.62±0.15 nmol min^{-1} m^{-2}; p=0.42). Maximal insulin response became supranormal at 8 weeks (1.37±0.27 vs controls 1.15±0.18 nmol min^{-1} m^{-2}). Pancreatic triacylglycerol decreased from 8.0±1.6% to 6.2±1.1% (p=0.03).
Conclusions/interpretation Normalisation of both beta cell function and hepatic insulin sensitivity in type 2 diabetes was achieved by dietary energy restriction alone. This was associated with decreased pancreatic and liver triacylglycerol stores. The abnormalities underlying type 2 diabetes are reversible by reducing dietary energy intake.

Keywords Insulin secretion · Liver fat · Low energy diet · Pancreatic fat · Type 2 diabetes

Abbreviation
ffm Fat-free mass

Introduction

Type 2 diabetes has long been regarded as a chronic progressive condition, capable of amelioration but not cure. A steady rise in plasma glucose occurs irrespective of the degree of control or type of treatment [1]. Beta cell function declines linearly with time, and after 10 years more than 50% of individuals require insulin therapy [2]. The underlying changes in beta cell function have been well described [3, 4], and beta-cell mass decreases steadily during the course of type 2 diabetes [5, 6]. Overall, there is strong evidence that type 2 diabetes is inexorably progressive, with a high likelihood of insulin therapy being eventually required to maintain good glycaemic control.

However, type 2 diabetes is clearly reversible following bariatric surgery [7]. The normalisation of plasma glucose

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concentration follows within days of surgery, long before major weight loss has occurred, and it has become widely assumed that the protective effects of gastrointestinal surgery are mediated by altered secretion of incretin hormones [8, 9]. Improved control of blood glucose in type 2 diabetes by moderate energy restriction has been demonstrated by others [10]. We have hypothesised that the profound effect of a sudden negative energy balance on the metabolism could explain the post-bariatric surgery effect [11] and, specifically, that the decrease in the intracellular fatty acid concentrations in the liver would lead to a lower export of lipoprotein triacylglycerol to the pancreas, with the release of beta cells from the chronic inhibitory effects of excess fatty acid exposure.

This study was designed to test the hypothesis that acute negative energy balance alone reverses type 2 diabetes by normalising both beta cell function and insulin sensitivity. We examined the restoration of first-phase and total insulin response as well as hepatic and peripheral insulin sensitivity. Additionally, to examine the mechanistic basis of observed outcomes, we quantified the change in fat content of the pancreas and liver.

Methods

Participants Individuals with type 2 diabetes (age 35–65 years, HbA_{1c} 6.5–9.0% [48–75 mmol/mol], diabetes duration <4 years, stable BMI 25–45 kg/m^2) were recruited. Participants were excluded if being treated with thiazolidinediones, insulin, steroids or beta-blockers, with a serum creatinine >150 mmol/l, with a serum alanine transaminase level >2.5-fold above the upper limit of the reference range, or if there were contraindications for MRI. Statin therapy was continued. The study protocol was approved by the Newcastle upon Tyne Ethics Committee No. 2, and all participants gave their informed consent. Sulfonylurea (two individuals) was discontinued 2 months, and metformin (seven individuals) 1 week, before the baseline study. Dietary adherence was assessed using capillary ketone levels (Xceed Optium; Abbott Diabetes Care, Maidenhead, UK). Three individuals failed to comply with the diet (two during the first week and one during weeks 4–8), and one left the study for an unrelated medical reason. Hence 11 individuals (nine male and two female, age 49.5±2.5 years) completed the study.

Nine control participants matched for weight, age and sex were also studied (seven male, two female, age 49.7±2.5 years; Table 1). These participants had no family history of diabetes, were taking no medication and had normal glucose metabolism as confirmed by a standard 75 g OGTT.

Experimental protocol Participants were asked to continue their habitual pattern of eating until the start of the study. Assessments of beta cell function, insulin sensitivity, liver and pancreatic fat content and total body fat were carried out at baseline immediately prior to dietary intervention (day −1), and after 1, 4 and 8 weeks of the very-low-energy diet. A group of matched non-diabetic controls were studied on one occasion only, without dietary intervention.

After the baseline measurements, individuals with type 2 diabetes started the diet, which consisted of a liquid diet formula (46.4% carbohydrate, 32.5% protein and 20.1% fat; vitamins, minerals and trace elements; 2.1 MJ/day [510 kcal/day]; Optifast; Nestlé Nutrition, Croydon, UK). This was supplemented with three portions of non-starchy vegetables such that total energy intake was about 2.5 MJ (600 kcal)/day. Participants were provided with suggestions of vegetable recipes to enhance compliance by varying daily eating. They were also encouraged to drink at least 2 l of water or other energy-free beverages each day, and asked to maintain their habitual level of physical activity. Ongoing support and encouragement was provided by means of regular telephone contact. At the end of the 8 week intervention participants returned to normal eating but were provided with information about portion size and healthy eating.

The striking results seen at 8 weeks demanded experimental follow-up, and additional ethics permission was obtained to repeat the MRI studies and carry out OGTTs 12 weeks after completing the dietary intervention.

Hepatic glucose production and insulin sensitivity After an overnight fast, cannulae were inserted into an antecubital vein for infusion and the contralateral wrist vein for arterialised

Table 1 Anthropometric data before and during the 8 weeks of dietary intervention in comparison with control individuals

Variable	Controls	Baseline	Week 1	Week 4	Week 8
Weight (kg)	101.5±3.4	103.7±4.5	99.7±4.5*	94.1±4.3*	88.4±4.3*†
BMI (kg/m^2)	33.4±0.9	33.6±1.2	32.3±1.2*	30.5±1.2*	28.7±1.3*†
Fat mass (kg)	36.2±2.7	39.0±3.5	36.6±3.6*	31.7±3.7*	26.3±4.0*
ffm (kg)	64.7±3.8	64.7±3.0	63.2±3.1	62.4±3.0*	62.1±3.0*
Waist circumference (cm)	105.0±1.5	107.4±2.2	104.4±2.2*	99.7±2.4*	94.2±2.5*†
Hip circumference (cm)	109.8±2.4	109.5±2.9	108.3±2.7*	105.0±2.6*	99.5±2.6*†
WHR	0.96±0.02	0.98±0.02	0.97±0.02	0.95±0.01	0.95±0.01

Data are mean ± SE

†$p<0.05$ individuals with type 2 diabetes vs controls; *$p<0.05$ type 2 diabetes baseline vs later

blood sampling. [6′6′-^{2}H]glucose (98% enriched; Cambridge Isotope Laboratories, Andover, MA, USA) was used to determine hepatic glucose production [12, 13], and basal rates were calculated during the last 30 min of the 150 min basal period. Preinfusion enrichment of isotope was insignificant throughout. An isoglycaemic–hyperinsulinaemic clamp (insulin infusion rate 40 mU m^{-2} min^{-1}) was initiated at 0 min [14]. Isoglycaemia was used to ensure that the true fasting condition of each participant could be observed at each study time point. Each participant was clamped at the glucose level observed at the end of the basal period. Whole-body insulin sensitivity was determined during the last 30 min of the hyperinsulinaemic glucose clamp as whole-body glucose disposal corrected for glucose space and urinary loss [14, 15]. Glucose metabolic clearance rates during steady-state conditions were calculated by dividing whole-body insulin sensitivity by steady-state plasma glucose.

Assessment of beta cell function Sixty minutes after the clamp test, two consecutive 30 min square-wave steps of hyperglycaemia (2.8 and 5.6 mmol/l above baseline) were achieved by a priming glucose dose followed by variable 20% glucose infusion [16]. Blood samples for determination of plasma glucose, insulin and C-peptide concentrations were obtained every 2 min for the first 10 min and every 5 min for the other 20 min of each step. An arginine bolus was administered during the second step of hyperglycaemia, followed by sampling every 2 min for 10 min. Insulin secretion rate was calculated using a computerised program implementing a regularisation method of deconvolution [17] and using a population model of C-peptide kinetics [18].

Magnetic resonance measurements A Philips 3.0 T Achieva scanner and six-channel cardiac coil (Philips Healthcare, Best, the Netherlands) were used to acquire three gradient-echo scans with adjacent out-of-phase and in-phase echoes (time to repetition/time to echo/averages/flip angle=50 ms/3.45, 4.60, 5.75 ms/1/30°, matrix 160×109, field of view 400–480 mm to suit participant size with 70% phase field of view). Six slices were acquired within a 17 s breath-hold to cover the liver with slice thickness 10 mm and the pancreas with slice thickness 5 mm. The data were analysed in MATLAB (MathWorks, Cambridge, UK) to produce separate fat and water images [19]. The fat content of the image was expressed as a percentage of the total signal per voxel. The intraorgan fat percentage was evaluated from five liver regions of interest and two pancreatic regions of interest, defined and averaged in a blinded fashion by one observer (K. G. Hollingsworth). The pancreatic fat data represent solely intrapancreatic fat, and the liver data avoid contamination from blood vessels and gallbladder. This was achievable because of the data processing after image acquisition, allowing manual selection of wholly intraorgan volume from the anatomical slice. The interscan Bland–Altman repeatability coefficients were 0.5% for the liver and 0.9% for the pancreas.

Body composition and anthropometry Percentage body fat was measured following an overnight fast using air-displacement plethysmography (BOD POD Express; Life Measurement, Concord, CA, USA). Waist and hip circumferences were measured with the participants in a relaxed standing posture. Waist circumference was taken at the mid-point between the anterior superior iliac spine and the lower edge of the rib cage, and hip circumference at the level of the greater trochanter. All measurements were made throughout the study period by a single observer (E. L. Lim).

Analytical procedures Plasma glucose concentration was measured by the glucose oxidase method (YSI glucose analyser; Yellow Springs, OH, USA), plasma insulin and C-peptide concentrations by ELISA (Dako, Ely, UK), plasma triacylglycerol by lipase with released glycerol measured by a Roche Cobas centrifugal analyser using a colorimetric assay (ABX Diagnostics, Montpellier, France) and HbA_{1c} by a Biorad HPLC (TOSOH Corporation, Tokyo, Japan). ^{2}H atom percent excess in plasma glucose was determined using a Thermo ‘Voyager’ single quadrupole mass spectrometer with Thermo ‘Trace’ gas chromatograph (Thermo Scientific, Waltham, MA, USA).

Statistical methods Statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SE. Statistical comparisons between diabetes and control groups were performed using the Student’s *t* test, while within-group differences were determined using a paired *t* test. Changes of sequential data within experiments were evaluated by repeated measures one-way ANOVA with post hoc Bonferroni testing where appropriate. Correlations were examined using the Spearman rank test. Statistical significance was accepted at $p<0.05$.

Results

Plasma glucose and insulin After 1 week of dietary intervention, fasting plasma glucose decreased from 9.2±0.4 to 5.9±0.4 mmol/l ($p=0.003$; Fig. 1) and was not significantly different from that of the non-diabetic control group (5.3±0.1 mmol/l; $p=0.18$). It remained stable for the rest of the 8 week study (5.7±0.5 mmol/l at weeks 4 and 8; $p=0.52$ compared with control). HbA_{1c} decreased from

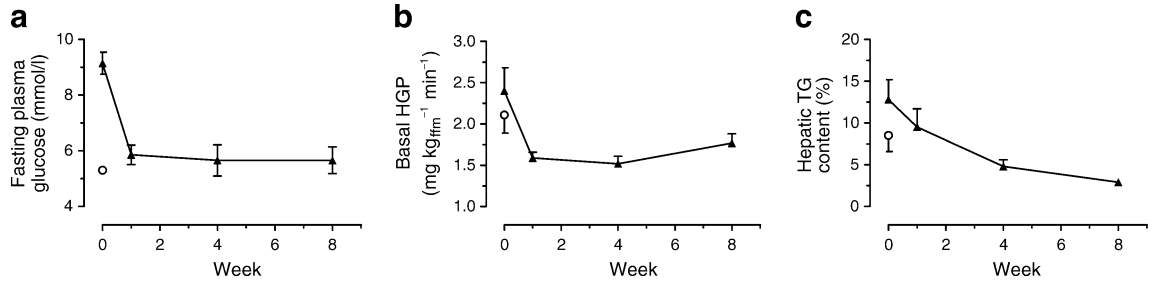

Fig. 1 Effect of 8 weeks of dietary intervention on (**a**) plasma glucose, (**b**) hepatic glucose production (HGP) and (**c**) hepatic triacylglycerol content (TG) for diabetic participants (black triangles). White circles indicate the mean for the weight-matched non-diabetic control group. Data are shown as mean ± SE

7.4±0.3% (57±3 mmol/mol) and at 8 weeks was not significantly different from non-diabetic control values (6.0±0.2 vs 5.7±0.1% [42±2 vs 39±1 mmol/mol], p=0.27) Fasting plasma insulin fell from 151±31 to 73±10 pmol/l after 1 week (p=0.03) and to 65±15 pmol/l by 8 weeks (p=0.03 vs baseline; p=0.04 vs control). Fasting plasma C-peptide decreased similarly (Table 2).

During the isoglycaemic clamp, plasma glucose was clamped at 4.6±0.1 mmol/l in the control group compared with 7.0±0.3 mmol/l in the diabetes group at baseline (p<0.001). At week 1, the achieved clamped plasma glucose level was 4.8±0.2 mmol/l (p<0.001 vs baseline) in the diabetes individuals and this was similar at weeks 4 and 8 (4.8±0.3 and 4.8±0.4, respectively, p< 0.001 vs baseline).

Hepatic insulin sensitivity and hepatic triacylglycerol content Basal hepatic glucose production decreased significantly during the first week of energy restriction (2.40±0.28 to 1.59±0.07 mg kg$_{ffm}^{-1}$ min^{-1}; p=0.05), remained decreased compared with baseline for the rest of the study, and at 8 weeks was not significantly different from that of the control group (1.71±0.11 vs 2.11±0.22 mg kg$_{ffm}^{-1}$ min^{-1} respectively; p=0.60; Fig. 1). At baseline, hepatic insulin sensitivity, assessed by the suppression of hepatic glucose production by insulin infusion, was 43±4% in the diabetic group compared with 68±5% in the control group (p=0.001). During the first week of energy restriction, there was a marked improvement in hepatic insulin responsiveness, with insulin suppression of hepatic glucose production increasing to 74±5% (p=0.003 vs baseline).

Hepatic triacylglycerol content decreased by 30±5% during week 1 of intervention (p<0.001), becoming similar to control values (p=0.75). It continued to decline throughout the intervention period to reach the normal range for non-obese individuals [20] (2.9±0.2%; p=0.003; Fig. 1), i.e. a total reduction of 70±5%. At baseline, hepatic triacylglycerol content was 8.5±1.9% in the control group compared with 12.8±2.4% in the diabetic group (p=0.14). Hepatic triacylglycerol at baseline correlated with BMI in

Table 2 Metabolic response to 8 weeks of dietary intervention in participants with type 2 diabetes in comparison with controls

Fasting concentration	Controls	Baseline	Week 1	Week 4	Week 8
HbA_{1c} (%)	5.7±0.1	7.4±0.3††	7.1±0.3*††	6.5±0.2**†	6.0±0.2**
HbA_{1c} (mmol/mol)	39±1	57±3††	55±3*†	47±3**†	42±2**
Plasma glucose (mmol/l)	5.3±0.1	9.2±0.4††	5.9±0.4**	5.7±0.6**	5.7±0.5**
Plasma insulin (pmol/l)	115±27	151±31	73±10*	57±11*	65±15*†
Plasma C-peptide (nmol/l)	1.06±0.12	1.21±0.20	1.14±0.16	1.19±0.19	0.86±0.11
Triacylglycerol (mmol/l)	1.8±0.1	2.4±0.5	1.2±0.1*†	1.0±0.1*††	1.3±0.3*†
NEFA (mmol/l)	0.57±0.07	0.69±0.06	0.93±0.05*††	0.81±0.08†	0.72±0.06
Cholesterol (mmol/l)	5.1±0.3	4.0±0.3†	3.3±0.3*††	2.8±0.2*††	3.2±0.3††
LDL-cholesterol (mmol/l)	3.2±0.3	1.7±0.2††	1.8±0.4††	1.0±0.2*††	1.3±0.2††
HDL-cholesterol (mmol/l)	1.1±0.1	1.1±0.1	1.0±0.1	1.1±0.1	1.1±0.1
ALT (U/l)	33±3	46±7	61±10*†	44±3†	33±3
Gamma GT (U/l)	39±5	62±12	49±8	25±4†	26±5**

Data are mean ± SE

$^{\dagger}p$<0.05 vs controls; $^{\dagger\dagger}p$<0.005 vs controls; $^{*}p$<0.05 vs type 2 diabetes baseline; $^{**}p$<0.005 vs type 2 diabetes baseline

ALT, alanine transaminase; gamma GT, γ-glutamyltransferase

the controls (Rs=0.71; p<0.05) but not in the diabetic group (Rs=−0.50; p=0.12).

Beta cell sensitivity to glucose and pancreas triacylglycerol content Fasting insulin secretion rate decreased from 0.10±0.01 to 0.06±0.01 nmol min^{-1} m^{-2} during the first week (p<0.05) and remained constant thereafter. During the insulin secretion test, the planned step increases in plasma glucose concentration of +2.8 and +5.6 mmol/l were achieved (Fig. 2). In the diabetes individuals, peak insulin secretion rate at 6 min was minimal at baseline (0.19±0.02 vs control 0.62±0.15 nmol min^{-1} m^{-2}; p<0.001; Fig. 2). The first-phase insulin response steadily increased and was significantly different from baseline by 8 weeks (0.29±0.05, 0.34±0.06 and 0.46±0.07 nmol min^{-1} m^{-2} at 1, 4 and 8 weeks; p=0.20, p=0.09, p=0.006, respectively). At 8 weeks in the individuals with type 2 diabetes, the insulin secretion rate was not significantly different from control (0.46±0.07 vs 0.62±0.15 nmol min^{-1} m^{-2}; p=0.42). There was an increase in arginine-induced insulin response after 1 week (from 0.72±0.11 to 0.95±0.19 nmol min^{-1} m^{-2}; p<0.006), and by 8 weeks it was completely normalised (1.37±0.27 vs 1.15±0.18 nmol min^{-1} m^{-2}; p=0.77 vs control; p<0.03 vs baseline; Fig. 2). It was 38% lower in the participants with type 2 diabetes at baseline compared with the controls (0.72±0.11 vs 1.15±0.18 nmol min^{-1} m^{-2}; p=0.04).

Pancreatic triacylglycerol content in the diabetic group was 8.0±1.6% and fell steadily to 6.2±1.1% after 8 weeks (p=0.03; Fig. 3). In control individuals, pancreatic triacylglycerol content was 6.0±1.3% (p=0.17 compared with participants with type 2 diabetes at baseline). There was no correlation with BMI in either the control or the diabetic group (Rs=0.31, p=0.36; Rs=0.01, p=0.98, respectively).

Peripheral insulin sensitivity There was no significant change in peripheral insulin sensitivity expressed as glucose disposal rates during the entire study. Insulin-stimulated glucose

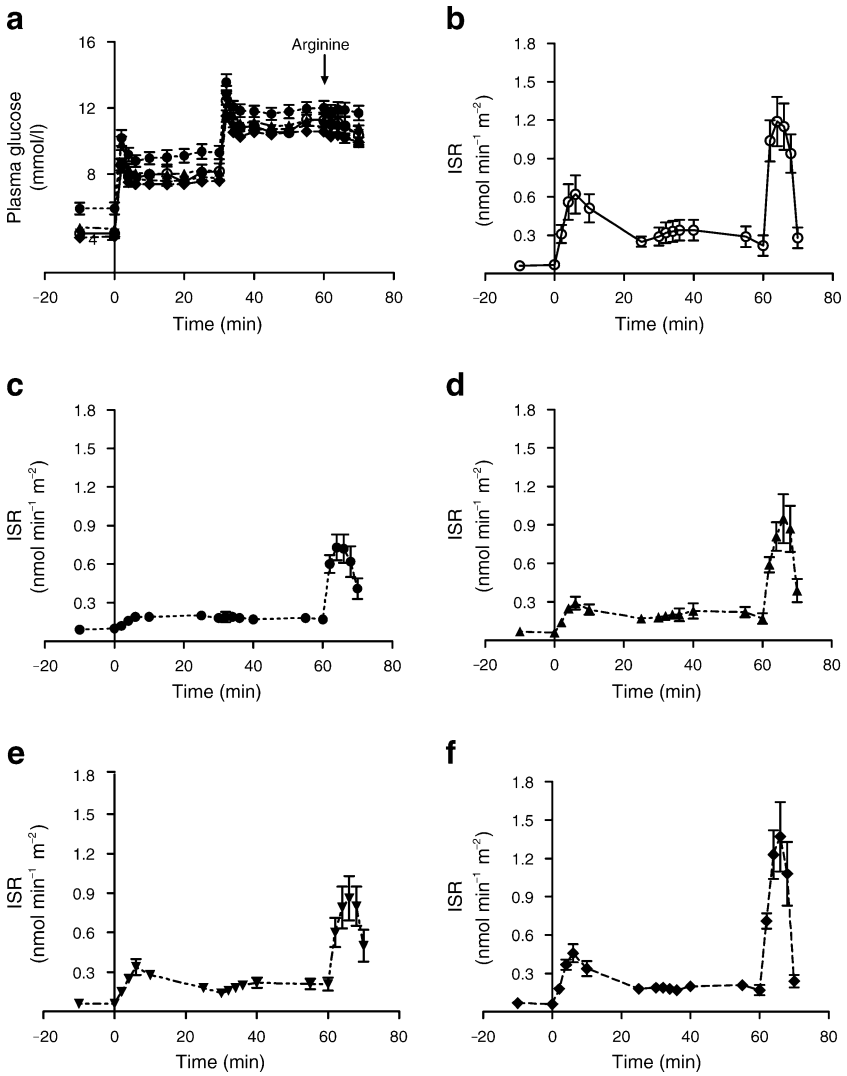

Fig. 2 Insulin secretion test data in controls and in diabetic participants at each time point. **a** Plasma glucose levels achieved in each group. Insulin section rate (ISR) obtained in (**b**) the non-diabetic control group, (**c**) the diabetic group at baseline, (**d**) the diabetic group at 1 week of the diet, (**e**) the diabetic group at 4 weeks and (**f**) the diabetic group at 8 weeks. Data are shown as mean ± SE

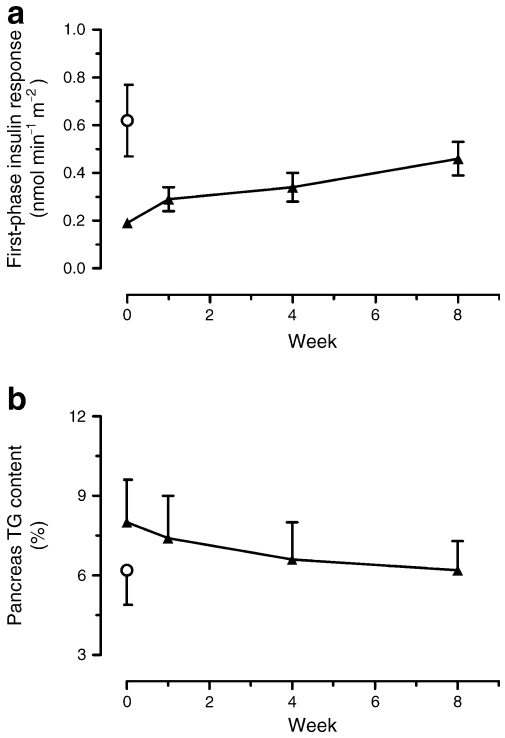

Fig. 3 **a** Change in first-phase insulin response, and (**b**) change in pancreas triacylglycerol (TG) content during the 8 week dietary intervention in diabetic individuals (black triangles). White circles indicate the mean for the weight-matched non-diabetic control group. Data are shown as mean ± SE

disposal was 3.83±0.23 and 4.36±0.36 mg $kg_{ffm}^{-1}min^{-1}$ (where ffm is fat-free mass) at baseline and 8 weeks, respectively (p=0.21). Change in glucose metabolic clearance rate was examined to correct for the difference in clamp glucose levels between study days. Fasting plasma glucose decreased between baseline and week 1. There was no significant effect of the dietary intervention on glucose metabolic clearance rate at either 1 or 4 weeks (3.1±0.3 vs 4.23±0.34 and 4.21±0.36 ml $kg_{ffm}^{-1}min^{-1}$, respectively), but improvement was demonstrable by week 8 (5.2± 0.5 ml $kg_{ffm}^{-1}min^{-1}$; p=0.003 for baseline vs 8 weeks; control group 5.2±0.4 ml $kg_{ffm}^{-1}min^{-1}$; p=0.98).

Weight and body composition Average weight loss during the 8 weeks of dietary intervention was 15.3±1.2 kg, equivalent to 15±1% of initial body weight (Table 1). Weight loss was 3.9±0.2 kg during the first week (61% of which was fat loss), 5.7±0.6 kg (86% as fat) between weeks 1 and 4, and 5.7±0.7 kg (94% as fat) during the final 4 weeks. Both waist and hip circumference decreased to the same extent and WHR remained unchanged during the 8 weeks (Table 1).

Plasma lipids Plasma triacylglycerol levels halved during the first week of dietary energy restriction (2.4±0.5 to 1.2± 0.1 mmol/l; p<0.02) and remained constant thereafter (Table 2). Total cholesterol also decreased, and HDL-cholesterol remained unchanged during the study period (Table 2). Fasting plasma NEFA levels were modestly but not significantly higher in the diabetic participants compared with the matched controls at baseline (0.69± 0.06 vs 0.57±0.07 mmol/l; p=0.24). During the study period, fasting NEFA in the participants with diabetes increased significantly at week 1 (0.93±0.05 mmol/l; p=0.03 vs baseline). With continued hypoenergetic intake, plasma NEFA declined steadily towards baseline values (0.81±0.08 and 0.72±0.06 mmol/l at weeks 4 and 8, respectively).

Post-intervention observation At follow-up 12 weeks after completion of the dietary intervention, mean weight gain was 3.1±1.0 kg. Hepatic triacylglycerol remained low and unchanged (2.9±0.2 vs 3.0±0.3%; p=0.80), and pancreatic triacylglycerol decreased further to a small extent (6.2±1.1 vs 5.7±1.1%; p=0.005). HbA_{1c} was unchanged (6.0±0.2 vs 6.2±0.1% [42±2 vs 44±1 mmol/mol]; p=0.10) and fasting plasma glucose increased modestly (5.7±0.5 vs 6.1± 0.2 mmol/l; p<0.01), with a 2 h OGTT plasma glucose of 10.3±1.0 mmol/l. Three participants had recurrence of diabetes as judged by a 2 h post-load plasma glucose >11.1 mmol/l. Fasting plasma insulin concentrations were unchanged (57±11 vs 65±15 pmol/l) and fasting plasma NEFA decreased further (0.72±0.06 vs 0.54±0.05 mmol/l; p<0.02). One individual was unavailable for retesting, having had surgery for an ovarian cyst (non-malignant).

Discussion

This study demonstrates that the twin defects of beta cell failure and insulin resistance that underlie type 2 diabetes can be reversed by acute negative energy balance alone. A hierarchy of response was observed, with a very early change in hepatic insulin sensitivity and a slower change in beta cell function. In the first 7 days of the reduced energy intake, fasting blood glucose and hepatic insulin sensitivity fell to normal, and intrahepatic lipid decreased by 30%. Over the 8 weeks of dietary energy restriction, beta cell function increased towards normal and pancreatic fat decreased. Following the intervention, participants gained 3.1±1.0 kg body weight over 12 weeks, but their HbA_{1c} remained steady while the fat content of both pancreas and liver did not increase. The data are consistent with the hypothesis that the abnormalities of insulin secretion and insulin resistance that underlie type 2 diabetes have a single, common aetiology, i.e. excess lipid accumulation in the liver and pancreas [11]. This provides a unified hypothesis to explain a common disease that previously

appeared to require separate disease processes affecting the pancreas and insulin-sensitive tissues.

Absence of rapid insulin secretion in response to a rise in plasma glucose is the hallmark of type 2 diabetes [3, 21], and the decline in beta cell function determines the progression towards a need for insulin therapy [2]. However, conventional therapy, even with sulfonylurea, fails to produce more than a small increase in the first-phase insulin response. As a consequence, the rapidity and extent of return of beta cell function in response to dietary energy restriction in the present study is striking. It supports the accumulating information on the inhibitory effect of fatty acids on insulin secretion in vitro and in vivo [22–24] and is the first direct evidence in humans that the beta cell defect of type 2 diabetes is reversible by sustained negative energy balance. Prolonged elevation of plasma fatty acids in humans decreases insulin secretion [25, 26], and it has previously been shown that there is an association between pancreatic fat content and type 2 diabetes [27–29]. Prior to the onset of spontaneous diabetes in rodents, both total pancreatic fat and islet triacylglycerol content increase sharply [30, 31]. In vitro, chronic saturated fatty acid exposure of beta cells inhibits the acute insulin response to glucose, and removal of fatty acids allows recovery of this response [32].

The present data provide clear evidence that decreasing total pancreatic fat is associated with a return of beta cell function. However, it is probable that the negative effect on beta cell function is exerted by toxic intermediaries such as diacylglycerol and ceramides, which change rapidly in response to acute metabolic changes [33], rather than by stored triacylglycerol per se, which acts as an index of fatty acid intermediary concentration. There was no correlation between indices of insulin secretion and pancreatic fat, which suggests that there are individual thresholds of tolerance for such toxic intermediaries rather than a simple dose–response relationship within the pancreas.

Fasting plasma glucose concentration is determined by the rate of hepatic glucose production, and hepatic insulin sensitivity is inversely proportional to intrahepatic lipid content [13, 34–36]. Moderate weight loss has previously been shown to be associated with a fall in intrahepatic fat content [10, 37]. Petersen et al. have previously reported improved liver and no significant change in muscle insulin sensitivity measured by euglycaemic hyperinsulinaemic clamps after 8 weeks of moderate energy restriction in type 2 diabetes [10]. A very low energy intake has been observed to lower liver fat content in healthy obese individuals within days [38]. The present study demonstrates for the first time the early time course with which both hepatic fat stores and hepatic glucose production fall in response to dietary restriction in type 2 diabetes. Change in peripheral insulin sensitivity played no part in the early return of normoglycaemia. It is possible that the sharp rise in plasma NEFA observed after 1 week of the hypocaloric diet could have prevented a change in peripheral insulin sensitivity even though this did not prevent the rapid improvement of hepatic insulin sensitivity.

We have previously hypothesised that establishing the pathophysiological changes that accompany the reversal of type 2 diabetes will illuminate the sequence of changes determining the onset of the disease [11]. This twin-cycle hypothesis was informed by observations following bariatric surgery, especially the demonstration that fasting blood glucose concentrations fell within days after biliopancreatic diversion [39]. As these changes occur before substantial weight loss, it has become widely accepted that bariatric surgery exerts such rapid effects by changes in incretin hormones. However, the extent of change in incretins is modest, not always present in type 2 diabetes, and absent after gastric banding [40–43]. Little attention has been paid to the potential role of a sudden negative energy balance on glucose metabolism after bariatric surgery, although a recent small study suggested that the improvement in insulin resistance in the first week after surgery can be attributed to negative energy balance alone [44]. Increased glucagon-like peptide-1 secretion following bariatric surgery compared with a hypocaloric diet has been reported, but the oral glucose load used in the presence of a gastroenterostomy is likely to explain the early rapid rise in both plasma glucose and the incretin, occasionally with the symptoms of early dumping [45].

The limitations of this study must be considered. First, the sample size was necessarily small to allow the application of gold standard methods for metabolic investigation and examination by magnetic resonance techniques. However, the participants were drawn from the population with type 2 diabetes, and their clinical and anthropometric characteristics were typical for the condition.

Second, pancreatic fat measurements included intraorgan adipocyte fat content because current methodology precludes the assessment of the more mechanistically important islet intracellular fatty acid content. Animal data suggest that the two variables are linked [31]. Although pancreatic fat content was 30% higher in the diabetic group, the study was powered to demonstrate responses to the dietary intervention rather than to test differences from weight-matched non-diabetic individuals. No correlation was observed between pancreatic fat and BMI within the restricted range of BMI examined in this study.

Third, the participants were selected to have had a relatively short duration of type 2 diabetes (up to 4 years), and further studies must establish the extent of reversibility with longer duration type 2 diabetes. In addition, further studies are required to determine the long-term outcome in respect of glucose regulation as the observations made after 12 weeks of return to a normal diet were necessarily limited.

Finally, the raised liver fat levels despite metabolic normality in some controls can be considered in the light of the recent description of the *PNPLA3* gene [46]. The G allele of this gene determines high liver fat levels, but in a form that is not associated with metabolic abnormality. This provides a clear genetic basis for the observed individual variation in susceptibility to insulin resistance despite raised liver fat content, and offers a partial explanation of the overlapping hepatic fat levels in type 2 diabetic and control groups. It is likely that other genetic factors yet to be defined underlie the differing individual levels of susceptibility to pancreatic fat accumulation in terms of inhibition of glucose-dependent insulin secretion.

This study demonstrates for the first time the time course of a return of normal beta cell function and hepatic glucose output by acute restriction of dietary energy intake in individuals with type 2 diabetes. The changes occurred in association with decreases in pancreatic and liver triacylglycerol concentrations. This new insight allows an understanding of the causality of type 2 diabetes in individuals as well as in populations. It carries major implications for information to be given to newly diagnosed patients, who should know that they have a potentially reversible condition and not one that is inevitably progressive.

Acknowledgements We thank L. Morris, C. Smith and T. Hodgson for magnetic resonance radiography, A. Lane for GC-MS analyses, J. Gerrard for research nurse support and K. Heron for dietary advice. We are most grateful to the volunteers for their enthusiastic participation. This study was funded wholly by project grant RD08/0003713 from Diabetes UK. Nestlé UK provided the Optifast on request but had no other input into the research.

E.L.L performed research, analysed data and wrote the manuscript. K.H. designed the research, analysed the data and edited the manuscript. B.A. analysed the data and edited the manuscript. M.J.C. performed research and contributed to the manuscript. J.M. designed the research and edited the manuscript. R.T. designed the research, analysed the data and reviewed/edited the manuscript. All authors approved the final version to be published.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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