Study Title: Targeting beta-cell failure in lean patients with type 2 diabetes

Short title: Lean-DM

Version: 2.1

Date: 23 Oct 2019

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2 Synopsis

Full Study Title	Targeting beta-cell failure in lean patients with type 2 diabetes	
Short Title	Lean-DM	
Study Design	A single center, open-label, randomized, cross-over study	
Study Participants	Lean and overweight type 2 diabetes (T2D) patients	
Planned Sample Size	28 lean and 28 overweight T2D patients	
Treatment duration	16 week treatment period with the first drug will be followed by 8-week washout and an additional 16-week treatment period with the second drug.	
Follow up duration	None	
Planned Study Period	40 week study period.	
	Objectives	Outcome Measures
Primary	To explore and compare the impact of enhanced insulin sensitivity to improved beta-cell function intervention strategy using Liraglutide or Pioglitazone to modulate myocardial perfusion.	Change in myocardial perfusion after treatment.
Secondary	To explore and compare the impact of Liraglutide and Pioglitazone on: 1. Myocardial energetics; 2. Myocardial steatosis; 3. Myocardial function; 4. Insulin resistance; 5. Physical performance; 6. Hepatic steatosis; and 7. Peripheral endothelial function.	After treatment change in: 1. Myocardial energetics (PCr/ATP ratio); 2.Myocardial steatosis (myocardial triglyceride content); 3. Myocardial function; 4. Insulin resistance (HOMA-IR); 5. Physical performance (6min walk test); 6. Hepatic steatosis (hepatic triglyceride content); 7. Peripheral endothelial function

Formulation, Dose, Route of Administration	Routinely prescribed doses of Pioglitazone 45mg od or Liraglutide 1.2mg subcutaneously once weekly

3 Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse event
AIR	Acute insulin response
AR	Adverse reaction
ATP	Adenosine triphosphate
BMI	Body mass index
CAD	Coronary Artery Disease
CI	Chief Investigator
CMD	Coronary microvascular dysfunction
CMR	Cardiovascular Magnetic Resonance
CRF	Case Report Form
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
GCP	Good Clinical Practice
GLP-1 RA	Glucagon-like peptide-1 receptor agonist
GP	General Practitioner
HbA1c	Glycated haemoglobin
HF	Heart failure
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HRA	Health Research Authority
ICF	Informed Consent Form
LFTs	Liver Function Tests
Ln; Ln-T2D	Lean; Lean Type 2 Diabetes
LV	Left Ventricle
MEN 2	Multiple Endocrine Neoplasia syndrome type 2
MPRI	Myocardial Perfusion Reserve Index
MRI	Magnetic Resonance Imaging

MRS	Magnetic Resonance Spectroscopy
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NRES	National Research Ethics Service
Ob; Ob-T2D	Obese; Obese Type 2 Diabetes
PAT	Peripheral Arterial Tone
PCr	Phosphocreatine
PIS	Participant/ Patient Information Sheet
PPAR- γ	Peroxisome proliferator activated receptor gamma
REC	Research Ethics Committee
RUSAE	Related and Unexpected Serious Adverse Event
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard Deviation
SUSAR	Suspected Unexpected Serious Adverse Reactions
T2D	Type 2 diabetes
UAR	Unexpected Adverse Reaction

4 Introduction

4.1 Background

Heart failure (HF) is a major cause of morbidity and mortality in patients with type 2 diabetes (T2D). While the focus of prevention in T2D is on obesity, the "lean" T2D variant (Ln-T2D) is not a rare condition. Ln-T2D patients exhibit similar degrees of cardiac concentric remodeling, myocardial steatosis and energetic impairment as obese T2D (Ob-T2D) patients at rest, and at stress they show more coronary microvascular dysfunction and greater reduction in myocardial energetics. Accumulating evidence suggests that these metabolic derangements in Ln- T2D are mediated by a defect in insulin secretory capacity through beta-cell dysfunction, as opposed to insulin resistance which dominates in Ob-T2D. Despite the known pathophysiological differences to Ob-T2D, current prevention and treatment regiments do not address Ln-T2D differentially. Glucagon-like peptide-1 receptor agonist (GLP-1RA) agents have favorable effects on beta-cell morphology and volume and a demonstrated cardiovascular safety profile and offer the potential to improve cardiac outcomes specifically for the Ln-T2D variant. We hypothesize that treatment with a GLP-1RA agent (Liraglutide) will promote beta-cell insulin secretion, restore coronary microvascular function, and modulate exercise metabolism in Ln-T2D patients compared to a peroxisome

proliferator activated receptor gamma (PPAR- γ) agonist (pioglitazone), which targets peripheral insulin sensitivity. These observations may identify a specific role for GLP-1RA therapy in Ln-T2D.

Ln-T2D shows significant differences in cardiac metabolic phenotype, characterised by worse metabolic reserve and myocardial perfusion reserve, in keeping with myocardial microvascular dysfunction when compared to obese patients with T2D, despite similar glycaemic control. The presence of these alterations in measures of stress metabolism might signify a distinct metabolic phenotype of 'lean diabetic cardiomyopathy' driven by a marked defect in insulin secretory capacity in these individuals. Current prevention and treatment strategies do not address Ln-T2D differentially. GLP-1RA agents have favorable effects on beta-cell morphology and volume and a demonstrated cardiovascular safety profile, thereby representing an opportunity to improve cardiac outcomes specifically for Ln-T2D. As GLP-1RA promote beta-cell insulin secretion, and insulin improves myocardial perfusion, treatment with Liraglutide should restore myocardial perfusion in patients with T2D. Furthermore, we have previously shown that in T2D coronary microvascular dysfunction (CMD) exacerbates myocardial energetic impairment during exercise. Improvement in myocardial perfusion with Liraglutide treatment should therefore also modulate exercise metabolism.

Accumulating evidence suggests that Ln-T2D patients represent a unique subgroup of the disease, characterized by disproportionally reduced insulin secretory capacity as the major pathology, rather than insulin resistance, which predominates in obese T2D. HF is a leading cardiovascular complication of diabetes. T2D contributes to the development of HF through a variety of mechanisms, including disease-specific myocardial structural, functional, vascular and metabolic changes. We have previously shown that Ln-T2D patients exhibit similar degrees of cardiac concentric remodeling, myocardial steatosis and energetic impairment at rest, but show more significant CMD, and associated with this a greater reduction in myocardial energetics with stress and lower plasma insulin levels compared to their obese counterparts. CMD in patients with T2D has emerged among the potential mechanisms leading to increased incidence of HF (3) and risk of cardiovascular mortality(4,5). In T2D patients, CMD exacerbates derangement of cardiac energetics under conditions of increased workload (6). Although CMD has been suggested as a target to improve cardiac function, the effect of modulating myocardial perfusion reserve on cardiac function and energetic status remains to be shown in T2D patients.

Progressive pancreatic beta-cell dysfunction and peripheral insulin resistance have been identified as the two fundamental features in the pathogenesis of T2D. Both beta-cell function and insulin resistance are amenable to pharmacological intervention; there is growing evidence that GLP- 1RA agents slow the progression of beta-cell failure while PPAR- γ agonists target peripheral insulin sensitivity. Clinical studies examining the effects of GLP-1RA on CMD are scarce; small scaled studies in patients with T2D suggested enhanced endothelial function and increased perfusion by GLP-1RA (7). Liraglutide, a GLP-1RA agent, ameliorated both high glucose- and lipid-induced endothelial dysfunction, and stimulated endothelial AMP-Kinase pathway activity, resulting in greater endothelial nitric oxide synthase activation and nitric oxide production (7).

4.2 Purpose of the study

We hypothesize that GLP-1RA offer specific benefits in treatment of Ln-T2D by preventing or delaying the decline in beta-cell function. In the proposed study we will test whether treatment with the GLP-1RA Liraglutide promotes beta- cell insulin secretion, restores myocardial perfusion, and modulates myocardial exercise metabolism in Ln-T2D. This study will for the first time compare the clinical efficacy of GLP-1RA and PPAR- γ agonists as second line treatment and specifically explore the pathophysiology of Ln-T2D; it will confirm whether insulin resistance or beta-cell dysfunction represents the primary target for early intervention efforts to lower the burden of cardiovascular

disease in this subgroup, and underscore the relevance of CMD as a target for reversing or preventing diabetic cardiomyopathy.

In this study, we will study the effects of pharmacological modulation of insulin secretion and insulin sensitivity on myocardial perfusion, energetics and function in lean T2D patients. In a cross-over study, lean patients with T2D and no known cardiovascular disease will receive both Liraglutide and Pioglitazone and undergo serial assessments, including, CMR imaging of quantitative dobutamine stress perfusion imaging, MRS assessment of myocardial energetics and lipid deposition, non-invasive evaluation of the peripheral vascular endothelial function, and 6-minute walk tests. As a control group, obese patients with T2D and no cardiovascular disease will receive the same drugs and undergo the same serial assessments. The results of these investigations will help determine if GLP-1RA agents could offer specific benefits to Ln-T2D patients.

5 HYPOTHESES, OBJECTIVES AND OUTCOME MEASURES

5.1 Original Hypotheses

1. The GLP-1RA (Liraglutide) will promote beta-cell insulin secretion;
2. Treatment with the GLP-1RA (Liraglutide) will restore myocardial perfusion, modulate metabolism in the diabetic heart, and reverse the cardiomyopathic process in Ln-T2D;
3. Treatment with a PPAR- γ agonist (Pioglitazone) will reduce the degree of ectopic and visceral adiposity, reverse cardiac steatosis and concentric remodeling and improve insulin sensitivity, but not influence myocardial perfusion and energetics.

5.2 Aim, Objectives and Endpoints

Overall aim: To better understand the exact pathophysiology of T2D in the absence of obesity.

Primary objective

To explore and compare the impact of enhanced insulin sensitivity to improved beta-cell function intervention strategy using Liraglutide or Pioglitazone to modulate myocardial perfusion.

Secondary objectives

To explore and compare the impact of Liraglutide and Pioglitazone on:

- Dobutamine stress myocardial energetics;
- Myocardial steatosis;
- Myocardial function;
- Insulin resistance;
- Beta-cell function;
- Physical performance;
- Hepatic steatosis;
- Peripheral endothelial function.

Primary endpoint

Change in myocardial perfusion after treatment.

Secondary endpoints

After treatment, changes in:

- Percent difference in myocardial PCr/ATP from rest to dobutamine stress;
- Myocardial lipid levels;
- Strain parameters;
- HOMA-IR;
- Acute insulin response to glucose;
- 6-minute walk distance;
- Hepatic triglyceride content;
- Endothelium-mediated changes on peripheral arterialtone (PAT) ratio measured by modified plethysmographic probes (Endo-PAT).

6 STUDY DESIGN

This is a single center, open-label, randomized, cross-over study in lean and obese T2D patients. Patients will be administered two drugs sequentially. Pioglitazone will be started at 15mg once daily and titrated to 30mg once daily after 2 weeks, then finally to 45mg daily 2 weeks after that if glucose levels permit. Liraglutide will be administered at 0.6 mg once weekly to start and titrated up to 1.2mg after 2 weeks as tolerated will be administered. Patients will be randomised to be administered either pioglitazone or liraglutide first, followed by a washout period. Then patients will be administered whichever drug they did not have first.

Study duration: 40-week study period. 16-week treatment period with the first drug will be followed by 8-week washout and an additional 16-week treatment period with the second drug.
Setting: Tertiary cardiac centre – Leeds General Infirmary and University of Leeds
Study population: 28 lean and 28 overweight T2D patients.

7 STUDY PARTICIPANTS

28 lean and 28 overweight T2D patients will be recruited. Adult T2D patients who are either drug-naïve (i.e., treated with diet and exercise) or treated with oral glucose lowering therapies for at least 12 weeks prior to screening, with HBA1c between 6.5 and 10% at screening will be recruited.

7.1 Inclusion Criteria

7.1.1 Lean cohort

1. Men and women>18 years of age;
2. Normal body weight (18.5 ≤ BMI ≤25 kg/m2);
3. T2D patients can be on treatment with oral glucose lowering therapies, and if they are, they must have been on these treatments for at least 12 weeks prior to screening;
4. 6.5≤HBA1c≤10% at screening;
5. Agreement to maintain prior diet and exercise habits for the duration of the study.

7.1.2 Overweight cohort

1. Men and women>18 years of age;
2. Increased body weight (BMI >27 kg/m2);
3. T2D patients can be on treatment with oral glucose lowering therapies, and must have been on these treatments for at least 12 weeks prior to screening;
4. 6.5≤HBA1c≤10% at screening;
5. Agreement to maintain prior diet and exercise habits for the duration of the study.

7.2 Exclusion Criteria

1. Any type of diabetes other than T2D;
2. Past history of significant CAD;
3. Known HF;
4. Significant renal impairment (eGFR<30ml/min/m2);
5. Participation in a clinical trial of an investigational medicinal product (CTIMP) in the preceding 12 weeks;
6. Known hypersensitivity to dobutamine or gadolinium or any other contra-indications to MRI;
7. Participants with obesity where their girth exceeds the scanner bore;
8. History of pancreatitis;
9. Any history of liver disease;
10. Patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2);
11. Prior or current use of thiazolidinediones (aka PPAR-γ agonists), fibrates, GLP-1RA or insulin;
12. Patients with high serum calcitonin levels at baseline;
13. Patients that are pregnant (female participants only);
14. Inflammatory bowel disease
15. Diabetic gastroparesis

7.3 Recruitment

There are three recruitment pathways in place:

1. With assistance from the NIHR Yorkshire and Humber Clinical Research Network (CRN), we will recruit participants with T2D from local GP practices via:
    a. Identification of potential participants from patient lists by a specialist nurse in the practice. Potential participants identified in this manner will be mailed an invitation letter with reply slip, and a Participant Information Sheet (PIS) by the practice or
    b. Verbal agreement to be approached for the study will be sought by the primary care team. Their verbal permission will be recorded in their medical notes at the practice. If the potential participant is interested in discussing the study further, their contact details will then be given to the research team by the primary care team. The research team will send an invitation letter and the PIS to the address provided by the primary care GP or the diabetes specialist nurse.

The invitation letter will indicate that the research team will contact them by telephone in the near future to answer any questions they may have about the study. If the potential participant is interested in participating, the research team will arrange a convenient time for the first study visit to take place.

2. The study team at the LGI will also contact those who have participated in previous observational ethically approved studies in the department (University of Leeds, Biomedical Imaging) and who have consented to have their contact details retained to be contacted if eligible to take part in other studies. We will consult the LTHT's EPRO database containing up-to-date information regarding deceased patients prior to contacting those who have participated in other ethically approved studies in the department and who have consented to have their contact details retained in order to be contacted if eligible to take part in other studies.

3. Additional awareness and interest in the study will be generated through locally organised events for GPs and diabetes specialist nurses. There is a REC-approved poster specifically to be used for this activity. Posters will only be placed in GP practices that are also participating as Participant Identification Centres (PICs).

7.4 Informed Consent

The participant must personally sign and date the latest approved version of the Informed Consent form before any trial specific procedures are performed.

Written and verbal versions of the Participant Information Sheet (PIS) and Informed Consent Form (ICF) will be presented to the participants detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as they wish to consider the information, and the opportunity to discuss with the Investigator, their GP or other independent parties to decide whether they will participate in the trial. Written informed consent will then be obtained by means of dated participant signature and dated signature of the person who presented and obtained the informed consent on the ICF. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed ICF will be given to the participant. The original signed and dated form will be retained at the trial site.

7.5 Contraceptive Protection

Women of childbearing potential participating in the study must use a medically-approved, highly effective acceptable birth control method to prevent pregnancy. Acceptable methods of contraception include the following:

- Barrier-type devices (e.g., female condom, diaphragm, and contraceptive sponge) used only in combination with a spermicide
- Intrauterine Devices (IUDs)
- Oral contraceptive agents started at least 90 days before start of study
- Depo-Provera (medroxyprogesterone acetate)

- Levonorgestrel implants
- Naturally or surgically sterile (amenorrheic for at least 1 year and no record of childbirth for naturally sterile persons)
- Male partner is sterile and is the only sexual partner.

Note that true or periodic abstinence, the rhythm method, or contraception by the partner only are not acceptable methods of contraception.

Male participants with female partners of childbearing potential do not have to use birth control methods.

8 Methodology

8.1 Randomisation

Eligible patients will be randomized in a 1:1 ratio to the order of receiving Liraglutide and Pioglitazone. Randomisations will be achieved using minimization incorporating a random element, via a computer-generated program, that will allocate patients in a 1:1 ratio after taking account of age, gender and BMI. Each treatment (Liraglutide and Pioglitazone) will be received for 16 consecutive weeks, in a random order, with 8 weeks of no treatment in between.

8.2 Study Visits

The assessments listed below will be carried out at each visit in the Advanced Imaging Centre at the Leeds General Infirmary. Participants will continue taking their previously prescribed medications throughout the study. Study assessments are described in detail in Section 8.3.

Visit 1 (Baseline; week 0)

- Review of medical history and concomitant medications
- Review of history of diabetes and complications
- Review of inclusion/exclusion criteria
- Collection of demographic data
- Height and weight
- Urine pregnancy test in women of childbearing potential
- Written informed consent

Baseline assessments

- Vital signs
- Physical examination
- 12-lead ECG

- Blood pressure
- Venepuncture (fasting sample): 20mls
- Multiparametric MRI
- EndoPAT testing
- 6 minute walk test
- Randomization
- Dispense study medication and issue patient diary
- Urine sample collection

Visit 2 (week 16, +/- 5 days)

- Vital signs
- Physical examination
- Blood pressure
- Weight
- Venepuncture (fasting sample): 20mls
- 12-lead ECG
- Multiparametric MRI
- EndoPAT testing
- 6 minute walk test
- Check current medication list and patient clinical status
- Check study medication compliance (diary review)
- Urine sample collection

Visit 3 (week 24, +/- 5 days, following 8 weeks of wash-out period)

- Vital signs
- Physical examination
- Blood pressure
- Weight
- Venepuncture (fasting sample): 20mls
- 12-lead ECG
- Multiparametric MRI
- EndoPAT testing
- 6 minute walk test
- Check current medication list and patient clinical status
- Check study medication compliance (diary review)
- Urine sample collection

Visit 4 (week 40, +/- 5 days)

- Vital signs
- Physical examination
- Blood pressure
- Weight
- Venepuncture (fasting sample): 20mls
- 12-lead ECG
- Multiparametric MRI
- EndoPAT testing
- 6 minute walk test
- Check current medication list and patient clinical status
- Check study medication compliance (diary review)
- Urine sample collection

End of the study

8.3 Study Assessments

8.3.1 Blood and Urine Tests

Blood will be taken via venepuncture at each visit and tested for the following: triglycerides, alanine aminotransferase, haemoglobin, haematocrit, creatinine, estimated glomerular filtration rate, N-terminal pro–B-type natriuretic peptide, insulin, free fatty acids, adiponectin, glucose and lipid profiles, glutamic acid decarboxylase antibodies, and Zinc transporter 8 antibodies.

Urine will be collected and spot tested for urine albumin/ creatinine ratio.

With permission from participants, plasma will be frozen at -80^{o}C and stored for up to 3 years for future research.

Upon initiation of Liraglutide treatment, calcitonin levels will be checked. Upon initiation of Pioglitazone treatment, participants' liver function tests (LFTs) will be checked, and urine will be checked for the presence of haematuria.

The study drug dose will be titrated to the target dose if glucose levels permit this stepwise dose increase as described in sections 9.1.3 and 9.2.3. While on Liraglutide, glucose assessments will be done 2 weeks after starting treatment. While on Pioglitazone, glucose levels will be assessed 2 weeks after starting treatment and then again 4 weeks after starting treatment. Glucose assessments will be performed by the study team at the Leeds General Infirmary. Participants will be told to continue their usual schedule of glucose monitoring at home while on the study drugs (no additional monitoring at home will be necessary).

8.3.2 Multiparametric MRI

Multiparametric MRI assessments will consists of 2 parts:

Magnetic Resonance Spectroscopy (MRS): The relative concentration of phosphocreatine to ATP (PCr/ATP) by 31P-MRS and cardiac and hepatic triglyceride content by 1H-MRS.

Cardiovascular Magnetic Resonance (CMR): Comprehensive investigation of structural, functional and ischaemic changes in the heart (LV size, function, strain, myocardial perfusion, fibrosis and scarring).

MRI and MRS scans will be carried out in the Advanced Imaging Centre in the Old Site at Leeds General Infirmary. Following written informed consent for the study, participants will be asked to complete a standard MRI safety questionnaire that is also used in daily clinical practice. The questionnaire will be checked by a member of the research team and if there are no contraindications to undertaking the MRI scan, the volunteer/patient will be asked to change into a gown. For MRI scanning, patients will be taken to the MRI scanning room after again checking they have no contraindications to MRI. They will then lie on the MRI scanner bed. MRI safe electrocardiogram (ECG) leads will be attached for most scanning protocols. A flexible receiver coil will be positioned over the body area of interest and headphones will be fitted. Once the volunteer/patient is comfortable, they will be moved into the MRI scanner bore. The radiographer will stay in constant contact with the participant via a 2 way intercom system. The MRI scanning will commence with localiser images to identify the region of interest. This is typically completed within 1-2 minutes. Thereafter, all scanning protocols include series of image acquisition in chunks lasting a few seconds to a few minutes. For the shorter acquisitions, breathholding is often used, communicated via the intercom system. All methods that will be used to acquire all data will comply with all standard manufacturer regulations for the safe operation of the scanner. The perfusion scans require the administration of MRI contrast agents. Only contrast agents that are in routine clinical use will be given and within clinically used dosing regimes. Specifically, we will use the contrast agent Gadovist® (Gadolinium) in doses of up to 0.2mmol/kg bodyweight. The contrast agent will be administered via an MRI contrast pump (Medrad Solaris) as per clinical routine.

Stress testing will be carried out with dobutamine. Dobutamine will be used from 10 up to 40mcg/kg/min. Blood pressure will be recorded every two minutes and continuous ECG monitoring will take place.

8.3.3 Homeostatic Model Assessment of Insulin Resistence (HOMA-IR)

HOMA-IR is a robust method used for quantifying insulin resistance and β-cell function from basal (fasting) glucose and insulin concentrations(68). HOMA-IR describes this glucose-insulin homeostasis by means of a set of simple, mathematically-derived nonlinear equations. Insulin levels depend on the pancreatic β-cell effect to glucose concentrations while glucose concentrations are regulated by insulin-mediated glucose production via the liver. Thus, deficient β-cell function will echo a diminished response of β-cell to glucose-stimulated insulin secretion. Similarly, insulin resistance is reflected by the diminished suppressive effect of insulin on hepatic glucose production. HOMA-IR will be measured via blood testing.

8.3.4 Endothelial function (EndoPAT testing)

The Endo-PAT 2000 will be used to assess endothelium-mediated changes in the digital pulse waveform known as the PAT (Peripheral Arterial Tone) signal, measured with a pair of novel modified plethysmographic probes situated on the finger index of each hand. Endothelium-mediated changes in the PAT signal are elicited by creating a downstream hyperemic response. Hyperemia will be induced by occluding blood flow through the brachial artery for 5 minutes using an inflatable cuff on one hand. The response to reactive hyperemia will be calculated automatically by the system. A PAT ratio is created using the post and pre occlusion values. These values will be normalized to measurements from the contra-lateral arm, which serves as control for non-endothelial dependent systemic effects. This will also be performed at the Advanced Imaging Centre.

8.3.5 6 minute walk test

Participants will be instructed to walk along a 30-meter corridor and cover the maximum distance in 6 minutes under the supervision of study investigators with medical training and with experience in conducting the test. The investigators will tell the patients how much time had elapsed every 2 minutes and encourage participants to continue at intervals of between 30 seconds and 1 minute. At the end of 6 minutes, patients will be asked to stop, and the distance walked will be measured in meters. This will also be performed at the Advanced Imaging Centre.

8.4 Expenses and Benefits

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

8.5 Discontinuation/Withdrawal of Participants from Trial Treatment

Each participant has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Pregnancy
- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- An adverse event which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
- Disease progression which requires discontinuation of the trial medication or results in inability to continue to comply with study procedures
- Withdrawal of Consent
- Loss to follow up

Withdrawal from the study will result in exclusion of the data for that participant from analysis. Withdrawn participants will be replaced. The reason for withdrawal will be recorded in the CRF. If the participant is withdrawn due to an adverse event, the Investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

8.6 Definition of End of Study

The end of study will be the date of the last visit of the last participant.

9 Study Drug Treatment

9.1 Liraglutide

Liraglutide is a GLP-1RA which is a once-weekly, injectable, extended-release formulation drug. Liraglutide promotes insulin release from β-cells in the presence of elevated glucose concentrations, decreasing glucagon release, increasing satiety, and slowing gastric emptying. The acute effect of GLP-1RA agents on β-cells is stimulation of glucose-dependent insulin release, followed by enhancement of insulin biosynthesis and stimulation of insulin gene transcription(34). The chronic action is stimulating β-cell proliferation, induction of islet neogenesis,

and inhibition of β-cell apoptosis, thus promoting expansion of β-cell mass, as observed in rodent diabetes and in cultured β-cells(34). GLP-1 receptors are also expressed in the heart, and administration of GLP-1 improves cardiovascular function in the setting of experimental cardiac injury(35). The safety of Liraglutide and its efficacy as a glucose lowering agent are well established (36). The incretin based drugs have also been shown to reduce the occurrence and degree of hepatic steatosis independent of their action on body weight(37) and cardiac steatosis(38). Currently, NICE guidelines recommend GLP-1RA drugs as a second line therapy in patients with T2D. ESC guidelines recommend use of a GLP-1RA with proven CVS benefit as a first line agent in T2D patients with high or very high cardiovascular risk.

9.1.1 Contraindications

Contraindicated in patients with diabetic gastroparesis, inflammatory bowel disease and severe congestive cardiac failure.

9.1.2 Side effects

Common side effects can include nausea, decreased appetite, asthenia, constipation, diarrhea, dizziness, dry mouth, gallbladder disorders, gastrointestinal discomfort, headache, insomnia, nausea, altered taste, toothache, vomiting and injection site erythema. Rare side effects include acute pancreatitis, angioedema, thyroid dysfunction and tachycardia. Participants will be educated on these symptoms and given the standard of care action plan on how to manage them in the unlikely event that they occur.

Serious and life-threatening cases of diabetic ketoacidosis have been reported in patients with type 2 diabetes mellitus on a combination of a GLP-1RA and insulin, particularly after discontinuation or rapid dose reduction of concomitant insulin. No participant on insulin treatment will be included in this study which diminishes this small risk. In addition, patients will be monitored for blood glucose levels after initiation and stepwise dose titration of Liraglutide and patients will also be instructed for careful blood glucose self-monitoring at home. Patients will be informed of the risk factors for and signs and symptoms of diabetic ketoacidosis, and advised to seek immediate medical attention if these develop.

9.1.3 Frequency and duration

Liraglutide will be prescribed at 0.6 mg inititially, then titrated up to 1.2 mg once weekly via subcutaneous injection. Participants that are willing and competent to inject themselves will be trained to do so. Those that are unable or unwilling to inject themselves will be given the option of coming in and having a qualified member of the study team (either a research nurse or a research fellow) do the injection. The dose will be titrated to the target of 1.2mg once daily after 2 weeks if glucose levels permit this stepwise dose increase. Glucose levels will be assessed 2 weeks after starting treatment. Glucose assessments will be performed by the study team at the Leeds General Infirmary (LGI).

9.2 Pioglitazone

Recent innovative treatment approaches target multiple pathophysiological defects present in T2D, including insulin sensitivity and the β-cell dysfunction. Pioglitazone is a peroxisome proliferator activated receptor gamma (PPAR-γ) agonist targeting peripheral insulin sensitivity and therefore glycaemic control(41). At the molecular level, pioglitazone is a ligand for PPAR-γ, a nuclear hormone receptor expressed predominantly in adipose tissue. Recently, it has been shown that compared with non-use, glitazones were associated with a 23% decreased risk of all-cause mortality, a 26% decreased risk of HF, and a 25% decreased risk of cardiovascular disease(8). Pioglitazone improves insulin resistance in T2D in association with mobilization of toxic lipid intermediates out of muscle(42).

Modulations of adipose tissue energy balance via PPAR-γ activation is the key contributing mechanism by which Pioglitazone exerts its antidiabetic effects(42). These include enhanced differentiation of preadipocytes into mature adipocytes and regulation of gene expression in adipose tissue leading to the coordinated regulation of lipid metabolism. Treatment with pioglitazone results in an increase in whole-body adiposity, but the shift in distribution of fat is such that the visceral fat decreases while the subcutaneous fat increases(43). The Piramid study has shown significant improvement in diastolic function with the use of Pioglitazone in patients with type 2 diabetes, however there was no significant change in myocardial energetics or myocardial steatosis.

9.2.1 Contraindications

Hypersensitivity to the active substance or to any of the other ingredients.

9.2.2 Side effects

Side effects can include bone fracture, increased risk of infection, numbness, visual impairment, and increased weight. There have been very rare reports of liver dysfunction and bladder cancer. Liver function will be checked by blood tests and urine samples will be evaluated for haematuria before commencing treatment. These potential side effects will be included in the PIS given to participants. Patients will be monitored for blood glucose levels after initiation and stepwise dose titration of Pioglitazone and patients will also be instructed for careful blood glucose self-monitoring. Patients will be informed of the risk factors for and signs and symptoms of diabetic ketoacidosis, and advised to seek immediate medical attention if these develop.

9.2.3 Frequency and duration

Pioglitazone 45mg once daily po. Pioglitazone will be started at the smallest dose (15 mg once daily) initially and the dose will be increased to 30mg once daily after 2 weeks. The dose will be titrated to the target of 45 mg once daily after 2 weeks if glucose levels permit this stepwise dose increase. Glucose levels will be assessed 2 weeks after starting treatment and then again 4 weeks after starting treatment. Glucose assessments will be performed by the study team at the Leeds General Infirmary.

9.3 Administration / handling of the study drugs

The study drug will be prescribed and dispensed by the study centre. Instructions as to how and when to take the prescribed medication in its licensed dose will be issued at the time of enrolment. A diary will be issued at the time of enrolment for subjects to record taking the medication to assess compliance with treatment. These medications will be accounted for by the Leeds General Infirmary Pharmacy Department.

9.4 Monitoring

Recommended monitoring for Liraglutide is to check calcitonin levels prior to initiation. Recommended monitoring for pioglitazone is to check body weight, liver function (by measuring LFTs in blood) and to check for haematuria (via urine testing) before commencing treatment.

Participants will be advised regarding potential side effects listed above as per usual clinical practice.

9.5 Drug Supply and Randomisation

The study drugs will be prescribed and dispensed by the study centre. Randomisation will be carried out by the dedicated clinical trials pharmacy team at the study centre using block randomisation. They will use a validated randomisation programme and will securely backup both the randomisation seed and the randomisation allocation. There will be no blinding.

9.6 Concomitant Medications

Fibrates are contraindicated medications to use with the study drug Pioglitazone. There are no contraindicated medications for Liraglutide. Patients taking Liraglutide and on concomitant insulin therapy are at risk of diabetic ketoacidosis with discontinuation or rapid reduction in the insulin dose.

9.7 Withdrawal of Treatment

Treatment will be withdrawn if contra-indications to continued administration develop. Unexpected reactions to the medication will be recorded and may lead to the patient being withdrawn from the study.

9.8 Post-study Treatment

There will be no provision of the study drugs beyond the study period.

10 Serious Adverse Events Procedures

10.1 General Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical trial subject which does not necessarily have a causal relationship with this treatment and can include:

- any unintentional, unfavourable clinical sign or symptom
- any new illness or disease or the deterioration of existing disease or illness
- any clinically relevant deterioration in any laboratory assessments or clinical tests.

An adverse reaction (AR) or adverse drug reaction (ADR) is any untoward or unintended responses to a study drug related to any dose administered to that subject. All AEs judged by either the reporting investigator or the Sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression “reasonable causal relationship” means to convey in general that there is evidence or argument to suggest a causal relationship.

An unexpected adverse reaction (UAR) is any adverse reaction the nature and severity of which is not consistent with the information about the study medication in question set out in the listed side effects.

A suspected, unexpected serious adverse reaction (SUSAR) is an adverse reaction that is both unexpected and serious. An adverse reaction is “unexpected” if its nature or severity is not consistent with the Reference Safety Information.

Most adverse events and adverse drug reactions that occur in this study, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this study and will be classified as expected adverse reactions. For a full list of expected side effects of Liraglutide and Pioglitazone, please refer to the lists previous sections in this document (9.1.2 Liraglutide ; 9.2.2 Pioglitazone).

In addition, the following criteria may be used in order to collect protocol-defined reportable adverse events which do not meet the criteria for serious (below):

- requires medical or surgical intervention to prevent permanent impairment of function or permanent damage to body structure.

A serious adverse event (SAE) or serious adverse reaction (SAR) is defined in general as "any untoward medical occurrence or effect that:

- results in death,
- is life-threatening*,
- requires inpatient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity,
- consists of a congenital anomaly or birth defect,
- may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

*the term life-threatening refers to an event in which the patient 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 was more severe.

A SAE occurring to a research participant, where in the opinion of the Chief Investigator the event is Related and Unexpected will be reported to the main Research Ethics Committee (REC). The National Research Ethics Service (NRES) defines Related and Unexpected SAEs (RUSAEs) as follows:

- Related: that is, it resulted from administration of any research procedures; and
- Unexpected: that is, the type of event is not listed in the protocol as an expected occurrence.

10.2 Causality

The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below. All adverse events judged as having a reasonable suspected causal relationship to the study medications (i.e. definitely, probably or possibly related) are considered to be adverse reactions. In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the REC and other bodies will be informed of both points of view.

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.

Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

10.3 Severity

Severity of all AEs and ARs will be graded on a three-point scale of intensity (mild, moderate, severe):

Mild	Discomfort is noticed, but there is no disruption of normal daily activities.
Moderate	Discomfort is sufficient to reduce or affect normal daily activities.
Severe	Discomfort is incapacitating, with inability to work or to perform normal daily activities.

Note: An AE or AR may be severe but not serious

10.4 Operational definitions and reporting for AEs and SAEs

10.4.1 Expected AEs/SAEs – Not reportable

The patient populations being studied here may have co-morbid disease along with type 2 diabetes. However, we expect these patient populations to be generally healthy, as the exclusion criteria excludes coronary heart disease. Because these patients have been diagnosed with type 2 diabetes, we expect some adverse events related to this condition.

In recognition of this, events fulfilling the definition of an adverse event or serious adverse events will not be reported in this study unless they are classified as 'expected' (section 10.4.2) or 'unexpected' and 'related' (section 10.4.3).

10.4.2 Expected AEs/SAEs – Reported within standard CRFs

The following SAEs are expected within the study population and will be reported by the clinical research team using standardised tests and follow-up CRFs including:

- Complications related to any study test that requires a specific treatment or hospital admission
- Hypoglycaemia
- Other known side effects of study medications
- Any pre-planned hospitalisations (e.g. elective surgery) not associated with clinical deterioration
- Elective or scheduled treatment for pre-existing conditions that did not worsen during the study

These events are expected within the study population and will not be subject to expedited reporting to the main REC. All non-serious or expected adverse events will be recorded on the study CRF at visits 2, 3 and 4. They will also be included in the annual safety report provided to the main REC.

10.4.3 Related and Unexpected SAEs – Expedited Reporting

In keeping with HRA guidelines, reports of Serious Adverse Events (SAEs) or Serious Adverse Reactions (SARs) that are:

- related to the study (i.e. they resulted from administration of any of the research procedures) and
- unexpected (i.e. not listed in the protocol as an expected occurrence)

will be submitted to the REC using the HRA Non-Clinical Trial of an Investigational Medical Product (CTIMP) safety report to REC form within 15 days of the chief investigator becoming aware of the event and will be reported to the sponsor within 1 working day of the research team becoming aware of the event.

Events will be followed up until the event has resolved or a final outcome has been reached.

11 STATISTICS

11.1 Description of Statistical Methods

For this randomized study, outcome measures at follow-up will be compared between groups using linear regression models, adjusted for baseline measures of the outcome. Data will be transformed if necessary to meet model distributional assumptions. Differences between randomised groups will be tested overall, and then pairwise differences between groups will be estimated separately. All analyses will be pre-specified in a detailed Statistical Analysis Plan, to be finalised prior to any data being analysed.

11.2 The Number of Participants

Previous work (3) found the mean (SD) MPRI for Ln-T2D patients to be 1.46 (0.43), compared to 2.24 (0.41) for healthy controls, a difference of 0.78. We proposed that increasing the MPRI for T2D patients by one third of this difference (0.26) would constitute a biologically significant effect. Assuming that both study drugs are associated with an increase of this size, and assuming the SD to be 0.43, a study with 22 participants in each group would have 90% power, based on a one-way analysis of variance at a 5% significance level. Allowing for a dropout of up to 20%, 28 patients will be recruited and randomized, split between the two randomized groups of treatment sequences, one group with the order: Liraglutide − Pioglitazone, the second with the order Pioglitazone − Liraglutide. We will also recruit 28 age and gender matching overweight patients for comparison between the lean and overweight cohorts.

11.3 The Level of Statistical Significance

Statistical significance will be considered at P value of 5% significance and a power of 90%.

11.4 Procedure for Accounting for Missing, Unused, and Spurious Data

Spurious and missing data will lead to data from that participant being excluded from the study.

12 DATA MANAGEMENT

12.1 Data Collection Methods

All personal data is stored on the NHS server as all the participants will be NHS patients. Anonymised results will be password protected and stored on the University of Leeds server. There will be a master cipher sheet, which will be the only place where participants will be linked to their study number. This will be encrypted and password protected and stored on the NHS server. A separate study spread sheet will contain the anonymised results of the analysis of all study investigations. All data will be anonymised.

Data will be entered into a locally developed electronic database stored on the University of Leeds server. All imaging data, blood results and urine results will be entered into this database. All data will be anonymised on the electronic database.

12.2 Types of data

Clinical measurements such as the following will be collected: demographics, medical history, relevant concomitant medications, 12-lead ECG, clinical laboratory tests, MR studies, 6 minute walk test, and endoPAT test as described previously for those that participate. These data will be spread across the baseline and follow-up visits. Safety reporting will be collected as per UK legislation requirements. Other data such as administrative data regarding attendance of visits and patient status in regards to withdrawal will also be collected.

12.3 Methodologies for data collection / generation

Once data is collected, it will be entered into the study specific CRF by site staff in accordance with the protocol requirements. Data will be held for 15 years.

12.4 Formal information/data security standards

Data will be secured in line with Data Protection Act 2018.

12.5 Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all trial documents and any electronic database, with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by trial staff and authorised personnel. The study will comply with the Data Protection Act 2018, which requires data to be anonymised as soon as it is practical to do so.

13 SERIOUS BREACHES

A serious breach is defined as "A breach of GCP or the study protocol which is likely to affect to a significant degree:

(a) the safety or physical or mental integrity of the subjects of the study; or

(b) the scientific value of the study".

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the Chief Investigator, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC, regulatory authority and the NHS host organisation within seven calendar days.

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Appendix A: Visit Schedule

	Visit 1 (Baseline; Week 0)	Visit 2 (Week 16, +/- 5 days)	Visit 3 (Week 24, +/- 5 days)	Visit 4 (Week 40, +/- 5 days)
Medical History	X			
Diabetes and complications history	X			
Inclusion/ Exclusion Criteria	X			
Demographic Data	X			
Vital signs	X	X	X	X
Blood Pressure	X	X	X	X
Physical examination	X	X	X	X
Weight	X	X	X	X
Height	X			
12-lead ECG	X	X	X	X
Urine Pregnancy test (if applicable)	X			
Informed Consent	X			
Blood collection[1,2,3]	X	X	X	X
Urine collection[4,5,]	X	X	X	X
Multiparametric MRI[6]	X	X	X	X
EndoPAT	X	X	X	X
6 MWT	X	X	X	X
Randomisation	X			
Dispense study medication	X		X	
Issue patient diary	X		X	
Check current medications	X	X	X	X
Check clinical status (AEs)		X	X	X
Diary review and collection		X		X

1= triglycerides, alanine aminotransferase, haemoglobin, haematocrit, creatinine, estimated glomerular filtration rate, N-terminal pro–B-type natriuretic peptide, insulin, free fatty acids, adiponectin, glucose and lipid profiles, glutamic acid decarboxylase antibodies and Zinc transporter 8 antibodies (every visit)

2= While participant is on Liraglutide- calcitonin levels to be checked upon initiation of drug. Glucose assessment to be done 2 weeks after starting treatment.

3= While participant is on pioglitazone- liver function tests to be carried out before commencing treatment. Glucose assessments to be done 2 weeks and 4 weeks after starting treatment.

4= Spot test for albumin/ creatinine ratio

5= While participant is on pioglitazone- haematuria to be evaluated before commencing treatment

6= Magnetic Resonance Spectroscopy (MRS), followed by Cardiac MR (CMR)