TYPE 2 DIABETES IN CHILDHOOD: UK NATIONAL COHORT

Short title: The JUMP cohort

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Chief Investigator:  Timothy Barrett

Principle Co-Investigators:
David Dunger
Jo Blair
Rakesh Amin
Tim Cheetham
Julian Shield
Sarah Ehtisham
Neil Wright,
Tabitha Randell
Fiona Campbell

Preface

The protocol for the Type 2 Diabetes in Childhood cohort describes the background, design and organisation of the study. The protocol will be maintained by the Study Coordinating Centre at The NIHR Wellcome Clinical Research Facility, a partnership between Birmingham Children’s Hospital and University of Birmingham over the course of the study through new releases of the entire protocol, or issues of updates either in the form of revisions of complete chapters or pages thereof, or in the form of supplemental protocol memoranda.
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1. PROTOCOL SYNOPSIS

This is a UK national, prospective, cohort study of children and young people with type 2 diabetes (T2D) characterised by anthropometry, biomarkers, and co-morbidities; and in whom other diagnoses (such as type 1 diabetes, maturity onset diabetes of the young (MODY)) have been excluded. The tight clinical characterisation will allow inclusion of patients into studies of novel interventions in this group, including evaluation of both glycemic control and risk factors for cardiovascular disease; targeted interventions in small scale open label studies; and enrolment into multinational clinical trials. The cohort will encourage academic and industry collaboration for patient benefit.

The overall objective of this study is to characterise a cohort of children and young people with T2D. This will include baseline and repeat assessments over time of the anthropometric, cardiovascular, metabolic and psychological status of individuals with T2D in order to:

- Describe the natural history of T2D and related co-morbidities in a multiethnic cohort of UK children with the disease.

Undertake academic Investigator led studies into the Pathophysiology of the disease.

- Support recruitment to Industry studies of novel agents

The study is divided into three phases:

- Screening (Phase 1)
- Baseline characterisation (Phase 2)
- Follow-up risk assessments (Phase 3)

An overview of the study design is presented in Figure 1 (Page 10).

Phase 1 involves screening for evidence of T2D (and exclusion of type 1 diabetes), and DNA collection. Individuals with diabetes by the WHO criteria who are overweight or obese; and have absence of pancreatic autoimmunity; in the absence of any secondary or monogenic diabetes (diabetes syndrome or confirmed MODY), will be eligible for entry into Phase 2 of the study, the baseline risk assessment. The purpose of DNA collection at this stage is to screen for monogenic forms of diabetes if suspected; and for mutations in novel candidate genes for diabetes as they are identified.

Phase 2 will involve baseline assessments of body composition, a short oral glucose tolerance test (OGTT) with insulin and C-peptide measurements; measurement of HbA1c, and screening for metabolic and microvascular complications.
Individuals who participate in Phase 2 will also be invited to enter Phase 3 for follow up risk assessments. They will be seen at annual intervals until the end of the study (3 years). At each review, anthropometric measures will be recorded, fasting blood samples taken, and surrogate markers for cardiovascular disease measured. Participants will be informed of their test results.

During each phase of the study, residual blood samples (and DNA samples in Phase 1) from consenting participants will be stored indefinitely at a core laboratory in Birmingham for future metabolic assessments that bear upon the mechanisms of beta cell failure and tissue insulin resistance; and to obtain additional genetic information about genetic markers associated with the development of T2D. Patients may still participate in all phases of the study even if they choose not to give consent for DNA storage.

Children with T2D will be recruited through referring paediatricians and paediatric diabetes specialist nurses. They and their parents/guardians will be asked to attend a study site to go through the information sheet with a research nurse, and to gain parental consent to take part. For children, information sheets will be provided in suitable language and they will be asked to give their assent. They will not be allowed to enter the study unless parental consent is obtained. A separate consent is required for Phases 1 and 2 of the study.

The primary outcome is the detailed anthropometric and metabolic phenotyping of the cohort together with surrogate markers for cardiovascular disease.

It is planned that T2D intervention trials will be available following Phase 2. Individuals who might be eligible for such studies will be informed of them. Those individuals who enter an intervention trial after Phase 2 will not enter Phase 3. However, data accrued from intervention trials may still be used for analyses pertaining to natural history of the disease.
2. INTRODUCTION

Paralleling the rise in childhood obesity worldwide there has been a rise in T2D in childhood. The rise in T2D has been documented in the United Kingdom from the first cases of childhood T2D in 2000 [1] to the successive BSPED [2] and BPSU surveys of non-type 1 diabetes [3]. T2D occurs when insulin secretion is inadequate to meet the increased demand posed by insulin resistance. Thus T2D is commonly associated with other features of the insulin resistance syndrome, in particular obesity. The rise of obesity and reduced activity endemic in British society, threatens to produce a continuing rise in the incidence of T2D in UK children. Whilst T2D has been reported in all ethnic groups in the UK, it is clear that children of South Asian and African-Caribbean origin are at particular risk. Awareness of the risks of T2D may be less in these communities. Besides the acute risks of hyperglycaemia, such as ketoacidosis and non-ketotic hyperglycaemic coma [4], children with T2D are at high risk of hypertension, nephropathy, dyslipidemia, and the associated conditions non-alcoholic fatty liver disease (NAFLD), ovarian hyperandrogenism, premature adrenarche and polycystic ovarian syndrome [5]. Additional health problems related to obesity include Obstructive Sleep Apnoea (OSA) with associated pulmonary hypertension [6], orthopaedic problems resulting in diminished physical activity [7,8] pancreatitis, cholecystitis, pseudotumour cerebri, and depression. In addition there is evidence of systemic inflammation: elevated C-reactive protein, inflammatory cytokines, and white blood cell counts are found in obese adolescents, and these abnormalities have been associated with increased risk for cardiovascular disease in adults [9]. Insulin secretion depends on disease status and duration and can vary from delayed but markedly elevated in response to a glucose challenge, to absolutely diminished [10]. The data is not yet present in children, but adults with symptoms have a 50% reduction in insulin secretion at the time of diagnosis and may become insulin dependent within a few years [11]. Furthermore despite being perceived by patients and parents as “mild” diabetes, T2D appears to have a more adverse complication profile than type 1 diabetes, with a clear link between T2D and accelerated micro- and macro-vascular complications of diabetes [12].

There are some children with the clinical picture of T2D but who have diabetes autoantibodies typical of type 1 diabetes (T1D) [13]. The pathophysiology of autoimmune ‘T2DM’ is unclear. It most likely represents autoimmune T1DM in overweight or obese individuals with underlying insulin resistance. It has been postulated that obesity and insulin resistance may promote an inflammatory response to antigen exposure caused by apoptosis of beta cells [14]. Concerns have been expressed that there may be diagnostic confusion between T1D and T2D. The following issues are relevant:

- Up to 15% of children newly diagnosed with T1D are diabetes autoantibody negative [15,16]. The correct diagnosis can be confirmed by an absolute insulin dependency outside the honeymoon period.
- With increasing obesity in childhood, as many as 15–25% of newly diagnosed T1DM (or monogenic diabetes) patients may be obese.
The significant number of pediatric patients with T2DM demonstrating ketonuria or ketoacidosis at diagnosis [17].

T2DM is common in the general adult population, with a random family history of ~15% or greater in minority populations, reducing the specificity of a positive family history.

Positive family history for T2DM is increased for patients with T1DM as much as threefold over the non-diabetic population, and T1DM is more frequent in relatives of patients with T2DM [18,19].

There is considerable overlap in insulin or C-peptide measurements between T1DM, T2DM, and MODY at onset of diabetes and over the first year or so. This overlap is because of the recovery phase of autoimmune-mediated T1DM (the honeymoon) and degree of glucotoxicity/lipotoxicity, impairing insulin secretion at the time of testing in both T1DM and T2DM. In addition, the insulin resistance of obesity raises residual C-peptide levels in obese adolescents with T1DM. The role of C-peptide may be more helpful in established diabetes as persistent elevation of C-peptide above the level of normal would be unusual in T1DM after 12–24 months.

In view of the above discussion, studies of children with T2D need to distinguish between those who are diabetes autoantibody negative and those who are positive, to avoid ‘dilution’ of a T2D cohort with type 1 diabetes.

In 2005, Haines et al. undertook a prospective monthly surveillance study of 2,665 consultant paediatricians in the UK and The Republic of Ireland through The British Paediatric Surveillance Unit (BPSU) of The Royal College of Paediatrics and Child Health (RCPCH) to identify cases of non-type 1 diabetes in 0-16 year old children [3]. The study ran from October 2004 to October 2005 inclusive, and was approved by The South West Multi Research Ethics Committee (04/MREC06/39). The increase in childhood obesity has caused concern that it may be difficult to distinguish between obese children with type 1 or type 2 diabetes (see above); however this concern has not been shared by all [20]. It has been particularly difficult to classify children with diabetes related auto-antibodies and also obesity and insulin resistance. The presence of auto-antibodies would suggest type 1 diabetes, but the obesity and insulin resistance would suggest T2D. We therefore defined T2D by a reported raised fasting insulin (>132pmol/L or equivalent) or C-peptide level (>600pmol/L) and/or the absence of autoimmune antibodies found in type 1 diabetes. We identified 76 cases of newly diagnosed T2D, representing 40% of all non-type 1 diabetes cases reported. Fifty seven percent of cases were girls, and the mean age at diagnosis was 13.3 +/- 1.7 years for girls and 14.1 +/- 2 years for boys (range 8.3-16.8 years). About 60% of cases were classified as white and 40% as an ethnic minority, predominantly South Asian (35%) and Black/Black British (45%). T2D is clearly impacting on ethnic minority groups who are already disadvantaged in UK society. We reported a minimum incidence of T2D of 0.53/100,000/year, but 3.9/100,000/year in Black children. We showed that 95% of children with type 2 diabetes were overweight, and 83% obese; 84% had a family history of T2D. In a follow-up study, we found no significant overall improvement in BMI-SDS during the first year after diagnosis: mean change of -0.11 (range: -1.53 to +1.37) [21]. In addition, 42% failed to achieve the ADA/EASD recommended treatment target (HbA1c < 7.0%). As this was an epidemiological study, ethical considerations prevented us from directly contacting the children or taking additional samples to characterise them more completely.
The long term outcome for children with T2D is poorly understood; youth onset T2D is associated with higher risks for complications than type 1 diabetes; yet there is no longitudinal data from the more obese UK child population. Whilst adults with T2D may develop complications late in their working lives, children are likely to develop complications in early adulthood at a prime time for establishing employment, reproducing and bringing up children [22,23]. The child with T2D therefore represents not just a potential cost to the NHS through developing complications, but also a loss to the work force of the UK, and an impact on the next generation through parental illness or death. This knowledge on outcome and management is vital not just for counselling individual patients, but for planning for utilisation of many adult services including renal dialysis, ophthalmic, and vascular surgery services.

In adults, there is a strong association between level of hyperglycemia and increased risk of macrovascular disease. Hyperglycemia, dyslipidemia, and hypertension are contributors to the acceleration of atherosclerosis in T2DM along with oxidative stress, glycation of vascular proteins, and abnormalities of platelet function and coagulation. Defective endothelium-dependent vasodilatation is an additional factor accelerating atherosclerosis in T2DM. It is an early sign of increased risk for cardiovascular disease and predictive of cardiovascular events [24] and occurs in obese children relative to their level of obesity and degree of insulin resistance [25].

There is almost no evidence base for effective treatments for T2D in childhood. Whilst lifestyle change has been shown in adults to be the best way of preventing T2D [13], studies in childhood have proved to have short term benefit only, and few patients are compliant with lifestyle changes [26]. Compliance with treatment is often hindered by the lack of severe symptoms at diagnosis in comparison to type 1 diabetes; many patients are in their teenage years and rebelling against authority; and most pharmacotherapy for T2D is not manufactured in paediatric formulations. Furthermore there is little information on which drugs should be used for treatment. The only drug with randomised control trial data to support use in children with T2D is metformin [27] [32]. Newer treatments such as Incretin mimetics and dipeptidyl peptidase 4 inhibitors which seem to show promise in adult studies have not as yet been trialled in childhood populations, and require phase two studies to demonstrate effect size. Extrapolating from adult data is unwise in the growing and/or pubertal child; growth, the attainment of peak bone mass, and progression through puberty are naturally insulin resistant states. The numbers of children currently with T2D in the UK are such that only multicentre trials of novel treatments will have sufficient power, and such trials would be facilitated by having access to a central register from which to select well characterised patients with clearly delineated co-morbidities.

Recent American Diabetes Association Guidelines [33] recommend testing for Type 2 diabetes in children and adolescents who are overweight and have two or more additional risk factors for diabetes:

- Overweight (BMI .85th percentile for age and sex, weight for height .85th percentile, or weight greater than 120% of ideal for height)

Plus any two of the following risk factors:

- Family history of type 2 diabetes in first- or second-degree relative
- Race/ethnicity (Native American, African American, Latino, Asian American, Pacific Islander). For the UK this includes UK South Asian origin or UK African-

Caribbean origin.
- Signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidemia, polycystic ovary syndrome, or small-for-gestational age birth weight)
- Maternal history of diabetes or GDM during the child’s gestation

Age of initiation: age 10 years or at onset of puberty, if puberty occurs at a younger age
Frequency: every 3 years

Criteria for the diagnosis of diabetes now include HBA1C equal to or greater than 6.5%.

The goal of this study is therefore to establish a UK-wide prospective cohort of children and young people with ‘pure’ T2D, who will be characterised by diabetes autoantibody status, anthropometry, markers of glucose and insulin metabolism, and markers for future cardiovascular disease; and in whom other diagnoses such as type 1 diabetes have been excluded. The aim is to create a resource from which to recruit patients into intervention studies. These may include both evaluation of glycaemic control and risk factors for cardiovascular disease; clinical experimental studies on small groups; and enrolment into multi-centre clinical trials.

3. OBJECTIVES

The overall objective of this study is to perform baseline and repeated measurements over time of the metabolic and cardiovascular risk status of children with T2D in order:

a) To describe the natural history of type 2 diabetes in Children. To investigate micro vascular and Neuropathic complications.

b) To collect tissue (serum & urine samples) for proteomics. To investigate carotid intima media thickness as a surrogate marker for cardiovascular disease.

c) To Investigate Serum Insulin methylated DNA as a marker of Beta cell death (Appendix 3)

d) To undertake exercise and nutritional assessments. (Appendix 4)

4. RATIONALE

The rationale for developing this cohort in the UK is primarily to attract recruitment into multi-centre clinical trials to permit evaluation of interventions in children/adolescents. A European Union Directive now in place, requires trials to include some young people under 16 years in clinical trials of new products. Currently there are few, if any, countries with well characterised cohorts of childhood onset T2D to enable recruitment. Allied to this, a further benefit will be the development of an evidence base for treatment and counselling of UK children with T2D and their
families. This cohort will enable clinical trials while at the same time supporting observational studies of the natural history and outcomes of childhood T2D. Finally, the advent of T2D in childhood poses new threats to health with an accelerated progression to complications. The establishment of this cohort will put the right structures in place and have a major positive impact on our ability to respond to this threat to our children’s health.

5. STUDY OVERVIEW

The T2D study is divided into three phases: Screening (Phase 1), Baseline assessment (Phase 2), and Follow-up assessments (Phase 3). Phases 1 and 2 will require separate consents for entry. A prospective cohort design will be used for the study. An overview of the study design is presented in Figure 1.

Phase 1 involves screening obese children with diabetes for the absence of auto-antibodies associated with type 1 diabetes; and DNA collection. Children who test negative for auto-antibodies will be eligible for Phase 2 of the study, the Baseline Assessment. Insulin antibodies (IAA) may be positive if the patient has had previous insulin treatment. It is possible that children who do not qualify for Phase 2 might still be eligible for other studies. Therefore, they may be contacted in the future so that they can be informed of new studies. In addition, they may be contacted periodically to learn if their diagnosis has changed to T2D. Some of the samples obtained at phase 1 will be analysed for Serum Insulin methylated DNA.

**Figure 1: Overview of the study design Please note changes to figure**

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<th>Phase 1 – Screening</th>
<th>Phase 2 – Baseline assessment</th>
<th>Phase 3 – Follow-up</th>
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<td>• Informed consent</td>
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<td>• Anthropometry</td>
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<td>• BMI&gt;85th centile</td>
<td>• 30 min OGTT</td>
<td>• Fasting C-peptide,</td>
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<td>• Autoantibody tests (GAD-65, ICA-512, IAA)</td>
<td>• Lipids, LFTs, etc</td>
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<td>• DNA</td>
<td>• Fasting glucose insulin and C-Peptide (if not on insulin treatment)</td>
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<td>• Stored samples</td>
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<td>• Exercise &amp; Nutritional Assessments</td>
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Phase 2 will include a fasting glucose and insulin and C-peptide to assess HOMA insulin resistance; and lipids, liver function, blood pressure, and an exercise & Nutritional Assessment. Children who participate in Phase 2 will be automatically included in Phase 3, follow-up assessments.

Phase 3 will occur as part of childrens’ routine diabetes annual review by their local paediatrician. They will be seen annually until the end of the study (3 years), with the possibility of extension should further funding become available. The assessments will include anthropometry, blood and urine samples and markers of cardiovascular risk.

During each phase of the study, residual blood and urine samples (and DNA samples in Phase 1) from consenting participants will be stored indefinitely at the core laboratory for future metabolic and genetic assessments that bear upon mechanisms of beta cell destruction, tissue insulin resistance, and the development of cardiovascular disease. Children may still participate in all phases of the study even if they choose not to give consent for storage.

Individuals who qualify for intervention studies based on their baseline assessments will be invited to participate in trials as they become available. Individuals who enter an intervention trial will be followed according to the protocol for that trial. However pertinent data accrued during that trial (conditional upon treatment status in the trial) may be incorporated into the database for this study.

The primary outcomes are the estimation of tissue insulin resistance, and markers of cardiovascular disease risk. For most participants, these will be based on the results of an IVGTT, biochemistry and cardiovascular risk assessments.

All health professionals caring for children with known or suspected T2D will be able to participate in Phase 1. Children qualifying for entry to Phase 2 will be referred to the Project research nurse covering their area.

6. STUDY PROCEDURES

6.1 Phase 1 – Screening

Phase 1 involves establishment of the diagnosis of T2D and the exclusion of other forms of diabetes. This will be undertaken by the referring clinicians, with support from dedicated specialist research nurses based in a regional centre. Samples for autoantibody assays will be sent to a central reference laboratory. The research nurses attached to this study will not be normally be involved in this stage beyond administration support (sending out of study paperwork and sample tubes, collation of data).

6.1.1 Participants

Children and young people with a diagnosis of suspected T2D will be invited to participate in the screening phase (Phase 1) of this study. We will begin by
approaching the BPSU cohort, with new ethics approval. Consultants declaring children to the BPSU survey of T2D will be asked to approach children and families. We will prospectively approach all consultants looking after children with diabetes through the British Society of Paediatric Endocrinology and Diabetes (BSPED) and Association of Clinical Diabetes Consultants (ACDC) to notify us of children currently under their care with T2D or suspected T2D; and newly diagnosed or suspected patients on a monthly basis for the duration of the study.

Childhood T2D will be defined as:

- diabetes fulfilling American Diabetes Association diagnostic criteria for diagnosis [16] under 18 completed years;
- absence of type 1 diabetes associated antibodies (Islet cell antibodies (ICA), antibodies to Glutamate Decarboxylase (GAD), Insulin autoantibodies (IAA).
- Absence of any known secondary (e.g. cystic fibrosis, post transplant, Downs syndrome); or monogenic cause of diabetes (e.g. MODY, syndrome).

Inclusion and exclusion criteria for Phase 1 are listed below.

### 6.1.2 Inclusion criteria

**The child or young person must:**

1. Be willing to give informed assent/consent (and parent/guardian give informed consent) for the screening procedure.

2. Be aged 5-18 completed years

3. Have diabetes according to the ADA criteria (laboratory determinations of fasting glucose ≥7.0mmol/l, or two-hour OGTT glucose ≥11.1mmol/l) documented and confirmed in the medical record. For asymptomatic children diagnosed with diabetes with a normal fasting glucose but an elevated two-hour glucose during an OGTT, the HbA1c must be ≥6%. Children previously diagnosed with diabetes and laboratory determination of HbA1c ≥8% at the time of diagnosis will be accepted as surrogate evidence of eligibility, if there was no documented laboratory determination of serum glucose.

4. Have a suspected diagnosis of T2D made by his/her local general practitioner or paediatrician.

5. Have BMI ≥85th centile for age and sex according to the Child Growth Foundation BMI charts documented at time of diagnosis or at screening.

### 6.1.3 Exclusion criteria

1. Genetically confirmed monogenic diabetes (e.g. MODY or diabetes syndrome) or other syndrome associated with diabetes (e.g. Downs, Prader-Willi).
2. Any secondary diabetes e.g. cystic fibrosis related diabetes, transplant-related or thalassaemia-related diabetes

3. Any existing evidence of pancreatic autoimmunity (any of anti-GAD65, anti-ICA512. Insulin antibodies (IAA) may be positive if the patient has had previous insulin treatment).

4. Other significant organ system illness or condition (including psychiatric or developmental disorder) that would prevent participation in the opinion of the investigator.

6.1.4 Procedures

Phase 1 (screening for T2D) will occur in secondary care (hospital paediatric departments) and in clinical research facilities at participating centres (Sheffield, Birmingham, Cambridge, Bristol, London). Written informed consent will be required from all study participants. Doctors notifying the centre of a suspected child with T2D, will be sent a study pack including information/consent forms, data collection sheets, and laboratory sample instructions.

Contact information, basic demographic information and information on the presence of a family history of diabetes and cardiovascular disease will be obtained at screening. A venepuncture will then be performed from which up to 12ml of blood will be taken. The blood sample will be utilized for assessments of the auto-antibodies and DNA extraction. The result of the auto-antibody test will determine eligibility for inclusion in the Phase 2 of the study.

The screening sample will initially be tested at a central laboratory for the presence of three auto-antibodies that are predictive of the development of T1D. These are anti-GAD65, anti-ICA512, and IAA. In addition, samples will be collected for plasma and DNA extraction and these will be stored at a central laboratory in Birmingham. Children who are suspected to have a monogenic form of diabetes will be offered selective genetic testing in an accredited NHS laboratory as clinically indicated. The DNA will also be used for future assessment of candidate genes for T2D and/or complications. Those patients who test negative for these auto-antibodies will be invited to participate in Phase 2.

6.1.5 Stored samples

With the participants’ consent, residual Phase 1 plasma and serum samples will be stored indefinitely at the core laboratory for obtaining new information about the pathogenesis of T2D, and for discovering new markers for T2D risk. Stored samples will be used to study T2D, its complications and other conditions for which individuals with T2D may be at increased risk.
Some of the samples we have stored already for this study will be analysed in Italy and will be sent by the central laboratory based at Birmingham Children’s Hospital.

Figure 2: Phase 1 (screening for T2D)

First blood sample
GAD65, ICA512, IAA; DNA

All antibodies -ve

Any autoantibody +ve
or
Other clinical exclusion

Enter Phase 2: baseline risk assessment

Leaves the study

6.2 Phase 2 – Baseline assessment

6.2.1 Participants

Children who have a confirmed diagnosis of T2D will be eligible to participate in Phase 2 (phenotyping of the cohort).

6.2.2 Procedures

A full personal and family history and examination including assessment of pubertal stage will be performed according to a standard protocol. We will collect information on complications including fatty liver, hypertension, nephropathy and retinopathy. Measures of height, weight, blood pressure and waist circumference will be taken. Presence or absence of acanthosis nigricans will be documented as a marker of insulin resistance. Acanthosis nigricans will also be staged according to the grading method of Burke et al which takes into account site, extent and texture [28]. Investigations to characterise the cohort will be undertaken locally and will include the following:
A short oral glucose tolerance test for calculation of the insulogenic index. This will be undertaken based on a standard 75g glucose load according to a protocol, to measure fasting and 30 minute glucose and insulin values. The insulogenic index is calculated as the ratio between the supra-basal increments at 30min or insulin and glucose concentration.

Fasting cholesterol, HDL, LDL and triglyceride (to establish dyslipidaemia rates)

 Follicle stimulating hormone and luteinising hormone, SHBG and testosterone (in girls only, to investigate biochemical evidence of polycystic ovarian syndrome)

25 hydroxyvitamin D (to establish whether vitamin D deficiency is a confounding factor in the development of atherosclerosis)

ALT, AST and gGT (as markers of fatty liver disease)

DCCT aligned haemoglobin A1c (HbA1c)

Children will be asked to provide 3 early morning urine samples for albumin creatinine ratio to establish the presence or absence of diabetic nephropathy.

All children will be offered local retinopathy screening in line with guidelines for the management of childhood diabetes (www.nice.org.uk)

Blood pressure measured 3 times using an appropriate sized cuff.

C-reactive protein (CRP) and high sensitivity CRP as generic markers of inflammation and risk for microalbuminuria [30]

Consideration will be given to measurement of carotid intima media thickness estimation by ultrasound if local expertise available (e.g. if study centres map on to AdDIT study), aortic pulse wave analysis and arterial stiffness (again if available locally).

A web based exercise & nutritional assessment including the use of accelerometers. See Appendix 4

Assessment for peripheral neuropathy

Annual follow-ups will be established for children by their local paediatrician based around the annual review for all other children with diabetes. New cases will be assessed at their first outpatient appointment after diagnosis. On annual review, anthropometric measures will be recorded, and blood samples will be taken for lipids, transaminases, and HbA1c. Blood pressure, and urinary microalbumin will also be measured. Responsibility for ongoing management and treatment decisions will remain with the local paediatrician.
6.2.3 Short Oral glucose tolerance test (OGTT)

During Phase 2, all participants will have a short OGTT. The purpose is to make an assessment of baseline beta cell function and first phase insulin response. The choice of any particular test is a compromise; although a full oral glucose tolerance test (OGTT) gives a more comprehensive assessment of beta cell function, it is more time consuming for the patient than a short OGTT and not required for the diagnosis of diabetes as this has already been made. The key information we require is the insulogenic index, as this will give us a measure of early phase insulin secretion. The protocol is as follows:

1) The patient arrives in the morning fasted since midnight the night before, and having omitted any insulin injection therapy that morning. Oral antidiabetic medication eg. Metformin, should be stopped for 3 days before an OGTT. The patient is asked to start by emptying his or her bladder before commencing the test.
   1) Fasting blood samples are taken for venous glucose, insulin and C-Peptide.
   2) A standardized glucose bolus (1.75g/kg of Anhydrous Glucose (maximum 75g). Patients weighing >45kg should receive 75g. over 5 mins) is dissolved in 300mls water and taken orally over 5 minutes.
   3) A further blood sample is taken at 30 mins for Glucose and insulin
   4) The first phase secretion is estimated by the ratio between the supra-basal increments at 30 mins of insulin and glucose concentration (insulogenic index).

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<thead>
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<th>Timing</th>
<th>Glucose</th>
<th>Insulin</th>
<th>C-peptide Proinsulin Preproinsulin</th>
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<tbody>
<tr>
<td>0'</td>
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<td>X</td>
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<tr>
<td>30'</td>
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From the 30 min OGTT the following parameters are estimated:
1) Sensitivity from fasting glucose and insulin (HOMA – see below)
2) Secretion from the acute insulin response (Insulogenic index)
3) Disposition index (degree of beta-cell decompensation for insulin sensitivity) calculated as sensitivity x secretion (product of 1) and 2).

HOMA (Homeostasis Model Assessment)

Individual fasting plasma glucose levels depend on a balance between hepatic glucose production and glucose utilization. The liver is responsible for providing 90% of glucose in the fasting state; about two thirds of this glucose is utilized by non-insulin dependent tissues, principally the central nervous system. The insulin-dependent tissues utilizing the remaining one-third are the skeletal muscles and the liver itself. Insulin therefore regulates hepatic glucose production and glucose uptake to prevent
hyper- or hypo-glycaemia. Elevated fasting levels of glucose or insulin are indicative of insulin resistance.

The commonest index of insulin resistance in the fasting state is the Homeostasis Model Assessment formula (HOMA-R). For the HOMA protocol, glucose and insulin are measured in the fasting state at one time point 0 mins in table above for OGTT).

\[
\text{HOMA} = \frac{\text{fasting glucose (mmol/L)} \times \text{fasting insulin (microUnits/ml)}}{22.5}. \quad [28]
\]

There are limits to the data obtained from HOMA: HOMA-R can be affected by errors in measurements, so is better used to assess populations rather than individuals. HOMA may not be adequate to study the role of insulin sensitivity in type 2 diabetes, where hyperinsulinaemia itself is a factor in the pathology. Despite this, HOMA-R is a reasonable compromise for the purpose of collecting baseline data and informing future intervention studies.

Participants who are taking insulin for diabetes will have to stop these before the test. For insulin, this will be omission of the morning dose on the day of the test. Oral antidiabetic medication eg. Metformin, can be continued if a fasting glucose and insulin are measured only.

Evidence from population studies suggest insulin resistance is a raised fasting insulin (pubertal stage 1 & 2 ≥15mU/L, ≥90pmol/L; pubertal stage 3 & 4 ≥30mU/L, ≥180pmol/L; pubertal stage 5 ≥20mU/L, ≥120pmol/L). These values are based on a large sample of US adolescents (n=1802) across a range of BMI, but without diabetes [29].

6.2.4. Stored Samples

Residual serum and plasma from consenting participants during Phase 2 together with DNA samples from phase 1 will be stored indefinitely for accruing additional information about metabolic aspects of the pathogenesis of T2D and discovering new genetic and other markers for T2D risk.

6.3. Phase 3 - Follow-Up Risk Assessments

6.3.1. Eligibility

All subjects who participate in Phase 2 will be invited to enter Phase 3.

6.3.2. Procedures

Visits for Phase 3 will be incorporated into the routine Annual Review visit to the local paediatrician. For each Phase 3 visit, a standardised assessment will be completed by the local diabetes team. A medical history, height, weight and vital signs, to include seated arm blood pressure, and seated heart rate will be obtained from participants. All participants will have HbA1c measured. The study follow-up intervals will be annually for all participants.
It is possible that intervention trials will be available during the course of Phase 3. Individuals who might be eligible for such studies will be informed of them. Those individuals who enter an intervention trial will not continue to have Phase 3 assessments incorporated into their annual review visits. However, data accrued from the intervention trials may still be utilised for analyses pertaining to natural history.

7.0 OUTCOMES EVALUATION

7.1 Primary outcome

The primary outcome is the confirmation of T2D, i.e. diabetes according to ADA criteria, autoantibody negative, with raised fasting serum insulin levels according to puberty-specific cut-offs; and in the absence of any monogenic or secondary cause of diabetes. The purpose is to create a resource of patients for recruitment into intervention studies.

7.2 Secondary outcomes

The secondary outcomes are the phenotyping of the cohort, i.e.:

- Quantification of HOMA analysis of tissue insulin resistance
- Glycaemic control estimated by measurement of HbA1c
- Anthropometric variables and body composition
- Biochemical variables including liver and renal function
- Cardiovascular disease risk variables including blood pressure, fasting lipids
- Storage of DNA and other samples for later identification of disease gene alleles and biochemical quantification of disease risk

8.0 STATISTICAL ANALYSES

The main purpose of this study is to create a resource of patients with confirmed T2D that can be recruited into multi-centre intervention studies. Therefore power calculations will relate to the individual intervention studies, not the recruitment of this cohort.

Analyses of study data will be conducted to address the primary and secondary objectives of the study, other stated objectives, and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study.

8.1 Primary analysis

The primary outcome will be the confirmation of T2D, i.e. diabetes according to WHO criteria, autoantibody negative, with raised fasting serum insulin levels according to puberty-specific cut-offs; and in the absence of any monogenic or secondary cause of
Participant characteristics, demographics, medical/diabetes histories, and other baseline measurements will be summarized for purposes of characterising the study populations and assessing overall cardiovascular risk characteristics. Descriptive statistics, including mean, standard deviation, median, range, frequency distributions etc as appropriate, will be presented for the overall population and for any risk cohorts identified.

8.2 Secondary analyses

A secondary aim of the study is to prospectively determine the risk for cardiovascular disease using different measures. We will use proportional hazards regression models using (a) the percentage fat mass or BMI from body composition studies or anthropometry as a time-dependent covariate, (b) hypertension as a time-dependent covariate, (c) raised LDL-cholesterol or lowered HDL-cholesterol as time-dependent covariates.

8.3 Participant drop-out

We have made an assumption that we can achieve 70% recruitment into Phase 2 (baseline assessment of children with confirmed T2D).

Some of the participants in this study will participate in intervention studies that could potentially alter the natural history of their diabetes. Including these patients in descriptive studies of the natural history of diabetes will yield biased results. However as the main purpose of this current study is to develop a cohort from which to recruit into intervention studies, studies into the natural history of childhood onset T2D will have a lower priority.

8.4 Sample size

There were 76 children with T2D identified in the BPSU survey of 2005, and this survey suggested an incidence of 0.53 cases/100,000 child population under 17 years/year. We have achieved a cohort of almost 200 children participating in the JUMP study; the study is funded to follow up this cohort over the next 2 years; and to recruit new patients as they are diagnosed.

9.0 HUMAN SUBJECTS

9.1 Research Governance

The development of the cohort will be conducted in accordance with the Declaration of Helsinki Ethical Principles for Medical Research involving Human Subjects (October 2000), and will be according to NHS R&D research governance standards as for all NHS projects. The project will be registered by each NHS R&D department. The applicants are familiar with undertaking research studies involving children, and studies are undertaken with the involvement of expert patients in their design. In obtaining and documenting informed consent, the investigators will comply with the
applicable regulatory requirements and will adhere to ICH GCP Guidelines and to the ethical principles that have their origin in the Declaration of Helsinki. Prior to the start of the trial, the investigators will obtain favourable ethical opinion for the written informed consent form and any other written information to be provided to patients and their parents.

The British Society for Paediatric Endocrinology and Diabetes (BSPED) Clinical Trials Unit (BSPED CTU) will coordinate this study and database with the Principle Investigators. Research proposals requiring access to the database are made to the CTU who review them and offer peer review. The CTU will ensure equity of access to researchers and monitor cohort usage, as guided by MRC patient access policies. The aim is to encourage academia and industry collaborations to study novel interventions and natural history. Priority of access will be given to studies that involve therapies, both industry sponsored and NIHR funded. The CTU will also monitor the balance of studies to ensure equality of access for industry and academia; to ensure that the opportunities for longitudinal observational studies taken up; and to act as joint custodians of the clinical data with the lead Principle Investigator (TG Barrett, University of Birmingham).The BSPED CTU has experience of large databases, data tracking, quality assurance and quality control (through PI David Dunger).

A Cohort Steering Committee (CSC) will comprise the principal investigators from each centre, together with statistical expertise and trial management expertise supplied from the BSPED CTU. This committee is in charge of monitoring all activities towards the objective of the project in order to deliver as promised, in due time and in the budget. The remit includes:

- Ensure MREC approval, processes in place for data protection.
- Control the execution of the project on a quarterly basis with regards to the workplan;
- Address and document external and internal risks which may impair the progress towards the objectives and suggest strategies to anticipate and minimise such risks whenever possible;
- Assess the performance of the project coordinator and staff in different centres and monitor corrective actions;
- Propose all significant modifications of the workplan;
- Set up a dedicated Ethical Advisory Board responsible for the project ethical framework;
- Approve the global and detailed provisional budgets;
- Decide upon all significant modifications of the planned financial operations;
- The chairman shall convene the CSC on a quarterly basis.
- All decisions of the committee shall be formulated through written documents.

The BSPED CTU, University of Cambridge, on the Addenbrooke’s Hospital site, will house the server for the patient cohort database. Data on participating children will be inputted into the password protected database. A unique identifier system will be used and an identifier will be allocated on all data collection sheets and clinical investigation reports. Personal contact details for each patient linking them to this identifier will be held locally on a study screening log. Computers will be password protected and hard copies of data will be held in locked offices. Data will be stored in the BSPED CTU for 20 years. The principal investigator in each unit will keep a patient identification list to link study records with hospital records. The patients will be informed a paper copy of
their data will be stored and in line with the Data Protection Act 1998. Anonymised data may be shared with other disease registries and research projects relevant to this condition.

9.2 Informed Consent

A two-step consent process will be used. The first consent (Phase 1 consent for screening) will be specific for screening procedures. The second consent (Phase 2 for baseline risk assessment and Phase 3 for ongoing monitoring as part of the routine annual review) will apply to procedures that determine the metabolic and cardiovascular characterisation of the children. Each of the consents will be in two parts, one consent sheet for participation in the study, and a separate consent form for storage of DNA. Refusal to consent to storage of DNA will not affect the patients’ eligibility to participate in the study.

Informed consent will be administered by the Research Nurse or Investigators at each site. Potential study participants (and their parents/guardians) will have sufficient time to fully read the consent forms and have any questions answered. It will be explained to them that that there will be separate consent for Phases 1 and 2 of the study and that any consent is not consent for further participation in intervention studies. In all cases, additional consents will be required for those studies.

An assent form has also been developed for each phase of the study. Children will be given the consent or assent forms depending on Gillick competence, and will have the opportunity to discuss the study apart from their parent(s) or guardian(s). This will allow these individuals to ask questions they might not have felt comfortable asking otherwise. In addition, the parent(s) or guardian(s) will be given the opportunity to discuss the study apart from their child or adolescent. All participants will be given a copy of each of their signed consent forms (and assent forms where applicable).

9.3 Gender and Ethnic Diversity

Both males and females, and members of all racial and ethnic populations will be screened. Enrollment is expected to reflect the known differences in frequency of T2D among different racial and ethnic groups. Prior studies of children with T2D have shown a female predominance. Therefore, it is expected that approximately over half of the participants will be females. Special efforts will be made to recruit among ethnic minority groups in order to attain enrollment proportional to the frequency of T2D in the ethnic minority populations of the UK. The distributions of gender, race, and ethnic group will be monitored by the Cohort Steering Committee. If the study population does not reflect recruitment targets, corrective actions will be taken.

9.4 Disclosure of results to participants

During the course of the study, there will be occasions when results of metabolic tests and genetic tests will be sent to the local clinician responsible for the care of the participant.
This is so that the clinical management of the participant can be optimized, and both local clinician and participant kept informed of the study findings.

### 9.5 Risks

The physical risks of participation in this protocol are those associated with venepuncture and the oral glucose tolerance test. Discomfort, bruising and infection can occur with venepuncture.

### 9.6 Monitoring for Adverse Events

A standardised case report form will be completed by study personnel as needed to report possible adverse events (AEs) and serious adverse events that may occur related to phlebotomy or other study procedures. Summaries of AEs will be provided to the CSC at regular intervals.

### 9.7 Benefits

The detection of increased future risk from T2D through screening and risk assessment could lead to the earlier diagnosis of treatable complications than would otherwise be the case. There is also benefit in identifying monogenic forms of diabetes as some of these have been shown to benefit from specific treatments. The research study might eventually increase knowledge regarding the natural history and prevention of T2D.

### 9.8 Confidentiality

Personal information that is obtained for the study will be maintained in a dedicated database at the BSPED CTU. All information obtained from this study will be identified with a unique study identifier, and will not be kept with the participant’s name. Data from study examinations and procedures will be sent to the coordinating centre in Birmingham. This information will be forwarded to the BSPED CTU for entry into the dedicated database for the study that will be used for statistical analysis.

Stored samples from all phases of the study will be kept at a core laboratory facility in University of Birmingham. The stored samples may be used by Investigators within or outside of this study for research related to T2D, its complications and other conditions for which patients with T2D may be at increased risk. The utilisation of these samples will be subject to MRC policies and procedures.

All samples will be coded with a unique study identifier. Linkage of the unique study identifier to the names of the participants will be maintained at the Coordinating Centre. However, the names of participants will not be disclosed to any of the Study Investigators or to any other individuals except for informing participants of test results or possible participation in future studies. If such disclosure is requested, approval by the responsible scientific official for the study at the CSC will be required.
9.9 Qualification for intervention studies

All individuals participating in the study will be eligible for consideration for participation in intervention studies as they become available. Individuals participating in the current study who subsequently enter intervention trials may continue to contribute data toward this study. In all cases of new studies, eligibility for those studies will require that the inclusion and exclusion criteria specific to those studies be met.

10. Samples

10.1 DNA Analysis

Blood samples will be collected in tubes (10mls) according to the standard operating protocol and labelled with a unique study identifier. All blood samples will be sent to Birmingham Children’s Hospital using regulation packaging and first class mail. After database entry DNA will be extracted. Blood samples should reach Birmingham within three days and should be stored at room temperature. DNA from patients where there is a high risk of monogenic diabetes will be sent to the Reference NHS Laboratory in Exeter for mutation analysis. If a diagnosis of monogenic diabetes can be made, the information will be fed back to the referring Consultant.

The DNA samples collected will provide a potential national resource for the study of T2D jointly owned by the Investigators, the MRC and Diabetes UK. Any approach for additional genotyping or collection of phenotype would need to be approved by the Cohort Steering Committee. Detailed appraisal of such applications would be provided by the BSPED Clinical Trials Unit who would then send their recommendation to the Cohort Steering Committee. Any additional studies will require separate ethics approval.

10.2 Plasma and Serum Storage

Plasma and serum saved from the DNA extraction will be stored in the HTA licensed tissue bank in the University of Birmingham. All samples will be labelled with a unique study identifier. Plasma and serum will provide a potential resource for the identification of biomarkers related to T2D and its complications. Any approach for additional analysis would need to be approved by the Cohort Steering Committee in the same way as for DNA.
REFERENCES

## APPENDIX 1 – STUDY SCHEDULE

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<th>Phase 3</th>
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## APPENDIX 2 – VISIT FLOWCHART
Flowchart of the MRC Type 2 diabetes patient cohort study

Phase 1: initial screening

Initial screening performed by local paediatrician with/without support of study nurse and includes confirmation of diagnosis of type 2 diabetes, measurement of diabetes autoantibodies; and DNA collection

Phase 2: baseline assessment

Baseline assessment performed. Includes IVGTT, assessment for diabetes complications...

Phase 3: follow-up assessments

Follow-up assessments performed.
APPENDIX 3

Serum Insulin methylated DNA - Preliminary data

Specific PCR-based assays have been developed to monitor cell death in-vivo based on the detection of circulating DNA molecules released into the peripheral circulation following death of cells [1, 2]. These assays are able to differentiate DNA methylation patterns as identifiers of cell-specific nucleic acids [3, 4].

The methylation pattern of the insulin gene in pancreatic β-cells is different from other tissues. Previous work has shown that cytosines downstream of transcriptional start sites are often demethylated in β-cells while they are methylated in other tissues [5]. When β-cells are destroyed by autoimmune response resulting in T1D or apoptosis during the development of T2D, their DNA is released into the circulation. Although the majority of this DNA is quickly cleared by the immune system, fragmented cell free (cf) DNA can be present at very low quantity (4-12 ng/ml in the serum)[6]. The differentially methylated regions of the insulin gene therefore have the potential to be used as a biomarker to monitor β-cell death after the onset of autoimmunity or during the development of obesity related T2D. β-cell cfDNA can be extracted from serum and detection of cfDNA has been proved feasible using a two-step nested PCR [5, 6]. As shown in Figure 1, in the first step, a set of methylation unspecific primers amplify an outer region of interest regardless of the methylation status of the gene. This increases the amount of DNA template. In the second step, two sets of methylation specific primers are used to detect either the methylated or demethylated insulin sequence.

![Figure 1: Schematic illustration of nested PCR to detect the differentially methylated insulin gene](image)

Preliminary experiments from the group of Kathleen Gillespie in Bristol University have shown that the detection of demethylated cfDNA extracted from 200µl serum is feasible using semi-quantitative PCR (Figure 2). In her preliminary experiments 4 blinded serum samples (two with recent onset T1D and two matched controls) were tested. Both the methylated and demethylated INS gene (129bp) can be clearly detected in the 2nd round of nested PCR in samples 1 and 4. This was confirmed by gel extraction followed by DNA sequencing. When the code was unblinded, individuals 1 and 4 have recent onset T1D. Her group is now in the process of using the sequencing data obtained to make a Taqman probe for a qPCR assay and the SYBR® Green assay described by Dr Herold’s laboratory will also be investigated [5, 6].
Collectively this indicates a potential role for β-cell derived DNA as a direct marker of β-cell death in the context of human diabetes. However the measurement of β-cell derived DNA together with detailed physiological evaluation in other diabetic populations have yet to be undertaken, particularly in T2D. Physiological evaluation is important in order to interpret such measurements, as insulin secretion is modified by insulin sensitivity in a hyperbolic manner \cite{Kahn1993} and many other factors.

References

Appendix 4

Physical activity measurement
Physical activity will be objectively measured using an Actigraph GT1M accelerometer worn on an elasticated belt around the waist. Participants will wear the accelerometer during waking hours for seven days, removing it only for bathing and other activities where it may get wet. The accelerometer will be set to record physical activity every 10 seconds, and data will be downloaded using Actilife software. The accelerometer data will then be processed to generate outcome variables describing the level of total physical activity (average accelerometer counts per minute (cpm)), and the spectrum of daily physical activity intensity (time spent in sedentary, light, moderate and vigorous activity). Intensity variables will be derived using threshold values validated in children.

Dietary habits
Dietary phenotyping will utilise the ‘UK on-line 24h dietary recall’ website developed by Professor Janet Cade and her team at the University of Leeds. MYFOOD24 is the first online dietary assessment tool to be designed in the UK which is validated against both biomarkers (in adults) and alternative dietary assessment tools. This internet based system will be coordinated from University of Bristol and will require e-mails of the JUMP cohort to be available to contact individuals and is therefore appropriate for adolescents. Patient email addresses will be sent to University of Bristol who will then create log in details for the patients. All the required administration to complete the dietary recalls is automatically generated by MYFOOD24 and delivered via e-mail to the participants e-mail account. This includes an initial welcome email, prompt emails containing the appropriate link for each dietary recall, reminder emails in the case of non-completion and a thank you message that is sent upon dietary recall completion. The system will be used to assess three to four days (24 hours) of dietary intake per individual (multiple recalls) to look at types of food consumed and nutrient intakes. Each dietary assessment will take approximately 20 minutes. Adolescent diets are often chaotic and this online tool attempts to reduce the risk of foods being missed such as late night snacks by reminding participants to record food from any time of day. It is common for obese participants to underreport dietary intake, particularly energy dense foods. MYFOOD24 has the potential to reduce this problem by providing an alternative to face to face 24 hour recalls where there is a tendency to report what is expected. Using an online tool where foods and nutrients are automatically analysed is also less burdensome to research staff in terms of time and money than 24 hr recalls where diet diaries need to be manually input into a system containing the Composition of Foods.