Title- **Evaluating the therapeutic efficacy and metabolic impact of a low energy diet (LED) in people with familial partial lipodystrophy and diabetes**

Short Title- Low Energy Diet and Familial Partial Lipodystrophy

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Contents

1. Background and Rationale

Lipodystrophy is a rare condition characterised by a paucity of body fat due to the absence or dysfunction of adipocytes (fat cells). It can be inherited or acquired in origin and can result in a total (generalised lipodystrophy) or partial loss of adipose tissue (partial lipodystrophy). It has been estimated that there are 1.3–4.7 cases of lipodystrophy per million people worldwide (Dhankhar 2017). Due to the lack of fat storage capacity, excess lipid accumulates in other tissues such as the liver, pancreas and muscle where it impairs insulin action and contributes to the pathogenesis of several metabolic disorders including diabetes, non-alcoholic fatty liver disease and pancreatitis.

As fat transplantation or correction of the underlying genetic defect have yet to become viable therapeutic options, current therapeutic approaches focus on preventing and managing the metabolic complications of lipodystrophy using standard approaches used to treat obese patients with similar metabolic problems including agents such as metformin, gliptins, statins, fibrates and/or insulin (Brown et al. 2016). Advice for the dietary management of lipodystrophy is based on the evidence available from studies in patients with type 2 diabetes, cardiovascular disease and high triglycerides (Stears & Hames 2014). The general advice for all patients with lipodystrophy includes calorie and fat restriction (Brown et al. 2016). However, this approach is currently based on clinical experience rather than experimental evidence. Four case reports on patients with congenital generalised lipodystrophy describe improvements in blood glucose and triglycerides after restricting calorie and fat intake (Joannie et al. 2017; Kazlauskaite et al. 2001; Santos & Chvng 2017; Keenan et al. 1980), however there are currently no published studies.

Our group runs the only specialist NHS England designated service for patients with lipodystrophy at CUHFT (Cambridge University Hospitals Foundation Trust; website https://www.cuh.nhs.uk/national-severe-insulin-resistance-service). Our current dietetic approach includes specialist input from two dietitians who have been working with the multidisciplinary team since its inception in 2012. They focus on assessing current energy intake and macronutrient balance, and then attempt to assist patients in significantly reducing total energy intake. In general this is very helpful in improving the metabolic consequences of lipodystrophy – specifically NAFLD, dyslipidaemia (particularly hypertriglyceridaemia) and glycaemic control – in patients who adhere to the dietary advice. However, dietary compliance remains very challenging as the lipodystrophy itself leads to total or relative deficiency of leptin, a key hormone in the regulation of appetite. Lack of leptin is well known to promote food intake, so our patients have to work very hard to adhere to dietary calorie restriction. We also currently lack published evidence to support our approach, hence our interest in undertaking a formal study to document the impact of dietary calorie restriction. However, we and others have shown that severely limiting calorie intake by means of bariatric (weight loss) surgery is safe and highly effective in alleviating metabolic disease in lipodystrophic patients (Melvin et al. 2017; McGrath & Krishna 2006; Utzschneider & Trence 2006; Ciudin et al. 2011; Kozusko et al. 2015; Grundfest-Broniatowski et al. 2017). Unfortunately access to bariatric surgery remains limited in the UK currently and it is an expensive, 'fairly radical' option.

Recent results from the DIRECT study (Lean et al. 2017) reported substantial improvements in HbA1c, triglycerides and a reduction in antidiabetic medication for people with type 2 diabetes that followed a structured weight management programme. The DIRECT study recruited people that had been diagnosed with type 2 diabetes within the last 6 years and who were not receiving insulin, they were then randomised into two groups; one group were provided with a weight management

programme while the other received best practice by care guidelines. The weight management programme comprised of a total diet replacement (825–853 kcal/day formula diet for 3–5 months), stepped food reintroduction (2–8 weeks), and structured support for long-term weight loss maintenance. Diabetes remission was achieved in 46% of the intervention group compared to 4% in the control group (Lean et al. 2017). This approach has now been validated in a series of carefully planned and run studies and is being rolled out into general practice in the UK currently. We typically advise calorie restriction to a similar level in our patients so would like to formally assess this intervention in a study in our lipodystrophic patients.

The fact that this dietary intervention is very likely to rapidly alleviate insulin resistance in patients with FPLD – based on what has been seen in obese patients with T2DM in response to LEDs and in FPLD patients in response to bariatric surgery (Melvin et al. 2017)– also provides a unique opportunity to evaluate underlying mechanisms of insulin resistance in these patients. Despite decades of research (Petersen & Shulman 2018) the molecular mechanisms responsible for human insulin resistance remain unclear. Suggested defects include diacylglycerol (DAG) or ceramide accumulation and subsequent activation of PKCtheta in skeletal muscle (PKCepsilon in liver) which is in turn proposed to phosphorylate the insulin receptor. However, considerable debate continues about this and other hypotheses. The fact that we expect insulin resistance to be rapidly and reliably reversed in compliant participants offers a rare opportunity to obtain muscle and adipose samples before and then during the intervention. The samples will then be analysed a) histologically to assess lipid accumulation and inflammation, b) genetically to document changes in gene (mRNA) expression, c) for changes in protein expression and phosphorylation and d) metabolically in order to evaluate changes in DAGs and ceramides as well as other candidate lipid intermediates.

2. Summary of study

To evaluate the therapeutic efficacy and metabolic impact of a low energy diet (LED) in people with familial partial lipodystrophy and diabetes. Participants will be provided with a LED (total diet replacement) for 12 weeks, before the introduction of a stepped food transition as was done by Lean et al (Lean et al. 2017) in patients with type 2 diabetes. Metabolic effects will continue to be assessed for one year. In order to better understand why this intervention changes insulin sensitivity, we will also collect adipose and muscle tissue samples at baseline and 12 weeks into the intervention in participants willing to have these procedures performed. The original study by Taylor et al (Lim et al. 2011) suggested that insulin resistance was significantly improved by this time point so we anticipate a similar response in our patient cohort. These samples will be used for histological, metabolite, gene expression and protein expression analyses.

3. Objectives and Outcome Measures

3.1 Aims

- To determine whether following a LED dietary intervention in people with familial partial lipodystrophy will improve:
    - Glycaemic control
    - Hypertriglyceridaemia
    - Hepatic fat content

  - Insulin sensitivity
- To assess whether there is any change in quality of life, anxiety and depression at the end of the intervention
- To assess metabolic control up until one year post intervention

3.2 Primary Endpoint

A reduction in HbA1c at 12 weeks

3.3 Secondary Endpoints

- A reduction in HbA1c at 1 year
- A reduction in fasting glucose at 12 weeks and 1 year
- A reduction in triglycerides at 12 weeks and 1 year
- A reduction in liver fat at 12 weeks and 1 year
- A reduction in pancreatic fat at 12 weeks and 1 year
- An increase in insulin sensitivity (based on an oral glucose tolerance test (OGTT) at 12 weeks and 1 year
- An improvement in quality of life, anxiety and depression scores (as measured by PHQ9, GAD7 and EQ-5D-3LQOL)
- A reduction in antidiabetic medication use at 12 weeks and 1 year

4. Recruitment

Patients with partial lipodystrophy referred to our centre and who meet the inclusion criteria will be invited to take part in the study. A computerised search of medical records of patients attending the National Severe Insulin Resistance (NSIR) Service will be conducted by the clinical care team and potentially eligible participants will be invited to participate in the study. Potential participants will be invited at clinical appointments, via telephone calls, letters or email. They will be provided with an invitation letter and a patient information sheet along with contact details of the study team. Potential participants will be contacted to answer queries and ascertain interest.

4.1 Inclusion criteria

- Familial Partial Lipodystrophy
- Age >= 18 yrs
- T2DM
- Willingness to check daily blood sugars
- HbA1c between 53mmol(7%)- 108 mmol(12%)
- Weight stable for 3 months
- Capacity to consent

4.2 Exclusion criteria

- Pregnancy
- Untreated thyroid dysfunction (patients who have been euthyroid on medication for at least 3 months can be included)
- Use of medication that adversely affects diabetes control (e.g. steroids/ immunosuppressants/ certain antipsychotics)
- Incapacity to give informed consent

- History of an eating disorder/ purging behaviour
- Previous gastric bypass/ banding
- Use of leptin therapy
- Untreated proliferative retinopathy

4.3 Screening

Once potential participants have confirmed they are interested in participating in the study a screening visit appointment will be booked via the telephone and a screening proforma will be completed (see Appendix A). A member of the research team will explain the diet intervention and the study plan to the participant. Participants will be asked about their willingness to follow the diet plan. Participants will be asked to keep a food diary for 7 days whilst on their normal diet before their baseline appointment.

4.4 Sample

20- 30 participants will be recruited to participate.

5. Study Intervention

Usual food will be replaced by a total diet replacement (TDR). This will come as a formula food which comes as sachets of powder and is reconstituted with water, this is consumed with ample liquids (of approximately 3L) for 12 weeks and a fibre supplement will be prescribed. The TDR will be a commercial micronutrient-replete liquid formula diet providing approximately 800-853 cals, and consists of formulas and shakes. Following 12 weeks of TDR a stepped food reintroduction will be commenced in which food will slowly be reintroduced back into the diet over 6 weeks. The weight loss maintenance phase follows food reintroduction and aims to maintain the changes to eating and activity behaviours that have been made over the previous months. If participants' metabolic control worsens due to non-compliance during the maintenance phase a rescue plan can be used, in which participants are supported to incorporate the TDR again.

6. Study Design

Participants that are eligible and consent to take part in the study will be given an appointment to attend the Cambridge Clinical Research Centre (CCRC) at Addenbrooke's Hospital to participate in the study.

Participants will be commenced on a 12 week total diet replacement after which they will follow a stepped food reintroduction phase (weeks 13-18) and will then be followed up through a weight loss maintenance phase (weeks 19-52). Participants will be monitored throughout the year and will be seen for investigations and study procedures at the Clinical Research Facility.

- Total Diet Replacement phase - Participants will be seen face to face for metabolic investigations at baseline, week 4, week 8 and week 12. During this time the participants will be closely monitored with weekly phone calls, which will be increased if needed – this is mainly to avoid hypoglycaemia following the introduction of the LED.
- Stepped Food Reintroduction - Participants will be seen at week 16 for metabolic investigations and will be closely monitored with two-weekly phone calls which will be increased if needed to

maintain optimal glycaemic control. At the start of stepped food reintroduction orlistat 120mg three times a day will be prescribed and commenced, in line with standard practice.

- Weight loss maintenance - Participants will be seen at week 24, 36 and 52 and from week 24 will be followed up with four-weekly phone calls which will be increased if needed.

See Appendix B for a schedule of investigations and procedures.

7. Study Procedures

7.1 Clinical Assessment

All participants will undergo baseline clinical assessment which includes a detailed medical, family and social history. Clinical information will be recorded in a case record file identified only by a unique study identifier that will have been assigned to that participant.

7.2 Consent Procedure

Individuals who fulfil the inclusion criteria and are agreeable to study participation will attend the research facility at a scheduled date and time. A unique identifier code will be assigned to the participant at this point. A named investigator of the research team will obtain written informed consent prior to the initiation of any study procedures.

7.3 Anthropometric measures

- Weight measurement

Study participants will have their weight measured on a digital equilibrated scale. The measurements will be taken after the participant has been asked to void. Participants will be weighed in their clothing with their shoes off and in bare feet or socks. Measurements will be recorded to the nearest 0.1Kg.

- Height measurement

Study participants will have their height measured using a stadiometer. This measurement will be taken in bare feet or socks. Participants will be asked to stand upright with their heels, buttocks and occiput touching the backboard of the stadiometer. The measuring arm of the stadiometer will be brought down to the top of the subject's head. Height is recorded to the nearest 0.1cm.

- Waist circumference

A non-stretch tape measure will be used to measure waist circumference. The measurement will be taken with the subject in a standing position with the abdomen relaxed, the arms at the sides and the feet together and breathing normally. The measurer faces the subject and places a measuring tape around the subject in a horizontal plane, at the mid-point between the lowest rib and the supra iliac crest. The measurement should be taken at the end of a normal expiration, ensuring the tape is taut around the subject's waist, without compressing the skin. Waist circumference measurements will be taken in duplicate and values will be recorded to the nearest 0.1cm. The mean value of both measurements will be recorded as the waist circumference.

7.4 Venepuncture

The participant will undergo venous catheter insertion and blood sampling with fasting samples being sent for baseline biochemical measurements at the biochemistry laboratory, Cambridge University Hospital. Serum and plasma samples will also be retained for more specialised assays. Participants will be asked to fast prior to testing from 10pm on the night prior to study.

7.5 Magnetic Resonance Imaging/ Magnetic Resonance Spectroscopy

Magnetic Resonance Imaging and spectroscopy studies will be undertaken to image subcutaneous and abdominal fat depots and quantify the triglyceride (TG) content in liver, pancreas and skeletal muscle. TG accumulation is strongly associated with insulin resistance and we anticipate this to be alleviated by the diet intervention. These will be taken at baseline, 3 months, 6 months and 1 year. These are non-invasive procedures. Studies will be conducted in the Wolfson Brain Imaging Centre (WBIC).

7.6 DEXA Imaging

Dual Energy X-ray Absorptiometry (DEXA) will be used to document body composition, quantifying fat mass as well as bone mineral density and lean mass. Participants will be required to lie supine for approximately 15 minutes without moving in order to obtain the images. The same DEXA scanner will be used for all visits. The total dose of 8 microSv is equivalent to that received, on average in the UK, from natural sources of background radiation in approximately 32 hours. This dose corresponds to a negligible risk of cancer induction, estimated as around 1 in 3 million

7.7 Indirect Calorimetry

Indirect Calorimetry will be undertaken to assess Basal Metabolic Rate (BMR) using a Gas Exchange Measurement instrument. BMR typically falls with weight loss in obese patients so we would like to see if this also happens in patients with FPLD. Participants are advised to avoid any vigorous exercise and caffeine on the day prior to calorimetry. A standardised evening meal based on their weight and predicted or known (DEXA) body composition will be provided to the participant on the day prior to calorimetry followed by overnight fasting. The measurement will be undertaken by the technician at 7.30 am in the participant's own room at the WTCRF.

7.8 Oral Glucose Tolerance Test (OGTT)

This procedure allows the measurement of glucose tolerance and Insulin sensitivity. It involves fasting from 10 pm and then having a venous catheter inserted into the upper limb. A base-line set of bloods will be drawn prior to a drink of 75g of anhydrous glucose dissolved in water. Blood sampling will be taken every thirty minutes for the duration of the 3 hour test. In total 120 ml of blood will be drawn (approximately 7 tablespoons).

7.9 Fat and muscle (vastus lateralis) biopsies

Fat and skeletal muscle biopsies may be undertaken for histological, genetic or biochemical analysis. The muscle biopsy is done under local anaesthetic on the outer side of the thigh and involves the use of a blunt hollow needle to get a piece of muscle tissue from the leg.

The fat biopsy is performed under local anaesthetic. The incision will be approximately 3cm long, in the left lateral peri-umbilical area 5cm to 10cm from umbilicus, but may vary a little from patient to patient depending on their vasculature, build and access to the fat layer. The tissue in the centre of

the incision will be grasped with forceps, pulled up and gently cut and an appropriately sized chunk of fat will be removed.

7.10 Questionnaires

Questionnaires will be undertaken to measure psychosocial factors. The following five separate self-administered questionnaires will be used:

- GAD-7 will be used to measure anxiety
- PHQ-9 will be used to measure depression
- EQ-5D-3L will be used to measure quality of life
- AEBQ (adult eating behaviour questionnaire) will be used to measure eating behaviour and appetite
- TFEQ (three factor eating questionnaire) will be used to measure the cognitive and behavioural domains (or 'factors') of eating: cognitive restraint (CR), disinhibition and hunger

7.11 Urine Sample

Participants will be asked to provide a urine sample. A 5-mL portion of the urine sample will be centrifuged for 5 min at 2500 rpm to remove all sediment and particulate matter. A 500 µL fraction of the centrifuged urine will then be pipetted into a 1.5-mL plastic microcentrifuge tube. The urine will be analysed for changes in 8-iso-PGF2a, a validated marker of reactive oxygen species accumulation. It is expected to be fall following weight loss.

8. Data Protection

At enrolment into the study, participants will be assigned a unique identifier code. An electronic copy linking identifiable personal details to the unique identifier code will be stored on a secure server on an NHS computer. A hard copy will be retained in the study site file located in a locked cabinet and office at the Institute of Metabolic Science (IMS). Access to the room is restricted to the clinical research team conducting the study and access to the facility is permitted to authorised personnel only allowing only members of the study team to retain access to the participant's true identities.

Biological samples collected and stored during the study will be labelled with the unique identity code, no identifiable personal details will be included. Samples will be stored at the Wellcome Trust-MRC Institute of Metabolic Science until the time of analysis. Data obtained from biochemical analysis/ultrasound investigations undertaken by core departments at Cambridge University Hospital will be accessed via the EPIC computer system. This will include identifiable information however it is only accessible to authorised CUH NHS personnel via password protection of the programme.

Magnetic Resonance scans performed at the Wolfson Brain Imaging Centre (WBIC) will be stored securely within the University of Cambridge. These scans will be stored for 10 years. Participant identifiers will be linked to the scans and accessible to WBIC staff.

REDCap is a mature, secure, web application for building and managing research databases and will be used to collect all demographic and other measures related to the study, no identifiable

information will be collected here. All data held in REDCap is anonymised and can therefore be accessed, in real time, by the research team outside of the Department once they have been issued with the appropriate logins.

Electronic files collating data will be anonymised using the unique identifier code; they will be retained in encrypted files on a password protected computer. Computers will be located at the Wellcome Trust-MRC Institute of Metabolic Science, within a locked office where access is restricted to the research team. Data analysis will be undertaken at the same location. Data collected during the study will be retained for 15 years.

9. Statistics

There is no control group in this pilot study; participants will serve as their own controls i.e. before and after analysis.

Statistical analyses will be performed using commercially available software. Parametric data will be presented as mean +/- standard deviation with statistical comparisons performed using the Student's t test. Non-parametric data represented as median (interquartile range) and comparisons will be performed using a Wilcoxon signed rank test. Changes of sequential data within experiments were evaluated by repeated measures ANOVA. Correlations will be assessed using the Spearman rank test. The null hypothesis is rejected at a significance threshold of $p < 0.05$.

10. Reimbursement

Participants will have travel and overnight accommodation costs covered.

11. Sponsor

The study is jointly sponsored by Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge.

12. Insurance

The design of the study will be insured through the University of Cambridge (joint sponsor). The University Insurance Office have advised that insurance for negligent and non-negligent harm under the University's Clinical Trials policy can be arranged if this trial is approved by the NHS Ethics Committee. The University's insurers are Newline, the insurance policy reference is B0823Q31000177/WD1600523 and the Limit of Indemnity under the policy is £10,000,000 each and every claim. In addition named members of the research team will have insurance cover provided through the NHS indemnity scheme.

13. Summary of Main Issues

13.1 Management of Incidental Findings

Throughout the course of the study there is a possibility that incidental findings of clinical relevance are made during clinical, biochemical and radiological and psychological studies. In this instance the

study doctor will interpret the findings, disclosing them to the participant and referring on for further clinical investigation if necessary. Participants will be asked to consent as to whether they would like their GP informed of these study findings.

13.2 Side Effects of Diet

- There are some common transient side effects related to coming off a normal diet with a considerable reduction in energy intake. These include headaches, some dizziness, feeling cold and constipation. A fibre supplement will be prescribed to alleviate constipation.

- Low blood sugars may occur due to the reduction in calorie consumption and antidiabetic medication may need to be adjusted. At the start of the diet medication will be reviewed by a clinical doctor and insulin will be reduced in anticipation of a fall in blood sugars and some antidiabetic medicine will be stopped. Insulin adjustments will be titrated as needed and there will be regular telephone and email contact between participants and the study team to review blood sugars and amend insulin doses as necessary.

14. Dissemination

The results will be published in scientific literature and presented at scientific conferences. All information that is published and presented will be anonymous. Results will be shared at the Lipodystrophy Support Group Day, co- hosted by the NSIR service and Lipodystrophy UK (a charity based in the UK that provides support and information to patients with lipodystrophy and their families) and in the annual SIR Service newsletter.

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Appendix A- Screening Visit Proforma

VLCD and Lipodystrophy Screening Visit

Patient Details

Name: **Date of birth:**
Sex: **Ethnicity:**

Pregnant

Yes ☐ No ☐

Clinical assessment at most recent clinical appointment

Weight(Kg): Height(cm):
BMI(kg/m^2):

Weight stable for last 3 months?

Yes ☐ No ☐

Biochemical assessment at most recent clinical appointment

Parameter	Value	Units	Date tested
HbA1c			
TSH			
T4			

Previous gastric bypass/ banding

Yes ☐ No ☐

History of an eating disorder/ purging behaviour

Yes ☐ No ☐

Diabetic Eye Screening

Date of most recent screening:
Results of most recent screening:

Retinopathy diagnosis

Yes ☐ No ☐

If yes:

Background retinopathy ☐ Pre-proliferative retinopathy ☐ Proliferative retinopathy ☐

Treatment for retinopathy:

Medication Review (check for steroids, immunosuppressant's, antipsychotics, leptin therapy)

Food Diary Given

Yes ☐ No ☐

Comments related to Food Diary

Screening conducted by:

Name: **Signature:**

Date:

Appendix B- Schedule of Investigations & Procedures

Dietary Phase→		Total Diet Replacement Phase (Weeks 0-12)												
Week Number →	Screening	Baseline 0	0 + 1	0 + 2	0 + 3	0 + 4 (1 month)	0 + 5	0 + 6	0 + 7	0 + 8 (2 months)	0 + 9	0 + 10	0 + 11	0 + 12 (3 months)
Study procedure ↓														
Face to Face Assessment		√				√				√				√
Telephone Assessment	√		☎	☎	☎		☎	☎	☎		☎	☎	☎	
Consent		√												
Food Diary	√													
Fasting Bloods		√				√				√				√
Height		√												
Weight		√				√				√				√
Wasit & Hip		√				√				√				√
Blood Pressure		√				√				√				√
Blood Glucose readings		√	√	√	√	√	√	√	√	√	√	√	√	√
Medication Review		√	√	√	√	√	√	√	√	√	√	√	√	√
Oral Glucose Test		√												√
GEM		√												√
MRI Liver & Pancreas		√												√
DEXA		√												√
Fat Biopsy (optional)		√												√
Muscle Biopsy (optional)		√												√
Urine sample		√				√				√				√
Questionnaires		√												√

Dietary Phase→	Food Reintroduction (Weeks 13-18)				
Week Number →	0 + 13	0 + 14	0 + 15	0 + 16 (4 months)	0 + 18
Study procedure ↓					
Face to Face Assessment				√	
Telephone Assessment	☎	☎	☎		☎
Consent					
Food Diary					
Fasting Bloods				√	
Height					
Weight				√	
Wasit & Hip				√	
Blood Pressure				√	
Blood Glucose readings	√	√	√	√	√
Medication Review	√	√	√	√	√
Oral Glucose Test					
GEM					
MRI Liver & Pancreas					
DEXA					
Fat Biopsy (optional)					
Muscle Biopsy (optional)					
Urine sample				√	
Questionnaires					

Dietary Phase→	Weight Loss Maintenance (Weeks 19-52)									
Week Number →	0 + 20	0 + 22	0 + 24 (6 months)	0 + 28	0 + 32	0 + 36 (9 months)	0 + 40	0 + 44	0 + 48	0 + 52
Study procedure ↓										
Face to Face Assessment			√			√				√
Telephone Assessment	☎	☎		☎	☎		☎	☎	☎	
Consent										
Food Diary										
Fasting Bloods			√			√				√
Height										
Weight			√			√				√
Wasit & Hip			√			√				√
Blood Pressure			√			√				√
Blood Glucose readings	√	√	√	√	√	√	√	√	√	√
Medication Review	√	√	√	√	√	√	√	√	√	√
Oral Glucose Test			√							√
GEM			√							√
MRI Liver & Pancreas			√							√
DEXA			√							√
Fat Biopsy (optional)										
Muscle Biopsy (optional)										
Urine sample			√			√				√
Questionnaires			√							√