Non-CTIMP Study Protocol

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Short title: The ketogenic diet in bipolar disorder

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1 INTRODUCTION

1.1 BACKGROUND

Bipolar disorder (BD) is a major chronic mental health condition, with a lifetime risk of 1-2%. It primarily affects mood and energy/activity levels. People with BD have episodes of depression where they feel very low and lethargic, and episodes of mania where they feel very high and overactive. Each episode can last for several weeks or longer, and the pattern of mood swings varies widely between individuals. The global burden of this disease, measured in disability-adjusted life years (one DALY represents the loss of the equivalent of one year of full health), rose by 14.9% over the period 2005-15 (GBD 2015 DALYs and HALE Collaborators, 2016). In 2015 BD ranked 21st among 310 diseases studied in terms of global years of life lost from disability (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). It usually begins in early adulthood and thus accounts for significant economic loss in all societies.

BD is a heritable disorder and stressful life events or physical illness can trigger the onset of the illness. The main pathophysiology is poorly understood and current treatment and prevention strategies are suboptimal (and many medications for BD have significant side effects). There is consequently a need to identify effective new interventions for clinical use.

In recent years, case studies have demonstrated the benefit of the ketogenic diet (KD; low-carbohydrate and high-fat) in psychiatric conditions. In a 2013 small case series of BD, patients reported significant mood stabilisation on a KD, exceeding that from medication. A 2019 case series documented complete resolution of symptoms in 2 people with schizophrenia, a condition significantly overlapping BD, where similar changes in metabolism are observed (increased pyruvate and lactate). In 2019, 165 self-reports of those adhering to the KD from online BD forums were analysed. 56% reported remission of symptoms or significant mood stabilisation and a further 29% reported some degree of mood stabilisation. This survey demonstrated that the diet is being used in the online BD community. Some psychiatrists are now recommending the KD as a treatment adjunct in their clinical practice. Several recent reviews have concluded that there is now some evidence of KD having beneficial effects on many of the major features of BD, including: reduction of oxidative stress, improved mitochondrial function and biogenesis, improved glutamate/GABA transmission and reductions of intracellular sodium and calcium. Calls for clinical trials have been made.

A novel hypothesis on the mechanism underlying BD has been described by our group and others, including how the KD may counter this. There is substantial evidence of impaired use of glucose in the mitochondria (parts of all human cells responsible for energy production) in people with BD. These developments have been paralleled by advances in understanding the metabolic component to mood disorders, as recently summarised by Brietzke (2018). Problems with glucose metabolism are relatively common in people with BD, as demonstrated by an increased frequency of type 2 diabetes, metabolic syndrome, and insulin resistance. Other conditions caused by problems with glucose metabolism, such as Polycystic Ovary Syndrome, are also associated with an increased frequency of BD.

We have described in detail a novel hypothesis on the mechanism by which hyperinsulinaemia disrupts the production of energy by mitochondria in the brain,

resulting in increased levels of specific biomarkers, including lactate and pyruvate. We reference our recent publications (Campbell 2019, 2020) on this topic and have attached a paper currently submitted for publication (Campbell 2021) which reviews this in detail. Higher levels of these metabolites have been described in BD compared to the general population and may be linked to mood instability. The KD can provide an alternative energy source to glucose (ketones) that can bypass this abnormal processing of energy and allow for more stable levels of energy production.

The KD has been established as a safe and effective therapy for seizure reduction in epilepsy through a Cochrane review of randomized controlled trials and almost a century of clinical use. NHS ketogenic diet services run throughout the UK, and there is potential to build on this experience. The diet's most significant biological effect is that it induces the state of "ketosis" wherein mitochondria in the body and brain preferentially oxidise fatty acids (ketones) instead of glucose. This metabolic shift reduces seizures by 50% in around 50% of subjects (Martin 2016). It is thought that this is due to ketones providing an alternate fuel source to dysfunctional mitochondria which cannot effectively use glucose for energy. In this respect, the underlying disease process may be similar to BD (there are many parallels between the clinical presentations of BD and epilepsy, and several medications are effective in both conditions). Several clinical trials have also trialled use of the KD for other conditions and, in general, relatively low levels of negative side effects are reported (although this has not yet been explored in BD).

The only clinical trial of the KD in BD registered in clinicaltrials.gov is a small pilot study [n=20] from Stanford University looking at a subgroup of both BD and schizophrenia patients who are overweight or obese. In comparison, our research proposal will only look at BD patients, and weight will not impact on our inclusion criteria. In addition to metabolic and psychiatric outcomes, we will also conduct brain imaging.

1.2 RATIONALE

If shown to be effective, the use of the KD to treat BD has the potential to open up a new treatment approach, which could be used alongside established treatments, and potentially reduce the number of medications patients need to take (as well as reduce the overall costs to health, community and society). Reducing the number of medications would reduce risks associated with polypharmacy, and negative side effects, and patients would have greater control over managing their illness. In addition, new insights on the metabolic component of BD may help reduce morbidity and mortality from obesity, diabetes and coronary heart disease.

It is important that an adequately powered and carefully designed randomised controlled trial is conducted to assess the above hypotheses and explore whether the KD results in improved mood stability in this population. There are no previous such trials. Together with an understanding of any negative effects of the KD in BD patients, this would help inform the increasing use of KD by significant numbers of individual patients and in an increasing number of clinical practices. If shown to be ineffective this will help direct research away from KD into other promising new interventions for BD. Prior to running a full clinical trial, it is vital that a pilot study is undertaken to assess the acceptability and feasibility of the KD intervention, including the level of support required for participants. This will allow us to understand how to introduce and maintain the diet effectively, investigate adherence rates and adverse effects of the KD in people with BD, and carry out detailed assessments as they would be performed in a future trial.

2 RESEARCH QUESTIONS

2.1 PRIMARY RESEARCH QUESTION

This pilot study aims to understand the feasibility and acceptability of a low-carbohydrate, high-fat ketogenic diet (KD) in 25 patients with bipolar disorder (BD) who are euthymic at time of presentation. This will also test the acceptability of data capture instruments, explore rates of compliance, adverse effects, the range and variability of outcome parameters (to inform a future clinical trial design), the level of support required to introduce the KD successfully in this population, and the most appropriate diet formulation and means of introduction and maintenance. The effect of a KD on the frequency and severity of mood instability, quality of life, and functional outcomes will be investigated. This feasibility work will help us to develop a protocol for a future clinical trial.

2.2 SECONDARY RESEARCH QUESTION

The study also aims to better understand the proposed mechanism of action of the ketogenic diet by investigating biochemical and more detailed metabolic evidence (through blood tests and brain imaging), and the relationship between these biomarkers and clinical outcome. Definitive conclusions will not be drawn due to the small sample size.

2.3 PRIMARY OBJECTIVES

- Recruit 25 patients with bipolar disorder [BD] who are euthymic at time of presentation.
- Support the introduction of a KD over an 8-week period and determine the most appropriate diet formulation and means of introduction and maintenance in this group.
- Investigate adherence rates with a KD intervention in this patient group and the level of support required to introduce KD successfully.
- Investigate evidence of short-term metabolic changes [from blood metabolomic and brain imaging studies] associated with KD consistent with underlying study hypothesis for mechanism of action.
- Report any adverse effects associated with use of KD in this patient group.
- Explore the feasibility and acceptability of the KD to patients and clinicians.
- Explore feasibility and acceptability of study processes for a future trial e.g. recruitment procedures, monitoring and outcome assessments.
- Document the range and variability of outcome parameter values (to inform future clinical trial design).
- Investigate preliminary signals of efficacy on mood instability, quality of life and functional outcomes in this patient cohort (in a before-after assessment).

2.4 SECONDARY OBJECTIVES

- Investigate biochemical and more detailed metabolic (metabolomic and CNS magnetic resonance imaging) evidence for the proposed mechanisms of KD action outlined in the study hypothesis and relationship between these biomarkers.

- Work collaboratively with and communicate results to diverse academic, policy, public and patient groups through multiple channels.
- Work with Bipolar Scotland and other patient groups at all stages of the research process from study design to interpretation and communication of results and appropriate translation of findings into patient care.

2.5 PRIMARY OUTCOME MEASURES

There is not a single primary outcome as this is a pilot study investigating the feasibility and acceptability of an intervention and gathering data on a range of other parameters as noted above. Therefore, all outcomes are equally important, and it will be essential for us to understand how to make all aspects of the study workable for a subsequent trial.

- Timeline estimates for future trial recruitment by measuring the number of interested participants and fully recruited participants from each avenue of recruitment during the estimated 4-month recruitment period
- Participant level of compliance with dietary interventions measured using fidelity checklists at weeks 0, 2, 4, 6, and 8, and daily ketone measurements during the 8-week intervention period
- Participant level of compliance with study assessments as recorded on the case report form at baseline and week 8
- Participant level of compliance with continuous study assessments measured using data from the study devices and apps (actigraph device, ketomojo device and app, and ilumivu app) or that sent directly to the research team by participants, during the 10-week study period
- Quality of life measured using the EQ5D-5L at baseline and week 8 Productivity and activity impairment measured using the Work Productivity and Activity Impairment Questionnaire at baseline and week 8
- Participant resource use measured using a Within Trial Resource Use Questionnaire at baseline and week 8
- Staff time and cost in delivering dietary interventions, measured by keeping contemporaneous records throughout the 10-week study period
- Attrition rate measured using the number of participants who consent to participate who remain in the study until the end of follow-up at 10 weeks, to help inform estimation of sample size for a future trial
- Acceptability and experience of the study participants evaluated via telephone interviews after the main study has ended as part of a process evaluation

2.6 SECONDARY OUTCOME MEASURES

- Mood stability measured by the Affective Lability Scale (ALS18) and Beck Depression Inventory (BDI) at baseline and at 8-week follow-upHypomania/mania symptoms measured using the Young Mania Rating Scale (YMRS) at baseline and 8-week follow up
- Glucose/ketone ratio measured using ketomojo devices daily throughout the 10 week study period
- Identification of specific metabolic changes in glucose, ketones and TCA metabolites associated with the KD that are predicted by the study

hypothesis, measured using serum and brain MRI measures at baseline and week 8
- Mood, energy, speed of thought, impulsivity and anxiety measured using visual analogue scales from 1-100 at daily ecological momentary assessments during the 10-week study period
- Episodes of bipolar depression and hypomania/mania occurring during the study period assessed by repeat MINI Neuropsychiatric assessment at baseline and week 8.
- Sleep duration and circadian activity/rest rhythmicity parameters assessed by actigraphs throughout the 10-week period (to include 2 weeks of stepping down the diet).

3 STUDY DESIGN AND METHODS

3.1 STUDY SETTING

Recruitment and subsequent face-to-face contacts will take place in the Department of Psychiatry (University of Edinburgh), and the Clinical Research Facility (CRF) and imaging department at the Royal Infirmary of Edinburgh (RIE). Participants will also have regular phone contact with members of the research team. They may have additional phone contact with the University of Glasgow if they decide to take part in semi-structed interviews as part of the process evaluation.

3.2 TYPE AND LENGTH OF STUDY

This is a pilot study which is estimated to last 12 months. Individual participants will be involved for 10-12 weeks.

3.3 KETOGENIC DIET INTERVENTION

All study participants will receive a low-carbohydrate, high-fat ketogenic diet intervention for an 8-week period, the first 2 weeks of which will be an adaptation period. The 8 weeks will then be followed by a 2-week cessation period. They will continue usual medical treatment from their regular care teams, including mental health services.

Based on the successful experience of the study dietitians in administering the KD to adults with epilepsy, we will identify a KD formulation associated with good levels of adherence. We will work closely with Patient and Public Involvement (PPI) representatives from Bipolar Scotland to develop and refine materials to support patients in adopting this diet.

Participants will have an initial meeting with a dietitian, lasting up to 2 hours, to discuss the details of the diet, food preferences, monitoring, and potential risks. They will request that participants complete a food diary to bring to their first appointment, to help plan what meals may be most appropriate for them to try on a KD. This will involve recording everything they eat and drink for 3 days prior to attending. International guidelines for the management of adults treated with ketogenic diet therapies (Mackenzie 2021) suggests that ‘preparation and training must be individualised to provide the greatest likelihood of success’, and we will endeavour to make this is a key part of our dietary initiation.

The diet will be gradually introduced over a 2-week period. Support will be via dietary supervision over the first few days to check adherence to prescribed diets, problem solve and identify any adverse effects, which will mainly occur over the phone or via email. Dietitians will provide guidance and practical support around establishing and characterising the diet, using recipe cards and planning, dietary booklets and providing advice on common problems. We will have support from a Professor of Behavioural Science to develop our approach to behavioural aspects of the intervention including barrier identification, problem solving, habit formation, social support, reducing negative emotions, and restructuring the environment.

The majority of contact with the dietitian will be by phone or email to reduce the burden on participants, but with the option of face-to-face appointments if needed. After initiation of the diet, contact will be weekly. A dietitian will be contactable during normal working hours and will aim to respond within 48 hours. Experience has shown that it takes 2-3 weeks to establish the diet. There will be a 2-week cessation period after 8 weeks on the diet whereby participants are supervised to gradually step down from the ketogenic diet, unless they chose to continue it.

Possible side effects associated with commencing the KD include fatigue, thirst, irritability, hunger, and altered bowel habits (particularly constipation) (Mackenzie 2021). Participants will be informed of these, and simple strategies to manage them. Other common side effects include, weight loss, hyperlipidaemia, leg cramps, menstrual irregularity (in women of childbearing age), nausea, and vomiting. Common side effects are typically mild and can often be managed with diet adjustments under the guidance of a dietitian or nutritionist and infrequently require medical intervention. Smaller meals, increased plant fibre intake, restriction of processed fat sources, exercise, and increased sodium and fluid intake can often prevent or alleviate common symptoms such as constipation (Mackenzie 2021). These strategies will be implemented in this pilot study if required. We will also introduce the diet slowly over a period of 2 weeks which reduces the chances of these side effects being experienced.

As the Ketogenic diet is high fat it may increase blood levels of cholesterol and triglycerides. These levels will be monitored before and after starting the diet. We will minimise this adverse effect of the diet by using unsaturated fats where possible. High levels of cholesterol and triglycerides usually return to normal when the diet is weaned. We will also introduce the diet slowly over a period of 2 weeks which reduces the chances of side effects being experienced.

In line with the international recommendations mentioned above (Mackenzie 2021), we will advise participants to increase their fluid consumption and take a multivitamin and mineral supplement that meets recommended daily allowances. Participants will only follow the diet for 8 weeks (plus a 2-week cessation period) during the pilot study and so the chances of deficiencies if they do not choose to take these is low. If they chose to continue to follow the diet, we will recommend they continue the multivitamin.

The dietitians will have a written protocol detailing what to do in specific situations, e.g. if participants glucose is too low or ketones too high. Participants will be encouraged to contact the dietitian if they have any concerns. It will be communicated both verbally and in written form by the dietitian to participants what adverse effects they may experience and how to manage these. Participants will be asked to notify the research team of any adverse effects they experience via email to allow the team to respond in a timely manner. .

If participants are unable to manage to prepare their own meals, we will consider providing pre-prepared meals on a case-by-case basis. We will also consider providing pre-prepared ketogenic meals for a 2-week period for all participants at the beginning of the study to support them when initiating the diet. Our main aim is to ensure participants achieve ketosis, and the methodology may need to be adapted within the pilot study to ensure we are enabling participants to do this.

In addition to support from the dietitian, participants will have the contact details of a psychiatrist on the research team, who will be available during normal working hours to answer any questions they may have about other aspects of the study, or if they have concerns about their mental state.

We will complete a focus group with patients to inform the refinement of the intervention.

3.4 PARTICIPANT EVALUATION (see appendix 1 and 2 for study flowchart and participant flow infographic)

Participants will be advised not to provide their personal details when completing any free text questions as part of the study questionnaires and/or during telephone interviews.

3.4.1 BASELINE ASSESSMENTS

Prior to commencing the ketogenic diet, participants will be required to attend for initial baseline assessments, to include venepuncture and brain imaging. It is likely that this will take place over two separate days, with the venepuncture and brain imaging occurring on the second attendance. The research team will try to minimise the number of journeys for patients and reimburse any travel costs. It will be important for the baseline assessments to all be within a few days of each other to allow initiation of the diet as soon as possible after venepuncture, brain imaging, and receiving advice on initiating the diet.

Participants will be seen by a psychiatrist at the clinical research facility (CRF) in Edinburgh, for an appointment lasting around 2 hours. They will gain written consent if the patient wishes to continue and after any questions or concerns have been addressed (around 10 minutes). They will then collect basic demographics to include age, sex, highest educational qualification, and total household income (around 10 minutes).

They will then administer the following questionnaires (around 1 hour):

- MINI Neuropsychiatric interview (modules A-D and L) – a structured diagnostic interview based on DSM-4 criteria to confirm BD diagnosis and current mood episodes
- Affective Lability Scale (ALS-18) - a self-reported measure of mood instability
- Beck Depression Inventory (BDI) - a self-reported measure of depressive symptoms
- Young Mania Rating Scale (YMRS) – a self-reported measure of hypomanic/manic symptoms
- EQ5D-5L – health economics Quality of Life instrument

- Work Productivity and Activity Impairment Questionnaire (WPAI) – health economics questionnaire
- Within Trial Resource Use Questionnaire – health economics questionnaire

After completing the questionnaires, participants will have their height, weight and blood pressure measured (around 10 minutes). This means they will have been at rest for around an hour when their blood pressure if checked. They will be provided with instruction on the use of a wrist-worn actigraph to record sleep and activity, which will be worn throughout the 10-week study period (around 10 minutes). They will be provided with education on the use of a glucose and ketone measuring device, to allow participants to measure both parameters every day during the10-week study period (around 10 minutes). This will allow us to monitor adherence to the diet and explore any correlation of these markers with regular mood assessments. We will ensure the participant is comfortable with using both devices, including how to dispose of used lancets into sharps boxes, which will be provided to them, and how and where to record the results, which will be in an app on their phone (around 10 minutes).

Participants will also be familiarised with the ilumivu app to allow them to record their mental state via ecological momentary assessments (a simple scoring system which should take about 5 - 10 minutes to complete). This will entail using a simple visual analogue scale from 1-100 to record their mood, energy, speed of thought, impulsivity, and anxiety on a daily basis, which will take 5-10 minutes. Participants will be taught to set a daily alarm in the app to remind them to do this. Participants may be asked to download both phone apps prior to attending their first face-to-face appointment if they are able.

If it is not possible to use the ketomojo or ilumivu apps, the study team will instead establish a texting or telephone call system. If this is the case, participants will be texted or called each day to ask them to provide their ketone and glucose readings, and elements of their mental state on a scale of 1-100. Even if the apps are used as the method for collecting this data, the team may still use texting as a way to remind participants to fill in these parameters on the apps if they are forgetting to do this. Participants will be informed of the correct process at their baseline appointment.

The participant will see a dietitian who will provide detailed information about establishing and maintaining the KD. Participants will receive education around how to do this, based on resources from the KD service already in place in Edinburgh.

Participants will also have venous blood samples taken to be sent for biochemistry and metabolomics, following the specific procedures required by each department. This will either be undertaken by a psychiatrist on the research team, or by staff at the clinical research facility, who will support the research team. Venous blood samples will likely be taken at a second visit (around 10 minutes), when participants attend for brain imaging (see below). They will need to fast prior to these.

Metabolomic blood samples will be taken and processed at the CRF and frozen at -80. Plasma and cell samples will then be sent to another CRF lab for extractions (see separate protocols). The extracts will then be sent to University of Edinburgh metabolomics department for analysis (see study protocol). Serum samples will also be kept in case we need these in future for more advanced metabolomic analysis. All of these samples will be labelled with participant ID number only, and the University of Edinburgh lab will not have access to any personal identifiable information.

Biochemistry blood samples will be sent to the Royal Infirmary of Edinburgh laboratory for U&Es, LFTS, lipid profile, HbA1c, CRP, beta-hydroxybutyrate, insulin and fasting glucose. The fasting glucose and insulin will be used to calculate HOMA-IR, a measure of insulin resistance. The samples will be processed by the local laboratory who will assign the study a number. They will be processed using the participants CHI number and will be available to view in their medical records, which participants will be informed of. Results will be reviewed and signed off by a member of the research team within 48 hours.

Participants may find the blood tests uncomfortable and/or distressing in the event of a needle phobia, and may experience minor bruising. It will be explained prior to taking blood what patients can expect. A chaperone will be offered if the patient would find this reassuring, provided by the Clinical Research Facility (CRF). The researcher will be an experienced phlebotomist and apply pressure to avoid bruising.

The participant will also attend the Royal Infirmary of Edinburgh for an MR spectroscopy (a type of brain scan) (around 1 hour). This is non-invasive and will not require injection of any substances. This will allow us to measure metabolic markers in the brain and compare these before and after the intervention. Please see imaging data analysis section for more information. All standard imaging service procedures will be followed.

Participants may find MRI brain scans anxiety provoking and may experience claustrophobia during the scans. The research team will ensure that the procedure is explained to participants, so they know what to expect. The research co-ordinator will attend this appointment with participants if necessary and appropriate. If any incidental findings occur, the participants GP will be informed.

Participants will be fully informed about what all the above assessments and interventions entail in both the PIS, and through discussions with the research team. The research team will spend time ensuring participants fully understand all aspects of the study, and how to use all the devices before they are expected to use them themselves. They will also be contactable by participants throughout the study.

3.4.2 FOLLOW UP ASSESSMENTS

Participants will attend for repeat venepuncture and brain imaging in week 6, 7, or 8 of the 8-week intervention period. They will need to be fasted prior to this and will follow the same process as the baseline venepuncture and brain imaging. A psychiatrist will then repeat the baseline questionnaires and physical parameters as detailed above. The dietitian will clarify with them if they plan to continue the KD. If they do plan to continue the dietitian will provide further advice about how to do this. The dietitian will also be available for a further 4 weeks following the end of the 10-week study period, to provide ongoing advice and support if participants plan to continue the KD. We will aim for all follow up assessments to take place during one appointment.

Participants will be asked to monitor glucose and ketone measurements every day, wear their actigraph device, and record mood daily for the full 10-week study period. This will apply if they decide to stop the diet after 8 weeks or if they have decided to continue the diet. This will allow us to see if there are any changes in these parameters related to stopping the diet.

3.4.3 DATA COLLECTION

Data from the ilumivu app will be accessible to the research team, and will be reviewed at least weekly for each patient. This is a research app, and the platform does not require any patient identifiable data, using only the participant's study number to enter into their mobile app.

The ilumivu app is produced by a company called ilumivu which is a decade old software company. The app is based on ecological momentary assessments, a method for collecting data from people as they go about their daily lives giving you access to what is really happening in the moment. It allows researchers to create their own surveys and notification schedules that can be standardised for a group. In this research study, participants will be asked to rate their mood, energy, speed of thought, impulsivity, and anxiety on a visual analogue scale from 0-100, on a once daily basis.

It is a cloud-based system which provides software maintenance, data security and backups. The technology logistics are managed by the ilumivu company. The platform does not require any personally identifying information from participants. It automatically generates a unique identifying code that the user enters into their mobile app. The key linking this code to personal identifiable information is stored outside the system by the research team. All data collected from the apps are encrypted before being pushed to the cloud-based storage database. No data are ever stored on the server's file server but always in the database. Access to the database is gated so entry is only permitted to users entering through the approved route (i.e. you can't hack your way into the database by guessing at the URL), and after supplying a verified user ID and password. For clients in Europe, there are servers in the Netherlands. Data are made accessible to the researchers only by directly accessing the secure site with their verified login credentials and downloading the dataset as a CSV file onto a secure server. Individual datasets will never be transmitted by email. An agreement is in place and the invoice for its use has been paid. Data collected via the Ilumivu app will be processed in the USA, outside of GDPR compliant zone, but will not include any personal identifiable data.

Participants will take ketone and glucose readings on their "KetoMojo" device. The device will be synced via Bluetooth to the KetoMojo phone app. This data is then synced to MyMojoHealth a secure, cloud-based, double encrypted, HIPAA-compliant data storage database with servers based in Europe. Practitioners can access and review patient data directly through a MyMojoHealth practitioner account. The ketomojo app will collect personal data, namely first name, second name and email address, and third parties will have access to general usage analytics such as metrics about what parts of the apps are used.

NHS Lothian cannot guarantee the safety of participants information on their own device when accessing the ketomojo and ilumivu apps.

If it is not possible to collect data via the above apps, a texting or telephone call system will be established by the research team to collect this data. Only UoE or NHS phones will be used, and participants numbers will be stored in these using their study ID number only. They will be sent generic messages which will not include personal identifiable information, and they will be reminded not to disclose any such information when sending replies. They will be asked only for data described above, namely ketone and glucose readings and ratings for aspects of their mental state. Once received, relevant data from text messages will be manually inputted into a UoE secure server as soon as practicably possible, and then text messages will be deleted.

Data from actigraph devices will be in an anonymised form (identifiable only by participant ID), and will be transferred to a University of Edinburgh secure server by plugging the device directly into the computer via a usb socket. This will then be stored securely and only accessible to members of the research team via a password. A third appointment will be arranged in week 11 for them to return these devices (and their ketomojo devices).

3.5 METABOLOMIC DATA ANALYSIS

To test the study hypothesis, a targeted metabolomics approach will be used to accurately measure the concentration of key metabolites in the TCA cycle and closely related pathways. The TCA cycle intermediates oxaloacetate, citrate, cis-aconitate, isocitrate, α-ketoglutarate, succinate, fumarate and malate, alongside pyruvate and lactate will be measured using our rapid HILIC-Z Ion Mobility Mass Spectrometry (RHIMMS) method. We will also monitor the composition and concentration of accumulating ketones [such as acetoacetate, β-hydroxybutyrate [βHB], β-ketopentanoate and β-hydroxypentanoate]. The data will be processed for peak identification, and metabolite annotation using the Agilent MassHunter software suite. The Unified CCS Compendium PCDL and METLIN accurate mass libraries will be used for reliable identification of these metabolites. Investigations will study repeated measures in individual patients, compare serum, and MRI values [we note that Wright 2018 reported a robust correlation between serum and MRI defined brain levels of βHB] and study the relationship to clinical study outcomes. Samples will be blocked and randomised to minimise the impact of measurement error. Data analysis will include the evaluation of confounding variables to accurately link the outcome measures with metabolite measures and will be conducted by the specialist analysis team in the EdinOmics centre.

3.6 IMAGING DATA ANALYSIS

We will conduct structural magnetic resonance imaging (MRI) and physiological imaging including proton magnetic resonance spectroscopy (1H-MRS) to study the relationship between ketogenesis and both glycolytic / TCA metabolic pathways in the brain specified in the study hypothesis, and spectroscopic and structural markers of neuronal health. We will study whether baseline levels of key metabolic parameters are correlated with circulating metabolite measurements and self-reported and measured mental health assessments. Participants will be investigated at baseline and again between weeks 6-8 (last weeks of dietary intervention). For the primary imaging outcome, we will administer non-invasive proton (1H) magnetic resonance spectroscopy to assess for levels of a selection of glycolytic / TCA metabolites directly related to our study hypothesis relating to the mechanism of action of KD in BD [see supplementary submitted paper and Campbell 2019] e.g. lactate and those related to ketogenesis (e.g. β-hydroybutyrate). We will employ procedures based on those detailed in Wright 2018 which has provided proof of principle for measuring these parameters in their study. We will seek to identify the most promising glycolytic / TCA metabolite levels / ratios and longitudinal changes for use as a clinical response biomarker. We will also assess spectroscopic N-acetyl aspartate (NAA) in preselected brain regions and structural grey matter using voxel based morphometry as indicators of neuronal health. As part of secondary / exploratory analysis and hypothesis generation, we will acquire complementary physiological MRI capturing microstructural parameters, regional brain perfusion, and resting state functional MRI. These investigations will be conducted under the

supervision of an experienced neuroradiology academic and honorary NHS Consultant Neuroradiologist [GT] and imaging data analysis will be conducted by the specialist imaging analysis team in Edinburgh Imaging. The Edinburgh Imaging Facility at the Royal Infirmary of Edinburgh / bioQuarter will be used which houses a state of the art 3 T neuro-optimised MRI scanner which is ideally suited to this study. The estimated scan time will be 45-60 minutes on each of the two occasions.

3.7 PROCESS EVALUATION

The process evaluation will explore the feasibility and acceptability of both the intervention, and elements of the study design, and it will be conducted following the Medical Research Council guidelines (Moore 2015). The process evaluation will assess: (i) intervention elements, i.e. acceptability, context, fidelity, exposure, and (ii) study related elements, i.e. acceptability of recruitment processes, outcome measures and contamination. It will also develop and refine the programme theory.

Quantitative process data will include adherence to the diet, measured via the finger prick blood tests to see if ketosis has been achieved. The dietitian will also complete weekly fidelity assessment of intervention delivery by the dietitians, using a fidelity checklist at the weekly update, which could be over the phone or via email. This will measure the extent to which the KD was delivered as intended.

Semi-structured telephone interviews will take place with 15 patients and 5 staff after the ten- week intervention period is complete and will take 40-60 minutes. Participants will be approached by telephone about taking part, and if they agree they will be sent an information sheet and consent form. At the interview the consent form will then be reviewed, and an audio recording of their consent to take part will be made and stored separately from the interview data. Patients will be purposively sampled to include a range gender, age, socioeconomic status and engagement with the intervention. Staff will be sampled to include dietitians from both centres and other health professionals including psychiatrists. Interviews will explore the acceptability of the evaluation methods and intervention; impact of the intervention; barriers and facilitators, contamination, suggestions for improvements as well as contextual factors influencing intervention impact. Interviews will be audio recorded on encrypted devices (to AES256 standard or above) and transcribed verbatim.

Qualitative data will be analysed using thematic analysis. Quantitative data will be analysed descriptively.

3.8 ECONOMIC EVALUATION

The economic evaluation will identify, measure and value the resource use and health related quality of life (HRQOL) impacts associated with the ketogenic diet in people with bipolar disorder. With input from PPI representatives a tailored health economics questionnaire for people with BD will be developed to measure typical service use patterns, employment, productivity, income levels, household food expenditure and health related quality of life using the EuroQol EQ-5D 5L instrument (www.euroqol.org) at baseline and all follow up. All staff time, equipment and consumable resources involved in delivering the intervention will also be identified and measured with input from the research team. For the intervention costs, all clinical (dietary and psychiatric) supervision (through checking adherence to prescribed diets, solving problems, and

identifying any adverse effects etc) will be measured and valued. The dietitian support over the 8-week dietary intervention period (plus 2-week cessation period) will be identified, measured, and valued.

In the future trial an NHS and personal social services (PSS) perspective will be adopted for the base case analysis as per NICE guidance however a sensitivity analysis will explore a full societal perspective so as to quantify the likely societal impacts of the intervention compared to TAU. Readily available unit costs such as NHS Reference costs (https://improvement.nhs.uk/resources/reference-costs/) and personal social services research unit (PSSRU) costs (Curtis 2019) will be attached to measured resource use. The within trial pilot analyses will be reported and presented as an exploratory incremental cost-utility ratio with the joint distribution of cost/utility pairs being represented on the cost-effectiveness plane and with a cost-effectiveness acceptability curve (CEAC) (Fenwick 2001) employing a range of UK willingness to pay thresholds.

3.9 PATIENT AND PUBLIC INVOLVEMENT

Public involvement has informed our planning and research design from the earliest stages through consulting with online and offline BD networks. We participated in several large public online BD forums and performed a text mining study to gather all posts mentioning experiences on a KD. We performed a formal text mining analysis of 274 individual reports [Campbell BJP Open 2019]. The many common experiences with KD in the BD population have informed our research design.

Dr Graeme Bowman from Bipolar Scotland (national charity for people who live with bipolar disorder) has provided key insights to inform our recruitment strategy and planning of proposed interaction with patient and public groups. Bipolar Scotland were consulted on strategies for raising awareness of the study, recruitment and intervention adherence and contributed an article summarising KD research for their newsletter ahead of a visit to give a talk and host a question-and-answer session. Dr Bowman will remain on the project team throughout the pilot study, which will have patient and public involvement at its centre, as ensuring all aspects of the study are acceptable to participants is vital.

We have given 4 talks to BD local support groups on the ketogenic diet including question and answer sessions. Recently we presented a talk on ketogenic diet at the Bipolar Scotland annual conference. Dr Iain Campbell has been in regular communication with members to discuss what they would like to see in a trial of KD.

Iain Claque, their Mental Health Food and Nutrition representative, has also agreed to give advice and support. We will form a study PPI group including representation from Bipolar Scotland and The Scottish Association for Mental Health (SAMH) groups and have identified a PPI Lead who will be funded 0.2 WTE throughout the project to lead PPI activities and regular meetings and to develop a 10 episode online video series with content (e.g. detailing experiences on a KD) to be defined following PPI consultations. Our PPI group will advise on all aspects of the study including the intervention, recruitment, retention, outcomes measures and dissemination.

3.10 PARTICIPANT REIMBURSEMENT

Study participants will be reimbursed any travel expenses. This is an issue that members of Bipolar Scotland raised during PPI meetings, and reimbursement will

hopefully alleviate their concerns regarding travel costs. We may provide 2 weeks' worth of ketogenic meals for participants at the beginning, or more for those unable to cook for themselves. This will be paid for by the research team due to the increased cost of pre-prepared meals which would not be fair to pass over to participants. We will pay for the use of any phone applications which are required to be used by participants, and they will not incur any costs. We will also pay for accurate kitchen scales for participants.

This is in line with the reimbursement model whereby participants should not have to make any financial sacrifices. They will not receive any incentive or compensation payments.

3.11 SAMPLE STORAGE

The metabolomics team will store sample extracts for 5 years in their department before destroying them. They will be kept in a pseudonymised form, identifiable only by participant study number and visit number to the metabolomics team. They will only be accessed by authorised personnel at the metabolomics department. The original samples taken for metabolomic extraction will be stored at University of Edinburgh laboratory for 5 years.. The remaining blood sample will be stored and may be used anonymously or pseudonymously in future for other research on the ketogenic diet and mental health, subject to review by a Research Ethics Committee.

Biochemistry samples will be kept in the biochemistry department at the Royal Infirmary of Edinburgh and destroyed after 5 days as per standard laboratory procedure. They will only be accessed by authorised personnel in the biochemistry department. These samples will be identifiable, as they will be labelled with the patients CHI number, their unique health record identifier in Scotland.

4 STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

This study will aim to recruit 25 patients meeting the study criteria and consenting to take part.

4.2 INCLUSION CRITERIA

- Meet the DSM-4 diagnostic criteria for bipolar disorder type 1 or 2, for at least 1 year, assessed using the MINI Neuropsychiatric Interview
- Clinically stable and currently euthymic, defined as 3 months with no major mood episodes (major depression lasting at least 2 weeks or hypomania/mania lasting at least 1 week), assessed using the MINI Neuropsychiatric Interview
- Aged 18 to 70 years (upper age limit set due to increased risk of age-related changes over 70 making interpretation of brain imaging more difficult – we will aim to increase the upper age limit in a full clinical trial).
- Able to provide informed consent to take part in the study
- Able to speak, read and understand English to a level whereby they can understand the participant information sheet (PIS). (This is due to the

practicalities of implementing translation services for such a small pilot study, however we would hope to include non-English speaking participants in a future larger clinical trial).
- Currently living in Scotland

4.3 EXCLUSION CRITERIA

- Pregnancy or breastfeeding (or those planning to become pregnant within 3 months)
- Active substance misuse with alcohol or illicit drugs
- Use of the ketogenic diet in the previous 2 months
- Currently following a vegan diet
- Admission to hospital within the past 3 months
- Current involvement in another research study (This feasibility study is of moderate intensity and requires daily monitoring and a significant dietary intervention requiring careful planning. As such it would not be appropriate for participants involved in other current research to take part due to the potential high level of burden on them. Those involved in recent research that has ended by the time of recruitment to the pilot study would be eligible, and will be made fully aware of the level of engagement required to allow them to make an informed decision)
- Inability to complete baseline assessments
- Liver or kidney disease
- Cardiovascular disease
- Severe hyperlipidaemia
- Type 1 diabetes
- Insulin dependent type 2 diabetes
- Currently training for or undertaking very high energy requirement activities (as judged by the study dietitian)

5 PARTICIPANT SELECTION AND ENROLMENT

5.1 IDENTIFYING PARTICIPANTS

Recent consultation with Bipolar Scotland members and our investigation of online BD fora suggests that there is considerable interest among patient groups in dietary interventions for BD.

The identification of eligible patients and recruitment will be supported by Bipolar Scotland and the Scottish Mental Health Research Network via a call for volunteers. We will present the study to Bipolar Scotland groups in central Scotland and tailor social media and conventional media approaches. This will involve promoting a social media poster. We will ask potential participants to contact the research team if they are interested in taking part, and only then will they be contacted directly.

If necessary, we will also identify potential participants through psychiatric outpatient clinics in NHS Lothian. This would be led by the Scottish Mental Health Network working with clinicians to facilitate the recruitment of patients from outpatient clinics. Participants would only be approached by their consultant or responsible medical officer inviting them to take part. Potential participants would be identified by clinicians reviewing their caseloads and potentially individual medical records to determine eligibility.

Potential participants will be sent a patient approach letter and a participant information sheet (PIS). They will be asked to contact the research team if they would like to take part, to arrange an initial online meeting (using the secure platform Near Me) or telephone call with a psychiatrist and/or dietitian. This will be to determine if they meet the eligibility criteria, and to answer any questions they may have about the PIS or the study. If they would still like to take part, they will be asked for some basic information (name, date of birth, and address, CHI number if known) to allow the Clinical Research Facility (CRF) to register them on NHS Lothian's clinical system (TRAK) and book them into the facility. An appointment will then be arranged at the CRF to sign the consent form and answer any further questions. If they would still like to take part, they will then undergo baseline assessments. Participants will be asked to compete a 3 day food diary and pre-ketogenic diet and nutrition history and send these back to the team prior to attending their first appointment. If they decide not to take part these will be immediately destroyed.

We aim to recruit a total of 25 patients over a 6-month period. There is very little experience of introducing a KD in this patient group, thus requiring a phased approach to tailor the intervention and gain experience. There will be no restrictions on recruitment by any of the protected characteristics recognised by NIHR. Potential participants will need to be able to understand English well enough to read the PIS and take part in interviews. This is due to the practicalities of implementing translation services for such a small pilot study, however we would hope to include non-English speaking participants in a future clinical trial.

Recruitment will take place at the Clinical Research Facility, Royal Infirmary of Edinburgh and in the Department of Psychiatry, University of Edinburgh.

5.2 CONSENTING PARTICIPANTS

The Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. Consent must be gained from each participant and recorded on a consent form. Participants must be provided with a participant information sheet (PIS) at least 3 days prior to their initial phone assessment, which will detail in full what they can expect from the study. They must be given an opportunity to discuss this during the initial phone consultation, and to have any questions about it answered. A summary of the research project must be provided by the researcher, and cover all elements specified in the PIS and consent form. At a subsequent face to face appointment with a psychiatrist, potential participants must again be given the opportunity to ask questions and check their understanding of any points on the consent form before signing this. It should be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled. Please see consent form for specific consent points (appendix 3).

The researcher taking consent must have completed core psychiatry training (Membership of the Royal College of Psychiatrists). They must also have undergone training in Good Clinical Practice. The principles of the Adults with Incapacity (Scotland) Act 2000 must be followed at all times. For the purposes of the Act, "incapable" means being unable to act on, make, communicate, understand, or retain the memory of decisions. If there are concerns that a potential participants capacity is borderline or likely to fluctuate, they must not be included in the study. All efforts must be made to avoid coercion and consent must be entirely voluntary, with potential participants allowed as much time as they need to decide. Potential participants must not have any identifiable dependent relationship to the researchers.

The participant will be informed and agree to their medical records being inspected by regulatory authorities and representatives of the sponsor(s). The Investigator or delegated member of the trial team and the participant will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The participant will receive a copy of this document and a copy filed in the Investigator Site File (ISF) and participant's GP.

5.2.1 WITHDRAWAL OF STUDY PARTICIPANTS

Participants will be informed via the Participant Information Sheet that their participation is voluntary, and that they are free to withdraw from the study at any time. Information already collected will still be used when preparing the results of the study, unless the participant tells us they don't want their information to be included. Once the study has finished and the data has been analysed for publication, participants will no longer be able to withdraw their information.

If a participant lost capacity during the study, it would not be appropriate to continue the study intervention as ongoing consent would be required. Data already collected would still be used.

5.3 CO-ENROLLMENT

In accordance with the co-enrolment policy (http://www.accord.scot/sites/default/files/POL008%20Co-enrolment%20v1.0.pdf), participant burden and the potential impact on the study endpoint has been considered. As such, being enrolled in another interventional study at the time of registration is not permitted, due to its potential impact on the participant and the outcome of the study. Participation in this pilot study requires moderately intensive input. Participants will be required to follow a completely new dietary pattern which will require planning and will impact on their day-to-day life. They will be required to engage in daily monitoring of several parameters and will need to attend lengthy baseline and follow up appointments. As such it would not be appropriate for participants involved in other current research to take part due to the potentially high level of burden on them.

Those involved in recent research that has ended by the time of recruitment to the pilot study would be eligible, and will be made fully aware of the level of engagement required to allow them to make an informed decision.

6 RISK ASSESSMENT

6.1 UNEXPECTED ADVERSE EVENTS

If unexpected significant adverse effects became apparent from the ketogenic diet in the pilot study, then immediate consideration would be given to stopping the study early by the principal investigators.

The research team will have regular contact with participants in the study. If any significant or potentially dangerous issues arise which may be of general relevance, then all participants will be informed by phone and letter. These include both self-

reported issues, and issues that may arise from the tests conducted or data collected as part of the study

7 DISTRESS PROCEDURE

Participants may find some questions in the questionnaires sensitive in nature, as they will be asked detailed questions about their mental health both past and present. Where there is any clinical risk to the participant (for example the disclosure of suicidal thoughts) the interviewing psychiatrist will ask for consent to contact the patient's GP and treating community mental health team so that appropriate monitoring and follow-up can be arranged.

8 DATA COLLECTION

Data will be collected by members of the research team at the following points:

- Baseline appointments
- Throughout the intervention period
- At follow up appointments
- During imaging and metabolomics analyses
- During semi structured interviews as part of the process evaluation

The following data will be collected and kept in a pseudonymised form:

- Age
- Gender
- Medical history
- Medication history
- Drug and alcohol use
- Mental health questionnaire scores
- Health economics questionnaire scores
- Height (before and after)
- Weight (before and after)
- BP (before and after)
- Biochemistry results – LFTs, U&Es, lipid profile, CRP, insulin, glucose, beta-hydroxybutyrase (before and after)
- Metabolomics analysis results (before and after)
- MRI brain scan analysis results (before and after)
- Fidelity testing scores
- Process evaluation interview content

8.1 SOURCE DATA DOCUMENTATION

Mental health questionnaires
Health economics questionnaires
Fidelity checklists
Actigraph reports
Ketomojo reports

Ilumivu reports

Text messages (may be used in place of ilumivu and ketomojo in some circumstances)

8.2 CASE REPORT FORMS

Certain data will be inputted by the research team onto a paper case report form. This will include age, gender, medical history, medication history, drug and alcohol use, mental health questionnaire scores, health economics questionnaire scores, height, weight, BMI, BP, biochemistry test results and fidelity checklist results.

9 DATA MANAGEMENT

9.1 PERSONAL DATA

Personal data collected in the study will include participant name, contact number, CHI number, address, and email address. These will be recorded alongside allocated participant study number. We will store these, along with signed consent forms, separately from pseudonymised data in a locked filing cabinet in a locked office at the Kennedy Tower, Royal Edinburgh Hospital (University of Edinburgh), and Clinical Research Facility, Royal Infirmary of Edinburgh, and they will be destroyed after 12 months. Only the research team members will have access to this, which includes investigators and collaborating groups who have been approved by the study investigators. University of Edinburgh policy regarding data management will be followed at all times (https://www.ed.ac.uk/information-services/about/policies-and-regulations/research-data-policy). The University of Edinburgh will need to share with the University of Glasgow basic participant personal data, including participant name, number, email and study ID, so that they can contact participants directly for the process evaluation. This will be sent in an encrypted form via a secure university file sharing platform, with participant consent, and deleted once the process evaluation is complete.

Any changes to the transfer or storage of NHS Lothian data will be approved by NHS Lothian IG/IT security.

9.2 DATA STORAGE

No data will be shared without explicit participant consent via the attached consent form. Data will be pseudonymised, and the principles of data minimisation will always be followed.

Pseudonymised data for research purposes will be password protected and stored on an encrypted University of Edinburgh or University of Glasgow (UoG) server, which will only be accessed by the research team using a secure username and password.

All identifiable paper records (e.g. signed consent forms and protocol number allocations) will be stored separately from blind data in a locked filing cabinet in a locked office at the Kennedy Tower, Royal Edinburgh Hospital (University of Edinburgh), and deleted 12 months after the end of the study. A copy of participants name and contact details will also be kept on a secure NHS Lothian server to allow

researchers to access this when needed during the study period. This may also be emailed using secure NHS email addresses to members of staff at the clinical research facility to allow them to correctly register new participants for the study, and identify them for phlebotomy. Personal data will also be emailed via secure NHS Lothian email to the imaging department as part of their standard procedure to correctly identify participants for MRI brain scanning.

Individual participant personal data will be shared with the UoG following participant completion of the study, to allow UoG to contact them for the process evaluation. As soon as the process evaluation is completed, this personal data will be deleted by the UoG.

Participant data used for the process evaluation and economic evaluation at the University of Glasgow (UoG) will also be stored in a password protected network drive at the UoG. Permissions will be set up so that only appropriate staff have access (named researchers and project members). All personal information (e.g. names, locations) will be anonymised and strict data protection policies will be followed as outlined in the University of Glasgow's data protection policy.

Audio recordings for process evaluation at the University of Glasgow will be stored on secure encrypted network drives until they are transcribed by the research team, and then they will be deleted. Transcriptions will be anonymised and retained for 10 years as per University of Glasgow policies.

Metabolomic blood test results will be identifiable only by participant identification number. Once results are collated they will be password protected and stored on a secure University of Edinburgh server, accessible only by members of the research team.

The research team will access participant's medical records using their unique health identifier (CHI number). This will allow them to access the results of any biochemistry blood tests that have been done as part of the study. Participants will be informed of this through the PIS and consent forms. MRI brain scans will also be available to view in participants medical records. They will additionally be backed up on secure University of Edinburgh back up servers in an identifiable form, and on a University of Edinburgh server in pseudonymised form, using participant ID, for the research team to access for analysis.

We will request that ketomojo and ilumivu physically delete all research data 3 months after the end of the study. Ketomojo have confirmed that all user information will be destroyed 30 days after a deletion request.

Any text messages with study data contained in them will be deleted from UoE or NHS work phones as soon as this data is entered onto the secure UoE server.

We will ensure published data does not contain any information that could disclose participant identity. Published results will be a descriptive analysis of aggregate results.

9.3 TRANSFER OF DATA

No data will be shared without explicit participant consent via the attached consent form.

Participants will be asked for their GP's name, and for consent to inform them that they are taking part in the study, and of any notifiable results. This information will be posted to the GP.

Metabolomic and imaging data will be processed separately in their respective departments, and the results shared via a secure University of Edinburgh server. All data will be fully anonymised, using only participant study number to identify them. Pseudonymised data from phone apps and actigraph device reports will be downloaded to a secure University of Edinburgh server by the research team, only accessible by them.

The University of Edinburgh will need to share with the University of Glasgow basic participant personal data, including participant name, number and study ID, so that they can contact participants directly for the process evaluation. This will be sent in an encrypted form via a secure university file-sharing platform, and with participant consent.

Collaborators at the University of Glasgow (UoG) will share pseudonymised data via a secure university file sharing platform with the University of Edinburgh, and vice versa. This will include the results of health economics questionnaires, fidelity checklist results, and final analyses from the UoG. This will be not shared outside of the research team.

9.4 DATA RETENTION

Personal data will be kept for a period of 12 months, and pseudonymised data for a period of 10 years. This is essential, as the research team hope to plan a future clinical trial based on this pilot study.

9.5 DISPOSAL OF DATA

Data will be deleted from University of Edinburgh and University of Glasgow secure servers or data stores following the retention period above.

9.6 DATA CONTROLLER

The University of Edinburgh and NHS Lothian are joint data controllers along with any other entities involved in delivering the study that may be a data controller in accordance with applicable laws (e.g. the site)

9.7 DATA BREACHES

Any data breaches will be reported to the University of Edinburgh and NHS Lothian Data Protection Officers who will onward report to the relevant authority according to the appropriate timelines if required.

9.8 DATA PROTECTION TRANSPARENCY

Participants will be fully informed about how their data will be used during the study, through the participant information sheet and the consent form. They will be informed that personal data will be kept separately and will be destroyed after 12 months.

9.9 DATA STATUS – APPROVALS/CONSENT

Participants will be asked to provide consent for their data to be shared between the University of Edinburgh (UoE) and the University of Glasgow (UoG), and between the UoE and NHS lothian. A contract will be in place between the UoE and UoG.

9.10 CONFIDENTIALITY

Biochemistry results from study blood samples will be accessed from NHS medical records and inputted into pseudonymised case report forms with participants consent.

All data collected as part of the study will be kept confidential, and will only be accessible to the research team.

10 STATISTICS AND DATA ANALYSIS

10.1 SAMPLE SIZE CALCULATION

No formal sample size was calculated as this is a small feasibility study. We are not planning to draw firm conclusions about the efficacy of the diet.

10.2 PROPOSED ANALYSES

This is a feasibility study assessing whether participants can adhere to the ketogenic diet and whether they can take part in the proposed baseline and outcome assessments. We will quantify and describe changes in the baseline and outcome assessments in this small sample of participants using descriptive approaches.

The analysis of the metabolomics data will be conducted by data analysis staff in the university metabolomics unit overseen by Dr Karl Burgess (Senior Lecturer in Biological Mass Spectrometry) and Dr Tessa Moses (Metabolomics Specialist and Facility Manager EdinOmics). The brain imaging data analysis will be conducted by data imaging analysis staff in the University imaging centre overseen by Dr Gerry Thompson. These will follow standard analytic pipelines established in these units. The process evaluation analyses will be conducted in the Social and Public Health Sciences Unit at the University of Glasgow, overseen by Professor Sharon Smith. The health economics analyses will be conducted in the Institute of Health and Wellbeing at the University of Glasgow, overseen by Professor Emma McIntosh.

Other statistical aspects of the study will be conducted by statistics staff in the Edinburgh clinical trials unit and Usher institute overseen by Professor John Norrie, Chair of Medical Statistics, University of Edinburgh, and grant holder. Since this is a small pilot study these analyses will be largely descriptions of response, adherence, and distributions of parameter values. Simple correlations between study parameters will follow standard statistical methods, which will be detailed in a comprehensive Statistical Analysis Plan authored by Professor Norrie.

11 ADVERSE EVENTS

The Investigator, or a delegated researcher, is responsible for the detection and documentation of adverse events that may be related to participating in the study and that meet the criteria and definitions detailed below.

11.1 DEFINITIONS

An **adverse event** (AE) is an untoward medical occurrence in a study participant.
An **adverse reaction** (AR), in the context of this study, is any untoward and unintended response which is related to the ketogenic diet administered to that participant.
A **serious adverse reaction** (SAR) is any AR that:

- results in death of the clinical trial participant; is life threatening*;
- requires in-patient hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity;
- consists of a congenital anomaly or birth defect;
- results in any other significant medical event not meeting the criteria above.

A **suspected unexpected serious adverse reaction** (SUSAR) is any AR that is classified as serious and is suspected to be caused by the ketogenic diet.

*Life-threatening in the definition of an SAE or SAR refers to an event where the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

^Any hospitalisation that was planned prior to randomisation will not meet seriousness criteria. Any hospitalisation that is planned post randomisation will meet the seriousness criteria unless it does not constitute an untoward medical occurrence (e.g. cosmetic elective surgery, social and/or convenience admission, etc.).

11.2 IDENTIFYING AEs AND ARs

All AEs and ARs will be identified from the time a participant signs the consent form to take part in the study until the completion of study follow-up.

11.3 RECORDING AEs AND ARs

AEs will be recorded as part of the outcome measures in the study CRF. There is no requirement to complete an additional AE form. When an AE occurs, it is the responsibility of the Investigator to review all documentation (e.g. hospital notes, laboratory and diagnostic reports) related to the event. To the extent the CRF permits, the Investigator will record relevant safety information in the CRF.

Any adverse reaction (AR) to the ketogenic diet that meets seriousness criteria (see section 9.1) will be recorded and reported in the study CRF and will also be recorded on an ACCORD SAE form, which will then be sent to the Sponsor via email (safety@accord.scot).

Pre-existing medical conditions (i.e. existed prior to informed consent) should be recorded as medical history and only recorded as AEs/ARs if medically judged to have unexpectedly worsened during the study. Events that are consistent with the expected progression of underlying disease should not be recorded as adverse events.

11.4 ASSESSMENT OF AEs, SAEs, ARs, SARs and SUSARs

Seriousness, causality, severity and expectedness will be assessed by the PI.
The Investigator is responsible for assessing each adverse event. The CI may not downgrade an event that has been assessed by an Investigator as a SAR or SUSAR, but can upgrade an AR, SAR or SUSAR if appropriate.

11.4.1 Assessment of Causality

The Investigator will make an assessment of whether an AE is likely to be related to the administration of the ketogenic diet (and therefore be considered an AR) according to the definitions below.

- Unrelated: Where an event is not considered to be related to the administration of the ketogenic diet.
- Possibly Related: The nature of the event, the underlying medical condition, concomitant medication or temporal relationship make it possible that the AE has a causal relationship to the administration of the ketogenic diet in this study.

11.4.2 Assessment of Seriousness

Subsequent to the assessment causality, the Investigator will make an assessment of seriousness as defined in Section 9.1.

11.4.3 Assessment of Severity

The Investigator will make an assessment of severity for each SAR, and record this on the ACCORD SAE form according to one of the following categories:
Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with every day activities.
Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.
Severe: an event that prevents normal everyday activities.
Note: the term ‘severe’, used to describe the intensity, should not be confused with ‘serious’ which is a regulatory definition based on participant/event outcome or action criteria. For example, a headache may be severe but not serious, while a minor stroke is serious but may not be severe.

11.4.4 Assessment of Expectedness of SARs

The Investigator will make an assessment of expectedness of any SARs identified following the ketogenic diet administration.

11.5 REPORTING OF SARs/SUSARs

As this trial is a non-CTIMP and involves procedures and interventions that are very well established in the medical community, with extensive information available regarding risks, only serious adverse reactions (SARs) and Suspected Unexpected

Serious Adverse Reactions (SUSARs) related to the ketogenic diet administration, will be onward reported to the Sponsor.

Once the Investigator becomes aware that a ketogenic diet related SAR/SUSAR, has occurred in a study participant, the information will be reported to the ACCORD Research Governance & QA Office within 24 hours. If the Investigator does not have all information regarding an event, they should not wait for this additional information before notifying ACCORD. The ACCORD SAE report form will be used to submit the event report, and can be updated when the additional information is received.

The SAE form will be transmitted by fax to ACCORD on +44 (0)131 242 9447 or may be submitted by hand to the office or sent via email to Safety.Accord@ed.ac.uk. Only forms in a pdf format will be accepted by ACCORD via email.

Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact the investigator and request the missing information.

All reports sent to ACCORD and any follow up information will be retained by the Investigator in the Investigator Site File.

11.6 REPORTING REQUIREMENTS

The ACCORD Research Governance & QA Office is responsible for reporting on behalf of the co-sponsors (Edinburgh University and NHS Lothian).
The Trial Manager will provide the ACCORD Research Governance & QA Office with quarterly safety reports based on the data collected on the CRF.

11.7 FOLLOW-UP PROCEDURES

After initially reporting a ketogenic diet related SAR/SUSAR, the Investigator will follow each participant until resolution or the completion of study follow-up. Follow-up information will be reported to the ACCORD office.

12 OVERSIGHT ARRANGEMENTS

12.1 INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit study related monitoring and audits on behalf of the sponsor, REC review, and regulatory inspection(s). In the event of audit or monitoring, the Investigator agrees to allow the representatives of the sponsor direct access to all study records and source documentation. In the event of regulatory inspection, the Investigator agrees to allow inspectors direct access to all study records and source documentation.

12.2 STUDY MONITORING AND AUDIT

The ACCORD Sponsor Representative will assess the study to determine if an independent risk assessment is required. If required, the independent risk assessment will be carried out by the ACCORD Quality Assurance Group to determine if an audit should be performed before/during/after the study and, if so, at what frequency.

Risk assessment, if required, will determine if audit by the ACCORD QA group is required. Should audit be required, details will be captured in an audit plan. Audit of Investigator sites, study management activities and study collaborative units, facilities and 3rd parties may be performed.

13 GOOD CLINICAL PRACTICE

13.1 ETHICAL CONDUCT

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP).

Before the study can commence, all required approvals will be obtained and any conditions of approvals will be met

13.2 INVESTIGATOR RESPONSIBILITIES

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of Good Clinical Practice (GCP), the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff.

13.2.1 Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants must receive adequate oral and written information – appropriate Participant Information and Informed Consent Forms will be provided. The oral explanation to the participant will be performed by the Investigator or qualified delegated person, and must cover all the elements specified in the Participant Information Sheet and Consent Form.

The participant must be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant must be given sufficient time to consider the information provided. It should be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The participant will be informed and agree to their medical records being inspected by regulatory authorities and representatives of the sponsor(s).

The Investigator or delegated member of the trial team and the participant will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The participant will receive a copy of this document and a copy filed in the Investigator Site File (ISF) and participant's medical notes (if applicable).

13.2.2 Study Site Staff

The Investigator must be familiar with the protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the protocol and their trial related duties.

13.2.3 Data Recording

The Principal Investigator is responsible for the quality of the data recorded in the CRF at each Investigator Site.

13.2.4 Investigator Documentation

The Principal Investigator will ensure that the required documentation is available in local Investigator site files ISFs.

13.2.5 GCP Training

For non-CTIMP (i.e. non-drug) studies all researchers are encouraged to undertake GCP training in order to understand the principles of GCP. However, this is not a mandatory requirement unless deemed so by the sponsor. GCP training status for all investigators should be indicated in their respective CVs.

13.2.6 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant. The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished information, which is confidential or identifiable, and has been disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

13.2.7 Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of the appropriate data protection legislation (including the General Data Protection Regulation and Data Protection Act) with regard to the collection, storage, processing and disclosure of personal information.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data and be of a form where individuals are not identified, and re-identification is not likely to take place

14 STUDY CONDUCT RESPONSIBILITIES

14.1 PROTOCOL AMENDMENTS

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant in the case of an urgent safety measure, must be reviewed and approved by the Chief Investigator.

Amendments will be submitted to a sponsor representative for review and authorisation before being submitted in writing to the appropriate REC, and local R&D for approval prior to participants being enrolled into an amended protocol.

14.2 MANAGEMENT OF PROTOCOL NON-COMPLIANCE

Prospective protocol deviations, i.e. protocol waivers, will not be approved by the sponsors and therefore will not be implemented, except where necessary to eliminate an immediate hazard to study participants. If this necessitates a subsequent protocol amendment, this should be submitted to the REC, and local R&D for review and approval if appropriate.

Protocol deviations will be recorded in a protocol deviation log and logs will be submitted to the sponsors every 3 months. Each protocol violation will be reported to the sponsor within 3 days of becoming aware of the violation. All protocol deviation logs and violation forms should be emailed to QA@accord.scot

Deviations and violations are non-compliance events discovered after the event has occurred. Deviation logs will be maintained for each site in multi-centre studies. An alternative frequency of deviation log submission to the sponsors may be agreed in writing with the sponsors.

14.3 SERIOUS BREACH REQUIREMENTS

A serious breach is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the participants of the trial; or

(b) the scientific value of the trial.

If a potential serious breach is identified by the Chief investigator, Principal Investigator or delegates, the co-sponsors (seriousbreach@accord.scot) must be notified within 24 hours. It is the responsibility of the co-sponsors to assess the impact of the breach on the scientific value of the trial, to determine whether the incident constitutes a serious breach and report to research ethics committees as necessary.

14.4 STUDY RECORD RETENTION

All patient identifiable documentation will be kept for 12 months. All pseudonymised data will be kept for 10 years from the protocol defined end of study point. When the minimum retention period has elapsed, study documentation will not be destroyed without permission from the sponsor.

14.5 END OF STUDY

The end of the study will be defined once all blood samples have been processed and all analyses have been completed. This will likely be around 4 months after the last participant contact.

The Investigators or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the REC and sponsor within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved. End of study notification will be reported to the co-sponsors via email to resgov@accord.scot

A summary report of the study will be provided to the REC within 1 year of the end of the study.

14.6 CONTINUATION OF TREATMENT FOLLOWING THE END OF STUDY

All participants will be free to continue with the ketogenic diet (KD) following the end of the study if they wish, as this is not something which needs to be prescribed and many members of the public already follow it. They will have had support around initiating the KD as part of the study from a dietitian and psychiatrist and will be able to keep menu plans and information they have been given. They will have had 10 weeks of support in total which should have allowed them to identify and manage any acute adverse effects. They will also receive education about the potential longer-term effects of continuing the diet, which will also be detailed on the Participant Information Sheet.

After the 10-week study period has ended we will provide ongoing dietitian support for a further 4 weeks, during which time participants will still be able to contact the dietitian within working hours with any queries or concerns they may have. The dietitian will continue to make weekly contact during this time if this is deemed desirable by both parties.

14.7 INSURANCE AND INDEMNITY

The Sponsors are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the co-sponsors' responsibilities:

- The Protocol has been designed by the Chief Investigator and researchers employed by the University of Edinburgh and collaborators.
- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the sites concerned. The co-sponsors require individual sites participating in the study to arrange for their own insurance or indemnity in respect of these liabilities.
- Sites which are part of the United Kingdom's National Health Service will have the benefit of NHS Indemnity.

15 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

15.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team.

15.2 COMMUNICATION AND DISSEMINATION OF RESULTS

We will communicate findings to academic, policy, patient and public audiences via diverse channels, including peer reviewed scientific journals, conference presentation and publication on our website. We will publish study protocols and results . We will ensure there is dissemination of findings to patients via patient organisations and professional groups and explore with them the opportunity for translation of findings.

Participants will be sent a report detailing their initial and repeat blood pressure and BMI measurements, a summary of their actigraph data, and their glucose and ketone measurements by post, as soon as possible after their involvement in the trial has ended. They will not routinely be sent biochemical blood test or MRI scan results, but if any results are out of the normal reference range, their GP will be informed to decide if any further action is required.

Participants will be sent a patient friendly report of the main findings/results from the study by post, once the results have been analysed. Additionally, we will provide feedback sessions to Bipolar Scotland groups in an easily understandable format. A lay summary of the study results will be posted on the study website.

16 REFERENCES

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17 Appendices

17.1 Appendix 1: Pilot study flowchart

17.2 Appendix 2: Participant pathway infographic

17.3 Appendix 3: Metabolomics extraction protocol – cell prep

17.4 Appendix 4: Metabolomics extraction protocol – serum prep