SGLT-2 INHIBITOR EMPAGLIFLOZIN EFFECTS ON APPETITE AND WEIGHT REGULATION: A RANDOMISED DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL

(SEESAW)

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Confidentiality Statement

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Protocol: SGLT-2 Inhibitor Empagliflozin Effects on Appetite and Weight Regulation: A randomised double-blind placebo-controlled trial (SEESAW)

Version: 7.0 (20/03/2018)

By my signature below, I confirm that I have read this protocol and its attachments, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with ICH Guidelines on good clinical practices and the applicable local laws and regulations. I will accept a monitor for the Sponsor overseeing this study. I will abide by the publication plan set form in my agreement with Boehringer Ingelheim Ltd and the Sponsor, including all statements regarding confidentiality.

Principal Investigator Name: _____

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Sponsor Representative Name: _____

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1. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	3.0	13/04/2016	Professor Melanie Davies Dr Sudesna Chatterjee Dr Danielle Bodicoat Dr Cat Taylor	Amendment to the stratification criteria to remove 'sex'. Update to safety reporting and safety reporting procedures following Direct Healthcare Professional Communication report dated 21st March 2016 regarding diabetic ketoacidosis and the use of Empagliflozin. Exclusion criteria updated to reflect safety information (addition of criteria 18-21. Instruction from Boehringer Ingelheim to include diabetic ketoacidosis as one of the Adverse Events of Special Interest. Several typographical errors corrected and update to page references following the addition of information.
2	4.0	29/09/2016	Professor Melanie Davies Dr Sudesna Chatterjee Dr Emer Brady Dr Cat Taylor Kyla Harrington	Removal of study contact details from the front cover of the protocol. Change of name from Project Management Committee to Trial Management Group Trial Summary updated to more accurately reflect visit length. Exclusion criteria updated to allow any future emerging safety concerns to be addressed without compromising participant safety. Amendment to the number and timing of blood samples collected during study visits 1-5. Addition of information regarding storage of blood samples for future research Section 9.1 updated to include

				information on acute kidney injury.
3	5.0	08/02/2017	Professor Melanie Davies Dr Sudesna Chatterjee Dr Emer Brady Dr Eleanor Taylor	Exclusion criteria amended to exclude only rotating day/night shift workers, as well as to allow investigators to use clinical judgement in assessing excessive alcohol consumption. Amendment to participant recruitment allowing for more strategies to be adopted. Amendment to the time required for inclinometer wear from 8 to 9 days. Amendment to the window in which screening bloods results can be used from 6 to 3 months prior to study visit. Addition of a follow-up telephone call within 1 week (+/-3days) of medication completion to capture any potential Adverse Events during the drug wash-out period. Clarification of the use of indirect calorimeter for determining participants' daily energy requirement. Addition of increased Lower Limb Amputation to the section on Adverse Events of Special Interest (AESI). Correction to formatting error in reproduction of VAS questionnaire.
4	6.0	29/06/2017	Professor Melanie Davies Dr Sudesna Chatterjee Dr Emer Brady Natasha Wileman	Inclusion and exclusion criteria amended to widen HbA1c range and increase upper age limit. Change to breakfast standardisation; standardised breakfast meal to

				represent 33.3% of daily energy requirement as opposed to 30%. Change to wording in section 8.5 (investigations) which references the timing of indirect calorimetry assessment; assessment performed at visit 1 not visit 0. Clarification provided regarding the timing of samples collected at visits 1-5; 1 fasting sample and 6 samples after the standardised breakfast meal. Change to the timing of provision of baseline activity monitors to participants; monitors to be given at visit 0 not visit 1. Study measures added to table 1 (scheduled of assessments) for clarity. Clarification providing regarding the visit window between visit 0 and visit 1. Boehringer Ingelheim added to the reporting procedure for SAE. Additional recruitment activity in primary care added to recruitment strategy section. Minor amendments to Author section based on title of authors. Minor amendment to randomisation and code breaking section to change description of placebo/IMP from 'capsules' to 'tablets'.
5	7.0	20/03/2018	Professor Melanie Davies	Visit window for visits 1, 2, 3, 4 and 5 amended (widened to +/-5 days) to

			Dr Sudesna Chatterjee Natasha Wileman	allow flexibility with appointment booking and reduce protocol deviations. Amendment to the wording within the 24 hour Meal Standardisation section to reflect current practice; all participants will consume a standardised evening meal provided to them at least 10 hours before their visit. Amendment to the physical activity section to allow for flexibility with provision of monitors. The study team wish to avoid these holiday periods, as it is not habitual behaviour. Amendment to inclusion/exclusion criteria in figure 1 – study design (page 19) as these were missed at the last amendment opportunity. Minor amendment to author section of the protocol to update job title of the senior dietician. Minor change to section 16 (study governance) to change Research Governance Framework to the UK Policy Framework for Health and Social Care Research which has superseded the Research Governance Framework.

2. PROTOCOL SYNOPSIS

Study Title	SGLT-2 Inhibitor Empagliflozin Effects on Appetite and Weight Regulation: A randomised double-blind placebo-controlled trial (SEESAW)
Internal ref. no.	0526
Clinical Phase	Phase IV
Trial Design	Single centre, prospective, randomised, double-blind placebo-controlled trial
Trial Participants	Male and postmenopausal female participants aged 30-75 years with type 2 diabetes mellitus which is controlled either by lifestyle or stable metformin dose
Planned Sample Size	76 participants
Follow-up duration	0
Planned Trial Period	21 months
Primary Objective	The aim of this study is to investigate the cause for the discrepancy in predicted and observed weight loss with Empagliflozin (Jardiance™) by measuring appetite regulation.
Secondary Objectives	Major secondary objectives are to determine the effects of Empagliflozin (Jardiance™) on energy expenditure and change in total body weight and body composition.
Primary Endpoint	Change in appetite hormone concentrations (specifically total PYY) between baseline and 24 weeks: - this will be measured by sequential blood sampling during visits 1-5.
Secondary Endpoints	Secondary endpoints, which are exploratory, are effect on appetite hormones (ghrelin and GLP-1), appetite perceptions, total body weight and fat and fat free mass, energy expenditure, appetite perception, physical activity and blood and urine biochemical parameters after Empagliflozin (Jardiance™) treatment for 24 weeks.
Investigational Medicinal Products	Empagliflozin (Jardiance™) and matched placebo
Form	Tablet
Dose	25mg daily
Route	Oral

3. ABBREVIATIONS

ADA	American Diabetes Association
AE	adverse event
ALT	alanine transaminase
AST	aspartate transaminase
AUC	area under curve
BMI	body mass index
BRU	biomedical research unit
CRF	case record form
CRP	C-reactive protein
CTU	clinical trials unit
DEXA	dual energy X –ray absorptiometry
DKA	diabetic ketoacidosis
DSMC	data safety monitoring committee
DPP-IV	dipeptidyl peptidase-IV
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunoassay
FBC	full blood count
FFA	free fatty acids
FPG	fasting plasma glucose
GLP-1	glucagon-like peptide-1
HbA1c	glycosylated haemoglobin
HCA	healthcare assistant
ICP-GCP	International Conference on Harmonisation Good Clinical Practice
IPAQ	international physical activity questionnaire
LFT	liver function tests
MHRA	Medicines and Healthcare Products Regulatory Agency
MSJE	Mifflin-St Jeor equation
MVPA	moderate to vigorous physical activity
PAL	physical activity level
PYY	peptide Y-Y
REC	research ethics committee
REE	resting energy expenditure
SAE	serious adverse event

SGLT-2	sodium glucose co-transporter-2
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
TMG	trial management group
TSC	trial steering committee
U+E	urea and electrolytes
UGE	urinary glucose excretion
ULN	upper limit of normal
VAS	visual assessment scale

4. BACKGROUND AND RATIONALE

Out of a family of six sodium glucose co-transporters, two have been extensively studied for therapeutic indications involving glucose reabsorption from the glomerular filtrate (Hasan, 2014). Sodium glucose co-transporter-1 (SGLT-1), which is mainly found in both the renal tubule and enterocytes lining intestinal villi, transfers glucose across a concentration gradient resulting in glucose absorption from the gut and accounting for 10% of the glucose reabsorption from the renal tubules. Sodium glucose co-transporter-2 (SGLT-2), which is mostly found in the kidneys, is responsible for reabsorbing 90% of the glucose that is filtered by the renal tubules (Tahrani, 2013). Compared with healthy individuals, SGLT-2 is over-expressed and over-activated in those with type 2 diabetes (Rahmoune, 2005).

SGLT-2 inhibitors are a new class of glucose-lowering drugs for the management of type 2 diabetes which reduce plasma glucose levels by increasing urinary glucose excretion (UGE) by up to 60-80g (240-320kCal) per day (Bailey, 2010). SGLT-1 inhibitors and combined SGLT-1 and -2 inhibitors are currently in development.

Several SGLT-2 inhibitors are commercially available and licensed for use in the UK including Dapagliflozin, Canagliflozin and Empagliflozin (Jardiance™). They all lower glycated haemoglobin (HbA1c) effectively compared with placebo (mean difference vs. placebo -0.66% (95% CI, -0.73% to -0.58%) (Vasilakou, 2013). They also result in weight loss of approximately 1.8kg compared with placebo and in combination with other oral hypoglycaemic medications (Vasilakou, 2013). Some of this weight loss can be attributed to fluid loss due to osmotic diuresis from UGE (Bailey, 2011). Weight is lost from both subcutaneous and visceral stores of adipose tissue (Bolinder, 2012).

Phase II studies have shown that the highly selective and potent SGLT-2 inhibitor Empagliflozin (Jardiance™) results in significant reductions in HbA1c level after 12 weeks of therapy compared with placebo (HbA1c 0.4-0.6% reduction with 5-25mg daily dose, $p<0.0001$) and reduction was similar to that seen with metformin (Ferrannini, 2013). Weight loss of between 1.81-2.33kg was also achieved depending on Empagliflozin (Jardiance™) dose along with reduction in fasting plasma glucose (FPG). Similar improvements in HbA1c, weight and FPG have been seen in phase III trials with Empagliflozin (Jardiance™) both as monotherapy and in combination with metformin, sulphonylureas or insulin (Roden, 2013; Haring, 2013; Rosenstock, 2014; Ridderstrale, 2014; Kovacs, 2014; Haring, 2014).

Empagliflozin (Jardiance™) is licensed in the UK for use in type 2 diabetes management. In this study, we will be using Empagliflozin (Jardiance™) according to its licensed indications and dosage. The commonest known side-effects include urinary tract and genital mycotic infections which are more frequent in women than men and generally mild in severity. Dehydration and postural hypotension may occur due to volume depletion and modest lowering of blood pressure. Increased urinary frequency may also occur. Increased risk of hypoglycaemia is observed when Empagliflozin (Jardiance™) is combined with sulphonylureas or insulin.

Weight loss from increased UGE and net energy loss with SGLT-2 inhibitors is less than expected as shown by studies in patients with Type 2 Diabetes (T2DM; Ferrannini, 2014). Endogenous glucose production is

markedly increased with shifting of substrate utilisation from carbohydrate to lipid (Ferrannini, 2014; Merovci, 2014).

Up to 90g of glucose are excreted per day with Empagliflozin (Jardiance™) which equates to 360kCal per day lost by glycosuria. A 90 week study of patients with Type 2 Diabetes treated with Empagliflozin (Jardiance™) 25mg daily showed that average weight loss was 3.2±4.2kg representing a calorie deficit of 51kCal/day (interquartile range [IQR] 112) (Ferrannini, 2015). This was 29%±41% of the expected loss of 11.3±3.1kg predicted by glycosuria of 206kCal per day using a validated mathematical model (http://bwsimulator.niddk.nih.gov). An increase in daily calorie intake of 269kCal/day [IQR 258] and daily energy expenditure appear to be the adaptive responses due to Empagliflozin (Jardiance™) therapy and the combination of SGLT-2 inhibitors and calorie restriction has been recommended by the authors of this study.

Appetite stimulation resulting in increased energy intake may be the underlying mechanism for this weight loss deficit. Furthermore, glucagon response is increased by SGLT-2 inhibition using Empagliflozin (Jardiance™) (Ferrannini, 2014) and dapagliflozin (Merovci, 2014).

Appetite hormones such as peptide Y-Y (PYY) and ghrelin are important in the control of appetite and weight regulation. They are secreted from intestinal L cells which are found in the distal small intestine. Ghrelin, which stimulates hunger, has been shown to increase with weight loss and energy restriction diets (Sumithran, 2011). Glucagon-like peptide 1 (GLP-1) is another hormone which is involved in the regulation of appetite and satiety along with other glucose homeostatic effects (Madsbad, 2014).

There are no studies that have investigated the impact of SGLT-2 inhibitors on appetite hormones and effect on body composition. It is essential to understand these mechanisms in order to maximize the weight loss achievable with these agents. They may need to be combined with appropriate dietary measures such as energy restriction diets and weight-lowering or weight-neutral hypoglycaemic therapies for optimal clinical benefit. The aim of our study is to explore the relationship between appetite hormones and Empagliflozin (Jardiance™) in order to understand the underlying mechanisms for observed weight loss which does not equate with that predicted for these agents.

5. OUTCOME MEASURES

5.1 Primary Objective

The aim of this study is to investigate the cause for the discrepancy in predicted and observed weight loss with Empagliflozin (Jardiance™) by measuring appetite regulation.

5.2 Secondary Objectives

To determine the effects of Empagliflozin (Jardiance™) on resting energy expenditure and change in total body weight and body composition.

5.3 Study End Points

The primary endpoint is effect on appetite hormones (specifically total PYY) with Empagliflozin (Jardiance™) after treatment for 24 weeks.

Secondary endpoints, which are exploratory, are effect on:-

(i) Appetite hormones ghrelin and GLP-1 and appetite perception
(ii) Total body weight and change in body composition (fat and fat free mass)
(iii) Resting energy expenditure
(iv) Physical activity
(v) Change in blood and urine biochemical parameters.

6. STUDY DESIGN

6.1 Trial Summary

This trial is a randomised, double-blind placebo-controlled trial conducted over 24 weeks in male and postmenopausal female participants with Type 2 Diabetes on lifestyle control or stable metformin dose only to compare the effects of Empagliflozin (Jardiance™) on appetite and weight regulation compared with placebo and energy restriction diet.

Participants will be randomised to one of four arms at baseline:-

(i) Empagliflozin (Jardiance™) 25mg once daily
(ii) Placebo once daily
(iii) Empagliflozin (Jardiance™) 25mg once daily and energy restriction diet
(iv) Placebo once daily and energy restriction diet

Participants will be stratified for age [1.≤50years 2.>50years] and body mass index (BMI; [1. BMI 25.0-29.9kg/m^2 2. BMI ≥30.0kg/m^2]).

The study design is illustrated in Figure 1 (pg. 17).

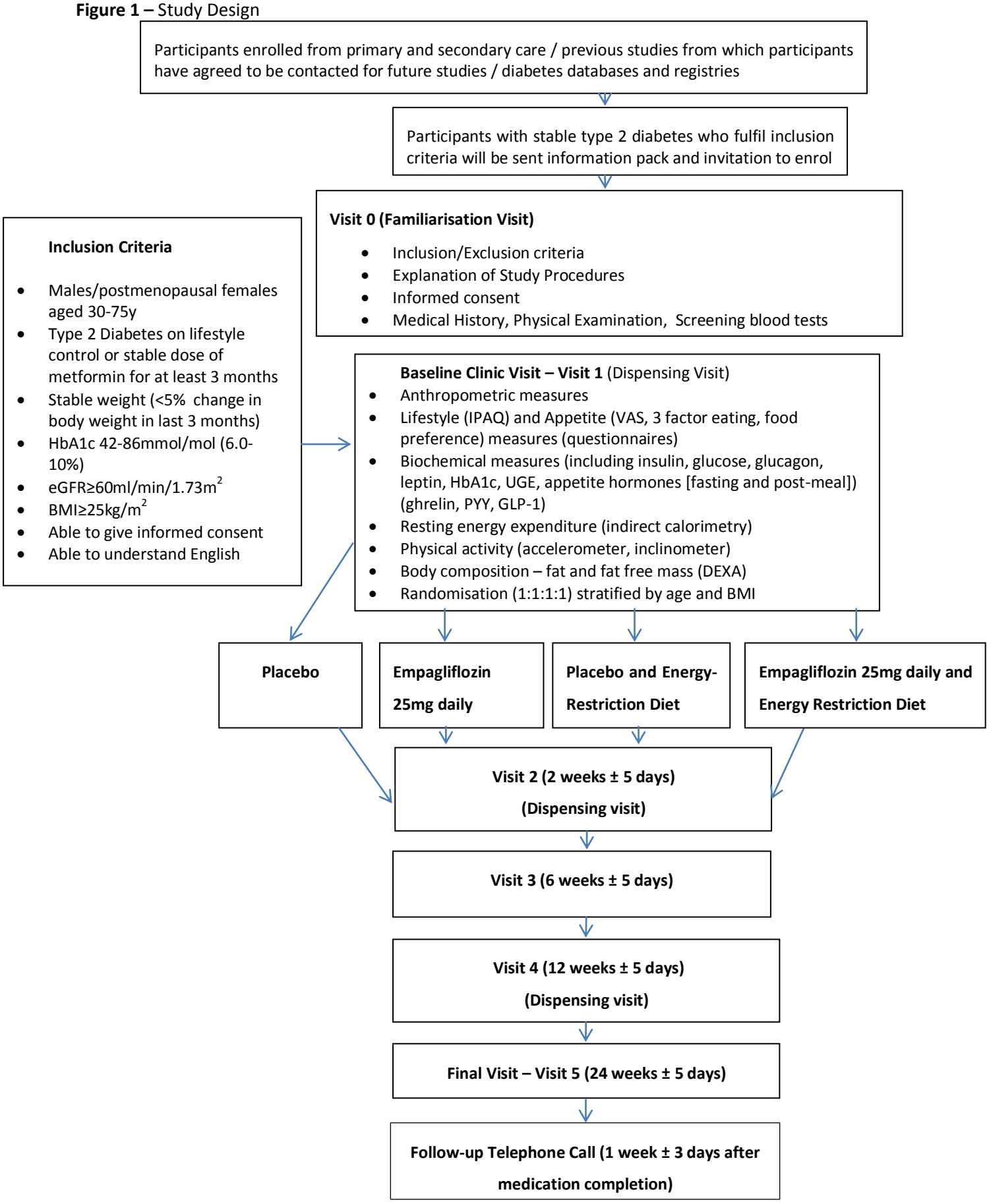
Participants will attend a screening (familiarisation) visit prior to the start of the study followed by 5 visits over 24 weeks.

The first visit (visit 0) is the Screening (Familiarisation) Visit and will occur not more than 1 week nor less than 2 days before the Visit 1 (Baseline visit). Visit 0 (approximately 2 hours) will involve a review of inclusion/exclusion criteria, an explanation of study procedures and obtaining verbal and written consent from participants by a medically qualified doctor. In addition, blood will be taken for HbA1c and renal function and participants will be issued with 2 physical activity monitors along with a log of wear, sleep and waking, and a food and drink diary for completion throughout the course of the study.

Visit 1 is the Baseline visit lasting between 6-8 hours divided into morning and afternoon sessions of procedures including blood and urine tests, completion of questionnaires, dual energy X-ray absorptiometry (DEXA) imaging and energy expenditure (indirect calorimetry). Randomisation to one of the four arms will also occur during this visit. Procedures will be conducted and supervised by the research team comprising of study clinician, trained research nurse and healthcare assistant (HCA).

Visits 2 (occurs 2 weeks (±5 days) after visit 1, lasting between 6-8 hours), 3 (6 weeks (±5 days), lasting between 6-8 hours), 4 (12 weeks (±5 days), lasting between 6-8 hours) and 5 (24 weeks (±5 days), lasting between 6-8 hours) will be divided into morning and afternoon sessions of procedures which will be supervised by the research team. There will be final review by a medically qualified doctor before the participant completes the study on this visit.

Figure 1 – Study Design



6.2 Study Setting

The study will be co-ordinated within the University Hospitals of Leicester NHS Trust and clinical measurement sessions will be co-ordinated by the appointed research team based at the Leicester Diabetes Centre, University Hospitals of Leicester NHS Trust, in collaboration with academic partner Loughborough University who are part of a Diet, Lifestyle and Physical Activity Biomedical Research Unit (BRU). This BRU is specifically aimed at using experimental research to extend the prevention and treatment options for chronic disease such as Type 2 Diabetes. The Leicester Diabetes Centre hosts this BRU; the added investment and infrastructure afforded by the award, totalling £4.5 million over 5 years, will be used to support and ensure the success of the proposed grant. In addition, there are state of the art laboratory facilities for analysing appetite hormones at the National Centre for Sports and Exercise Medicine at Loughborough University run by Dr David Stensel and Dr James King. The Leicester Clinical Trials Unit will also be supporting this study.

6.3 Primary and Secondary Endpoints

Primary Endpoint

Change in appetite hormone concentrations (specifically total PYY) between baseline and 24 weeks: - this will be measured by sequential blood sampling during visits 1-5.

Secondary Endpoints

The secondary endpoints below will be measured at the time points defined in Table 1 (pg. 28, below). We will be analysing between group differences in the change of these variables post therapy allocated at the predefined time-points.

1. Change in appetite hormone concentrations (ghrelin and GLP-1) and appetite perceptions from baseline to study end and between groups: - this will be measured by blood sampling and questionnaires at visits 1, 2, 3, 4 and 5

2. Change in weight and body composition from baseline to study end and between groups: - this will be measured by DEXA scanning at visits 1 and 5

3. Change in resting energy expenditure from baseline to study end and between groups: - this will be measured by indirect calorimetry at visits 1, 2, 3, 4 and 5

4. Change in physical activity from baseline to study end and between groups: - this will be measured using physical activity monitors at visits 0, 3, 4 and 5.

5. Change in blood and urine biochemical parameters to study end and between groups: - this will be measured using blood and urine sampling at visits 1, 2, 3, 4 and 5

The measurements undertaken above are described in detail in Section 8.4 (pg. 22).

7. TRIAL PARTICIPANTS

7.1 Overall Description of Trial Participants

Male and postmenopausal female participants with Type 2 Diabetes Mellitus on lifestyle only or stable metformin management only will be recruited to the study. Pre-menopausal female participants will not be recruited to this study as the menstrual cycle can affect appetite hormone concentrations. Recruitment strategies will include identifying participants who meet the following inclusion/exclusion criteria from within primary and secondary care settings (see Section 8.1, pg. 21).

7.2 Inclusion Criteria

1. Male and postmenopausal female participants aged between 30-75 years of age inclusive
2. Type 2 diabetes on diet and lifestyle control or stable dose of metformin only for at least 3 months
3. Stable weight (less than 5% change in body weight in last 3 months) – determined by self-reporting or documentation in clinical records
4. HbA1c 42-86mmol/mol (6.0 - 10%)
5. eGFR≥60ml/min/1.73m^2
6. BMI ≥ 25kg/m^2
7. Able and willing to give informed consent
8. Able to understand English

7.3 Exclusion Criteria

1. Females who are not postmenopausal (as menstrual cycle can affect appetite hormone concentrations) which is defined as “2 years post last menstrual period <50 years of age or 1 year post last menstrual period >50 years of age.”
2. Type 2 diabetes on any other glucose lowering treatment except metformin
3. Patients with Type 1 diabetes
4. Patients on loop diuretics
5. Age <30 years and >75 years
6. BMI <25kg/m^2
7. Not able to give informed consent
8. Not able to understand English
9. Moderate to severe renal impairment (eGFR<60ml/min/1.73m^2)
10. Unstable diabetes i.e. HbA1c >86mmol/mol (10%), recent hospital admission with diabetic emergency in last 3 months
11. Patients with familial renal glycosuria
12. Patients with recurrent balanitis, vaginal or urinary tract infections
13. Shift workers with rotating night/day shift patterns
14. Patients who have participated in another study of an investigational medicinal product in the last 3 months
15. Active malignancy
16. Serious illness with a life-expectancy of less than 1 year
17. Hypersensitivity to Empagliflozin (Jardiance™) or to any of the excipients
18. Patients with latent autoimmune diabetes in adults (LADA)

19. Patients with a history of chronic pancreatitis
20. Evidence of conditions that lead to restricted food intake or severe dehydration
21. Patients with a history of excessive alcohol consumption that in the opinion of the investigator or any sub-investigators would make implementation of the protocol or interpretation of the study results difficult or would preclude the safe participation of the subject in this protocol.
22. Patients on a severely calorie restricted diet (i.e., ≤800 calories per day)
23. Any contraindications to IMP

If the study clinician and/or Investigator deems it clinically inappropriate to include a potential participant in the study, that potential participant will deemed 'not eligible' and will not be enrolled/consented to the study (i.e., they will be classed as a screen failure and recorded as such on the visit CRF and in their medical notes). Clinicians will consider any emerging safety concerns throughout the duration of the study and subsequent eligibility of participants.

8. STUDY PROCEDURES

8.1 Screening and Eligibility Assessment

The outline of the patient flow through the study is highlighted Appendix 1 (pg. 51). Patient recruitment, which will not commence until Sponsor 'green light' has been given, will be co-ordinated via the research team at the Leicester Diabetes Research Centre with support from divisions 2 and 5 of the Clinical Research Network for the East Midlands. As the Leicester Diabetes Centre is located within a secondary care facility with specialist diabetes clinics we have access to more than 8000 patients through the University Hospitals of Leicester NHS Trust. Many more patients with Type 2 Diabetes are being treated within primary care across GP practices in Leicester City and Leicestershire County.

The study will recruit men and postmenopausal women with Type 2 Diabetes who are on stable dose of metformin or managed by lifestyle modification only. Pre-menopausal women are not being recruited to this study as the menstrual cycle can affect appetite hormone concentrations.

8.2 Recruitment Strategy

The recruitment phase will commence as soon as ethical, research governance and regulatory approval has been granted and the Sponsor green light has been given. Please see Appendix 1 (pg. 52) for participant identification pathway. Potential participants will be identified and/or contacted using direct and opportunistic marketing, using both verbal and written information about the research study, including invitation packs and a call to participants approximately 2 weeks after each mailing. If GP practices are willing to add notes and/or reminders to patient records within SystemOne to facilitate recruitment, then the study team will work with the CRN and GP practices to add notes and/or set-up reminders on eligible patient records for the GP or Diabetes Specialist Nurse to inform about the trial during routine appointments.

The following recruitment activities will be used:

1. Primary care
    a. GP practices
    b. Retinal screening clinics

2. Secondary care
    a. Attendance at UHL Outpatients Clinics, for example:
        i. Diabetes
        ii. Cardiology and Hypertension
        iii. Obstructive Sleep apnoea
        iv. Ophthalmology
        v. Lipid

    b. Community Clinics
        i. Hinckley
        ii. Market Harborough

c. Secondary Care Databases

3. Previous research participants

   a. At the Leicester Diabetes Centre, around 4200 adults have recently been screened for two diabetes prevention studies, namely "Let's Prevent Diabetes" and "Walking Away from Diabetes". Of these, around 5% (~210) of participants had Type 2 Diabetes at baseline and were withdrawn but who had given consent to be contacted to take part in future studies. We will recruit from this accessible pool of newly diagnosed Type 2 Diabetes patients.
   b. Leicester Diabetes Centre also has a volunteer database of people with Type 2 Diabetes who have given consent to be contacted and we will contact these individuals.
   c. CODEC study participants who have given consent to be contacted for future research.

4. Key people in community/community events and meetings
   a. Identify and engage key people in the community including pharmacists, GP mentors, other Healthcare professionals and community workers to distribute both verbal and written information about the research study.

5. Recruitment/health fairs in community
   a. Participate in community events and open days to publicise the study and distribute information. This will consist of having a stand with all the study information and/or presenting the study at these events.

6. Study advertisement
   a. Publicise the study through the local and social media including local radio stations and press releases, Twitter and Facebook.
   b. Distribute posters to publicise the study in primary and secondary care waiting rooms and within the community e.g. supermarkets, libraries, gyms and community centres.
   c. Advertise the study on the University Hospitals of Leicester NHS Trust and University of Leicester intranet which will include the study acronym and logo, a description about the study and contact details of the research team.

8.3 Participant Flow

Visit 0 (Screening Visit, -1 week to – 2 days), Visit 1 (Baseline Visit- 0 weeks), Visit 2 (2 weeks ± 5 days), Visit 3 (6 weeks ± 5 days), Visit 4 (12 weeks ± 5 days), Visit 5 (Final Visit - 24 weeks ± 5 days), Follow-up telephone call (1 week ± 3 days after medication completion).

8.4 Informed Consent (Screening Visit, Visit 0, only)

Informed written consent will be obtained from all participants prior to any study procedures and only after they have had sufficient time (at least 48 hours) to read through the patient information sheet and ask any questions related to the study. The study clinician who has undergone consent training and holds an up-to-

date Good Clinical Practice (GCP) certificate will obtain consent and this will be in the form of the participant's and consent taker's dated signatures. The participant will be given a copy of the signed informed consent form along with a copy of the patient information sheet, and a copy retained in the medical records and the original retained in the Investigator Site File. Consent for ongoing participation will be checked at each study visit and documented in the clinical notes and CRF by the study clinician.

8.5 Investigations

Primary Outcome Measurement

Appetite Hormones (visits 1, 2, 3, 4, 5) - Ghrelin, PYY, GLP-1

The primary outcome is change in appetite hormones from baseline to week 24 and between groups.

For this, participants are required to fast for 10 hours before study visits 1-5, and prior to the measurement of appetite hormones at each visit, there will be dietary standardisation. Specifically, there will be a 48 hour standardisation plan incorporating a 24 hour meal standardisation plan, and on the day of their study visit a standardised breakfast. The standardisation plans are described below:

- **48 Hour pre-visit Standardisation**

For the 48 hours before study visits 1-5, participants will be asked to refrain from:

- completing any structured form of physical activity,
- consuming alcohol
- consuming caffeine

Participants will be issued with food and drink diaries (see Section 8.7, pg. 26) at the familiarisation visit (visit 0) within which to record their intake and this will be reviewed at each visit to ensure that they are adhering to the 48 hour restriction period. Participants will be asked whether or not they have completed any structured form of physical activity at each visit.

- **24 Hour Meal Standardisation**

In addition to the 48 hour pre-visit standardisation described above, for the 24 hours before study visit 1-5, participants will be asked to:

- standardise their food and drink intake (i.e., to consume the same food and drinks before each study visit)
- consume the standardised evening meal provided to them at least 10 hours before their visit. This will be based on their energy requirements**. A set meal will be provided dependent on whether their energy requirement is above or below a 1500 Kcal threshold. .
- continue to fast until their visit (they will be provided with a standardised breakfast at their visit)

Participants will be issued with food and drink diaries (see Section 8.7, pg. 26) at the familiarisation visit (visit 0) within which to record their intake and this will be reviewed at each visit to ensure that they are adhering to the 24 hour meal standardisation period.

**participants daily energy requirement will be estimated at their familiarisation visit (visit 0) using the Mifflin St Jeor Equation (MSJE).

- **Breakfast Standardisation**

On the morning of study visits 1-5, participants will be provided with a standardised breakfast meal representing 33.3% of their daily energy requirement* with a macronuritent content of:

- 50% carbohydrate
- 15% protein
- 35% fat

*Note that participants' daily energy requirement will be estimated at the baseline visit (visit 1) using indirect calorimetry and multiplying by the appropriate physical activity level (PAL). If for any reason indirect calorimetry cannot be used, energy expenditure will be estimated using the Mifflin St Jeor Equation (MSJE) instead.

The appetite hormones acylated ghrelin (hunger stimulating) and GLP-1 (appetite-inhibiting) will be measured using commercially available ELISA kits. Total PYY (appetite-inhibiting) will be measured using ELISA. All hormones will be measured in fasting conditions and after the standardised breakfast meal at 7 time points during each visit. Specifically, using cannulation of the participant's forearm, blood samples will be collected at baseline (i.e., pre-standardised breakfast) and then at regular 30 minute intervals starting from the 'time of the last mouthful of breakfast' at 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes. A total of 7 samples will be taken during each visit. A total of 130ml (approximately 18ml per sample) will be collected at each visit. Blood will be collected into pre-cooled blood collection syringes containing necessary preservatives to ensure sample viability for hormone/metabolite biochemical analysis. Blood collection syringes will be prepared and kept on ice prior to their use. After each blood withdrawal the cannula will be flushed with saline to maintain patency. Once blood samples have been collected they will immediately be spun in a refrigerated centrifuge (4°C) and the plasma will be obtained and aliquoted into eppendorf tubes. These samples will then be frozen (initially at -20°C but then transferred to -80°C on the same day as collection) until required for analysis. The appetite hormone samples will be transported to Loughborough University for subsequent analysis using dry ice.

At 30 minute intervals and in conjunction with blood sampling, the participants will complete a set of appetite rating scales to assess subjective perceptions of hunger, fullness, satisfaction and prospective food consumption.

Secondary Outcome Measurements

Body Composition (visits 1, 5) – DEXA scan

Body composition (fat mass and fat free mass) will be assessed by DEXA scanning at baseline and study end visits using a scanner situated at the Leicester Diabetes Centre according to standard operating procedures.

Biochemical Analysis (visits 1, 2, 3, 4, 5)

Blood tests for FBC, U+E, LFT, lipid profile, HbA1c, FPG, eGFR, glucagon, insulin, leptin, C-peptide, FFA and CRP will be taken (see Abbreviations pg. 11). Urinary glucose excretion (UGE) will be quantified by timed collection to match with appetite hormone sampling. FBC, U+E, lipids, HbA1c, FPG, eGFR and CRP will be

measured once during each visit. Insulin, glucagon, glucose, leptin and C-peptide will be measured at 7 time points specifically at baseline (pre-standardised breakfast), 30 minutes post-breakfast and then 60 minutes, 90 minutes,120 minutes, 150 minutes and 180 minutes post-breakfast to correspond with the appetite hormones. These blood samples will be analysed at Leicester General Hospital either by the main hospital diagnostic laboratory or by the Research Scientist based at Leicester Diabetes Centre and then disposed of in accordance with the Human Tissue Authority's Code of Practice.

Following analysis, where a participant has consented, samples will be stored indefinitely for future research use.

Energy Expenditure (visits 1, 2, 3, 4, 5)

Indirect calorimetry will be used to assess resting energy expenditure (REE) at baseline, 2, 6, 12 and 24 weeks. This will be measured using a ventilated hood system at the Leicester Diabetes Centre where the participant will place their head under the hood to enable measurement of all expired gases over a predetermined time period according to standard operating procedures.

Physical Activity (visits 0, 3, 4, 5)

An ActiGraph accelerometer will be provided to the participant to be worn for 8 days (including the clinic day and 7 full consecutive days thereafter) for assessment of step count as a measure of physical activity at visit 0, 6, 12 and 24 weeks. An activPAL inclinometer will also be used to assess posture (i.e., sitting/lying, standing and stepping) for 9 days (including the clinic day and 8 consecutive days thereafter) at visit 0, 12 and 24 weeks. Should study visits occur directly before or during certain holiday periods i.e. Easter, Christmas and New Year, monitors will be sent to participants via the postal system after the holiday period. The study team wish to avoid these holidays periods as it is not habitual behaviour. These will be applied according to Leicester Diabetes Centre standard operating procedures. A log of sleep, wake and monitor removal times will be completed by the participants.

Study Questionnaires (visits 1, 2, 3, 4, 5)

The participant will be asked to complete the following validated questionnaires (see Appendix):-

- **Three-Factor Eating Questionnaire** – this will assess the participant's current eating habits
- **International Physical Activity Questionnaire (IPAQ)** – this will assess habitual physical activity in several domains including occupation, leisure, housework
- **Visual Analogue Scale (VAS) -** which will measure perceived hunger, fullness, satisfaction and prospective food consumption ratings

Other Measurements and Data Collection

Anthropometric Measures (visits 0, 1, 2, 3, 4, 5)

Body weight will be measured to the nearest 0.1kg, body fat percentage will be measured to the nearest 1% and muscle mass will be recorded to the nearest 0.1kg using bioelectrical impedance equipment (i.e., Tanita™ scales) while the participant is wearing no shoes and socks and after the removal of any heavy items of clothing.

Height will be measured using a portable stadiometer to the nearest 0.5cm with the participant wearing no shoes.

Waist circumference will be measured to the nearest 0.5cm as the midpoint between the lower costal margin and iliac crest. Hip circumference will be measured to the nearest 0.5cm at the maximum extension of the buttocks ensuring the tape measure is at the same level around the body. Both measures should be taken over underwear or light clothing with the subject standing with their feet together and breathing lightly.

Arterial blood pressure will be measured using an automated sphygmomanometer for the arm whilst the patient is seated after resting quietly for five minutes. Three measurements will be obtained for blood pressure and an average of the last two will be used. Standing blood pressure will also be collected. The measurement will be taken after the patient has been standing for three minutes with their feet hip-width apart and their arm supported at their side i.e. not hanging freely.

Demographics (Visit 0)
The date of birth, gender, race, smoking and drinking habits will be recorded by the doctor at the familiarisation visit (visit 0).

Medical History (Visit 0)
Details of any history of disease or surgical interventions will be recorded by the doctor at the familiarisation visit (visit 0).

Physical Examination (Visit 0)
A general physical examination will be performed by the doctor at the familiarisation visit (visit 0).

Randomisation to Study Medication (Visit 1)
At visit 1, participants will be randomised to either (i) Empagliflozin (Jardiance™) 25mg once daily, (ii) placebo once daily, (iii) Empagliflozin (Jardiance™) 25mg once daily and energy restriction diet, or (iv) placebo once daily and energy restriction diet using a web-based randomisation system supplied by the 3rd party company (Sealed Envelope Ltd.).

8.6 Energy Restriction Diet Prescription (Visit 1)

The daily energy restriction diet prescription for each participant will be determined using indirect calorimetry to calculate resting energy expenditure and multiplying by the appropriate activity factor (Mifflin 1990). If for any reason indirect calorimetry cannot be used, energy expenditure will be estimated using the Mifflin-St Jeor equation (MSJE) which uses height, weight and age to calculate resting energy expenditure. The factor will depend on level of activity categorised as sedentary, lightly active, moderately active, very active and extra active. The participant will be advised using standard written dietary information on how to maintain this daily energy restriction throughout the study with regular encouragement from the study team by face-to-face and telephone contact. Target energy intake will be reassessed at visit 3 (6 weeks) and visit 4 (12 weeks).

8.7 Randomisation and Codebreaking

Randomisation will be in a 1:1:1:1 format with stratification by age [1.≤50years 2.>50years] and BMI [1. BMI 25.0-29.9kg/m^2 2. BMI ≥30.0kg/m^2] at the Baseline visit (Visit 1).

Empagliflozin (Jardiance™) and the placebo will be formulated and supplied in identical tablet form sealed in identical medication packs by Boehringer Ingelheim and supplied to the third-party company ALMAC. ALMAC will then organise blinding, packaging and labelling of both Empagliflozin (Jardiance™) and the placebo. The finished stock will be sent by ALMAC to Leicester General Hospital Pharmacy. The allocation to placebo or Empagliflozin (Jardiance™) will be randomly assigned.

Randomisation will take place at the level of the individual using an independent online computerised randomisation system (Sealed Envelope Ltd.). In the event of an emergency, the investigator will decide on the necessity of unblinding the subject's treatment assignment. The blinded treatment assignments will be accessible to the investigator and Trial Manager should a subject need to be unblinded in an emergency using the Sealed Envelope (Ltd.) system. If unblinding occurs, the investigator must record the reason for unblinding, as well as the date and time of the event. Corresponding information will be recorded on the CRF by the investigator.

8.8 Subsequent Assessments

Table 1 (pg. 27) shows the visit numbers and window periods for the visits. A detailed description of each assessment is provided in Section 8 – Study Procedures (pg. 21).

Visit 0 (Screening Visit) – clinic visit (- 1 week to - 2 days)

During this visit, there will be an eligibility check of inclusion/exclusion criteria, concomitant medications recorded and a safety assessment including physical examination performed. Screening blood tests will be performed if not checked in the last 3 months. Participants will be provided with a diary within which to record their consumption of food and drink throughout the course of the study. Specifically, participants will be asked to record all food and drink consumed, including details about the amount/portion size consumed, a description of what was consumed (i.e., the brand) and the time that it was consumed for a minimum of four days to include the 48 hour restriction period. The diaries will be used to determine whether or not participants have adhered to the 48 hour pre-visit restriction period (see Section 8.4. pg. 22) and their daily energy restriction diet prescription (if applicable; see Section 8.5, pg. 25). The diaries will be checked by a member of the research team at each visit.

Visit 1 (Baseline Visit, 0 weeks) – clinic visit

Dispensing Visit

During this visit, there will be a check for ongoing consent to participate in the trial, record of concomitant medications and baseline blood tests performed including renal and liver function tests for safety. Study drugs will be dispensed following randomization. Energy restriction diet will be prescribed if participants are randomized to this intervention.

Visit 2 – clinic visit at 2 weeks (- 5 days to + 5 days)
Dispensing Visit
During this visit, there will be a check for ongoing consent to participate in the trial, record of concomitant medications and blood tests performed for safety including renal and liver function tests. There will be assessment of study drug compliance and/or energy restriction diet compliance. Drug accountability will be performed.

Visit 3 – clinic visit at 6 weeks (- 5 days to + 5 days)
During this visit, there will be a check for ongoing consent to participate in the trial, record of concomitant medications and blood tests performed for safety including renal and liver function tests. There will be assessment of study drug compliance and/or energy restriction diet compliance. Drug accountability will be performed.

Visit 4 – clinic visit at 12 weeks (- 5 days to + 5 days)
Dispensing Visit
During this visit, there will be a check for ongoing consent to participate in the trial, record of concomitant medications and blood tests performed for safety including renal and liver function tests. Study drugs will be dispensed again at this visit. There will be assessment of study drug and/or energy restriction diet compliance. Drug accountability will be performed.

Visit 5 (Final Visit) – clinic visit at 24 weeks (- 5 days to + 5 days)
During this visit, there will be a check for ongoing consent to participate in the trial, record of concomitant medications and blood tests performed for safety including renal and liver function tests. There will be assessment of compliance with study drugs and/or energy restriction diet. Drug accountability will be performed.

Telephone Consultation – 7 days after medication discontinuation (- 3 days to + 3 days)
Participants will be contacted by telephone within 3-7 days after study medication discontinuation in order to capture of any potential Adverse Events.

Table 1 – Measures at each Study Visit

Procedure or assessment during experimental period	**Visit 0 (Familiarisation Visit)** **-1 week to – 2 days**	**Visit 1 (Baseline) 0 weeks**	**Visit 2 (2 weeks)** **- 5 days to + 5 days**	**Visit 3 (6 weeks)** **- 5 days to + 5 days**	**4th Visit (12 weeks)** **-5 days to +5 days**	**5th and Final Visit (24 weeks)** **- 5 days to + 5 days**	**Follow-up Telephone Call (1 week after medication completion)** **- 3 days to + 3 days**
Inclusion/Exclusion criteria Explanation of Study Procedures Informed consent	X						
Confirmation of continued will to participate in the study		X	X	X	X	X	
Randomisation		X					
Demographics	X						
Smoking status and alcohol intake	X						
Medical history Physical examination	X					X	
Concomitant Medication review	X	X	X	X	X	X	
Height, weight, waist and hip circumference Resting heart rate and blood pressure	X	X	X	X	X	X	
Dispensing of Study Drug		X	X		X		
Study drug accountability			X	X	X	X	

Physical activity questionnaire (IPAQ)		X	X	X	X	X	
Appetite questionnaires (VAS, 3-Factor Eating)		X	X	X	X	X	
Screening blood test U+E, LFT, lipids, CRP, eGFR, HbA1c	X						
Urine samples		X	X	X	X	X	
Biochemical measures FBC,U+E,LFT,lipids,HbA1c,CRP,eGFR, UGE,FPG,FFA (once per visit), insulin, glucose, glucagon, leptin, PYY, ghrelin, GLP-1 (7 times per visit – pre and post meal)		X	X	X	X	X	
Resting energy expenditure (indirect calorimetry)		X	X	X	X	X	
Physical activity (Accelerometer [A], Inclinometer [I]	X [A,I]			X [A]	X[A,I]	X[A,I]	
Log of wearing monitors		X	X	X	X	X	
Body composition (DEXA scan)		X				X	

AE reporting		X	X	X	X	X	X
Food and Drink Diary	X	X	X	X	X	X	
Daily Energy Requirement estimation		X					
Standardised Breakfast Meal calculation		X					
Standardised Breakfast Meal		X	X	X	X	X	
48hr Pre-visit Standardisation		X	X	X	X	X	
24hr Meal Standardisation		X	X	X	X	X	
Energy Restriction Diet Prescription Assessment		X		X	X		
Energy Restriction Diet compliance check		X	X	X	X	X	

8.9 Definition of End of Trial

The end of trial is the date of the last visit of the last participant.

8.10 Discontinuation/Withdrawal of Participants from Study Treatment

Each participant has the right to withdraw from the study at any time without needing to give a reason. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:-

- Participant intolerance to study medication, as assessed at each visit by the study team, will necessitate withdrawal of participant from the study as study drug dose will not be reduced during the trial
- Ineligibility (either arising during the study or retrospective 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 study medication or results in inability to continue to comply with study procedures
- Consent withdrawn
- Lost to follow up

The reason for withdrawal will be recorded in the CRF and medical records if known. If the participant is withdrawn due to an adverse event, the investigator will arrange for safety follow-up visits or telephone calls until the adverse event has resolved or stabilised. The duration of safety follow-ups will be at least five half-lives of the study medication. The half-life of Empagliflozin (Jardiance™) is 12.4 hours, thus the duration of safety follow-ups will be rounded up to be at least 72 hours (or 3 days).

The participant will be withdrawn from the study if they lose capacity and data collected up to that point will be used for analysis. As analysis will be on an intention-to-treat basis, there will be analysis of all data of participants receiving study medication (e.g. most safety analyses) and data will be admitted to the database even after the participant has withdrawn from the study.

Certain circumstances will necessitate the stopping or interruption of the study medication for a particular participant. Adverse event review and other safety/acceptability assessments will provide the information for the study clinician to withdraw or interrupt the study drug at any time during the trial. Specifically, it may be necessary to interrupt treatment with SGLT2-ingibitors (such as Empagliflozin (Jardiance™)) in participants who are hospitalised for major surgery or acute serious illness; treatment may be restarted once the participant's condition has stabilised and only once the necessary safety assessments have been performed. The decision to stop or interrupt and subsequently re-start the study treatment Empagliflozin (Jardiance™) will be documented in the participant's medical notes.

Any decisions regarding the withdrawal of participants from the study will be made by the study clinician and Principal Investigator.

Each participant will have a copy of the consent form and patient information leaflet placed in their hospital medical records. This will also include explicit notice of their in-study medication and a standard label will be used on the front of the medical notes to highlight to any reviewer that this individual is taking part in the study and any issue regarding contra-indication of a procedure or medication outside of the study should be discussed with a study clinician.

The standard label template is provided by the local trust and should contain the following information:

PATIENT TAKING PART IN A CLINICAL TRIAL
Study Name. **SEESAW**
Patient ID No. . . . . .
Investigator: . . . . . .
Telephone: . . . . . .
Date Consent Form Signed . . . . . .
TRIAL START <date> TRIAL FINISH <date>
Don't destory the records before <date>

8.11 Source Data

Source documents are original documents, data, and records from which participants' CRF data will be obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication will be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no other written or electronic record of data). All documents will be stored safely in a secure office environment and will be listed on a source data agreement. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code only, and not by name.

9. TREATMENT OF TRIAL PARTICIPANTS

9.1 Description of Study Treatment & Pharmacy Process

The study medication is Empagliflozin (Jardiance™) which is an oral film-coated tablet manufactured by Boehringer Ingelheim pharmaceutical company. Placebo will also be manufactured and supplied by Boehringer Ingelheim. Both Empagliflozin (Jardiance™) and the placebo can be described as follows: pale yellow, biconvex film-coated tablets, one side debossed with the Boehringer Ingelheim company symbol and the other side debossed with "S25". The dosage will be 25mg once daily for Empagliflozin (Jardiance™), which is the maximum dose, licensed for the treatment of Type 2 Diabetes in the UK. The placebo will be labelled as '25mg once daily' to match. There is a lower dose of Empagliflozin (Jardiance™) 10mg daily. However, this dose will not be used in the study and participants who do not tolerate Empagliflozin (Jardiance™) 25mg daily will be discontinued from the study.

The ingredients of the placebo are:

- Tablet core
- Lactose monohydrate
- Microcrystalline cellulose
- Hydroxypropylcellulose
- Croscarmellose sodium
- Colloidal anhydrous silica
- Magnesium stearate

- Film coating
- Hypromellose
- Titanium dioxide (E171)
- Talc
- Macrogol (400)
- Iron oxide yellow (E172)

The licensed product Empagliflozin (Jardiance™) will be handled according to UHL pharmacy in-house protocols. Specifically, the employment, dosing, handling, storage and destruction of Empagliflozin (Jardiance™) and its placebo will be conducted in accordance with the specified conditions of its approved labelling and in line with our UHL pharmacy guidelines. All study medication dosage will not exceed the stated recommendations of local guidelines and the British National Formulary. All contra-indications will be checked as part of the eligibility criteria.

Boehringer Ingelheim will supply both Empagliflozin (Jardiance™) and the placebo to the third party company ALMAC who will arrange the packaging, blinding and labelling of both Empagliflozin (Jardiance™) and the placebo prior to delivery and storage of the finished blinded stock to Leicester General Hospital Pharmacy.

Study medication may be suspended or stopped by the study team if they become aware of the participant undergoing any contra-indicated procedure for out of study clinical care. This will be documented in the individual's study and medical notes and decision on any potential withdrawal from the study on this basis will reside with the PI. Recommencing of the study drug will follow individual safety assessments in-line with the procedure in question.

Prior to starting treatment with empagliflozin, the following factors will be considered as they may predispose patients to acute kidney injury:-

- decreased blood volume
- chronic kidney insufficiency
- congestive heart failure
- on diuretics, ACE inhibitors, ARBs, and NSAIDs

Kidney function will be assessed prior to starting treatment with empagliflozin and at each study visit. If acute kidney injury occurs, empagliflozin will be promptly discontinued and kidney impairment treated using standard guidelines.

9.2 Storage of Study Treatment

The study medication Empagliflozin (Jardiance™) and placebo will be stored at room temperature in a designated, restricted, clinical trials area within the pharmacy department at Leicester General Hospital. ALMAC will manage drug distribution with 50% of the drugs required (plus 10-20% for waste) being provided in the first instance. Thereafter the drugs will be automatically ordered and distributed to the Pharmacy as this is part of the functionality available on the randomization system.

9.3 Compliance with Study Treatment

The participants will be instructed to return all unused or part-used medication and packaging from used medication at each visit. The Investigator may withdraw the participants if they consider dose compliance is unsatisfactory.

9.4 Accountability of the Study Treatment

The study medication will be supplied by Boehringer Ingelheim to Leicester General Hospital pharmacy. All movements of study medication between Boehringer Ingelheim and pharmacy will be documented.

The Investigator will use a trial specific prescription form and a member of the Investigator team will collect the medication Empagliflozin (Jardiance™) or placebo.

The participant will be asked to bring all unused medication and packaging back to the clinic at each visit where it will be returned to pharmacy and the clinical trial pharmacists will be responsible for destroying the returned stock.

9.5 Concomitant Medication

Throughout the study, Investigators will be able to prescribe any concomitant medications or treatments

deemed necessary to provide adequate supportive care except for those listed in the exclusion criteria. If these are required, the participant will be withdrawn.

Any medication, other than the study medication taken during the study will be recorded in the CRF.

Contraindicated medications during the study will include any other glucose-lowering therapies apart from metformin. Loop diuretic therapy will also be contraindicated.

As study drug Empagliflozin (Jardiance™), placebo and metformin do not cause hypoglycaemia, participants will not be expected to perform home blood glucose monitoring during the study.

10. SAFETY REPORTING

10.1 Definitions

Adverse Event (AE)

An AE or adverse experience is:

Any untoward medical occurrence in a patient or clinical investigation participants administered a medicinal product, which does not necessarily have to have a causal relationship with this treatment (the study medication).

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study medication, whether or not considered related to the study medication.

Adverse Reaction (AR)

All untoward and unintended responses to a medicinal product related to any dose. The phrase "responses to a medicinal product" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

Severe Adverse Events (SAE)

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Serious Adverse Event or Serious Adverse Reaction (SAE or SAR)

A serious adverse event or reaction is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which 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.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Other important medical events*

*Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Expected Serious Adverse Events/Reactions (SAE)

The most common adverse events associated with Empagliflozin (Jardiance™) are urinary tract and mycotic fungal infections (e.g. balanitis) and volume depletion leading to dehydration, postural hypotension and dizziness. If these events lead to hospitalisation of the participant, they will require immediate reporting to the study team. Otherwise, they will be treated as an AE and need reporting to the study team within 2 working days.

Abnormalities of renal and liver function will also be closely monitored as part of the usual study visits and any significant clinical deterioration in renal and/or liver function will be reported as an SAE. A serious deterioration in renal function to be classified as an SAE will be a reduction in eGFR to less than 30ml/min/1.73m^2. A serious deterioration in liver function will be classified as an SAE if ALT or AST is 3x greater than the upper limit of normal (ULN).

Ketoacidosis and Diabetic Ketoacidosis (DKA) have recently been reported as a possible serious SAE occurring with SGLT-2 inhibitor use by the US Food and Drugs Administration. Participants will be closely monitored for the development of this SAE and will be advised to seek medical attention immediately if they experience symptoms such as difficulty breathing, nausea, vomiting, abdominal pain, confusion, unusual fatigue or sleepiness, anorexia, excessive thirst, dehydration, low food intake, weight loss, infection, poor control of diabetes, and/or only moderately increased glucose levels.

In patients where DKA is suspected or diagnosed, treatment with SGLT-2 inhibitors will be discontinued immediately. Restarting SGLT-2 inhibitor treatment in patients with previous DKA while on SGLT-2 inhibitor treatment is not recommended unless another clear precipitating factor is identified and resolved. Treatment will also be interrupted in patients who are hospitalised for major surgical procedures or acute medical illnesses. In both cases, treatment with SGLT-2 inhibitors may be restarted once the patient's condition has stabilised and only once the necessary safety assessments have been performed. The decision to stop or interrupt and subsequently re-start the study treatment Empagliflozin (Jardiance™) will be documented in the participant's medical notes. Any decisions regarding the withdrawal of participants from the study will be made by the study clinician and Principal Investigator. Further details about the discontinuation/withdrawal from, or interruption of, the study treatment can be found in Section 8.9, above (pg. 30).

Suspected Unexpected Serious Adverse Reactions (SUSAR)

A serious adverse reaction, the nature or severity of which is not consistent with the Summary of Product Characteristics for Empagliflozin (Jardiance™) will be reported as a SUSAR.

Adverse Events of Special Interests (AESI)

- Decreased renal function: creatinine value shows a ≥ 2 fold increase from baseline and is above ULN

- Hepatic injury defined by the following alterations of liver parameters after randomization at visit 1:-
  - Elevation of AST and/or ALT≥3 fold ULN combined with an elevation of total bilirubin≥2 fold ULN measured in the same blood draw sample
  - Isolated elevation of AST and/or ALT≥5 fold ULN irrespective of any bilirubin elevation

- Diabetic Ketoacidosis (DKA)
  - DKA is defined by the diagnostic criteria in the table below, and as defined by the American Diabetes Association (ADA).

Investigators should note that not all criteria in Table 2 (below) need to apply for the diagnosis of DKA, and clinical judgment should also be taken into consideration. Due to its mechanism of action, Empagliflozin (Jardiance™) may potentially modify the clinical presentation of DKA which may occur at lower plasma glucose levels than stated in the table below.

- Events involving Lower Limb Amputation
  - This definition includes amputation (i.e. resection of a limb through a bone), disarticulation (i.e. resection of a limb through a joint) and auto-amputations (i.e. spontaneous separation of non-viable portion of the lower limb).
  - Not included in this definition are debridement (removal of callus or dead tissue), procedures on a stump (like stump revision, drainage of an abscess, wound revision etc.) and other procedures (e.g., nail resection or removal) without a concomitant resection of a limb (amputation or disarticulation).
  - Each lower limb amputation, disarticulation, or auto-amputation should be reported separately. The SAE report should include the date of the procedure, the level of amputation or disarticulation, the medical condition(s) leading to the procedure and if the patient had some of the known risk factor(s) for lower limb amputation.

If any AESIs occur they will be reported to the Sponsor and Boehringer Ingelheim using the same procedure as reporting Serious Adverse Events (SAEs; see Section 10.3, pg. 38).

Table 2 – Diagnostic criteria for DKA

	DKA		
	Mild	Moderate	Severe
Plasma glucose (mg/dL)	>250	>250	>250
Arterial pH	7.25-7.30	7.00-7.24	<7.00
Serum bicarbonate (mEq/L)	15-18	10 to <15	<10
Urine ketones*	Positive	Positive	Positive
Serum ketones*	Positive	Positive	Positive
Effective serum osmolality (mOsm/kg)**	Variable	Variable	Variable
Anion gap***	>10	>12	>12
Alteration in sensoria or mental obtundation	Alert	Alert/drowsy	Stupor/coma

* Nitroprusside reaction method

** Calculation: 2[measured Na (mEq/L) + glucose (mg/dL)/18

*** Calculation: (Na+) – (Cl- + HCO3-) (mEq/L)

10.2 Reporting Procedures for All Adverse Events

All AEs, including AESI, occurring during the study observed by the investigator or reported by the participant, whether or not attributed to study medication, will be recorded on the CRF and medical records.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

AEs considered related to the study medication as judged by a medically qualified investigator or the Sponsor will be followed until resolution or the event is considered stable. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs. The duration of safety follow-ups will be at least five half-lives of the study medication. The half-life of Empagliflozin (Jardiance™) is 12.4 hours, thus the duration of safety follow-ups will be rounded up to be at least 72 hours (or 3 days).

It will be the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from treatment (see Section 8.9, pg. 30).

A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable. The duration of safety follow-ups will be at least five half-lives of the study medication. The half-life of

Empagliflozin (Jardiance™) is 12.4 hours, thus the duration of safety follow-ups will be rounded up to be at least 72 hours (or 3 days).

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

The relationship of AEs to the study medication will be assessed by a medically qualified investigator.

10.3 Reporting Procedures for Serious Adverse Events

All SAEs, except those expected ones defined in Section 10.1 (pg. 35) that do not require immediate reporting will be reported to the Sponsor and Boehringer Ingelheim within 24 hours of discovery or notification of the event. The SAE will be reported using appropriate forms and the immediate report will be made in writing and shall be followed by a detailed written report of the event. Additional information can be provided if requested to the Sponsor and main Research Ethics Committee (REC) (e.g. in the event of a death). The Principal Investigator or another delegated physician is responsible for the review and sign off of the SAE, or in their absence, another member of the team (in order to avoid a delay).

The Sponsor will perform an initial check of the information and ensure that it is reviewed at the next R&D management meeting. All SAE information must be recorded on an SAE form and sent to the Sponsor. Additional information received for a case (follow-up or corrections to the original case) needs to be detailed on a new SAE form and sent to the Sponsor.

The Sponsor will report all SUSARs to the MHRA and the REC concerned. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. The Chief Investigator will inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants. The Sponsor will use the Summary of Product Characteristics (SmPC) as the Reference Safety Information (RSI) when determining the expectedness of any untoward medical occurrences and thus SAE/SUSAR reporting.

In addition to the expedited reporting above, the Chief Investigator will submit once a year throughout the clinical study or on request a Developmental Safety Update Report (DSUR) to the MHRA and REC. The PI will inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

The investigator site file will contain documentation for:-

- SAE, SAR and SUSAR reports
- Evidence of submission of SAEs to the Sponsor within 24 hours of the team becoming aware of an event
- Evidence of timely SUSAR submission to the MHRA and main REC.

11. STATISTICS

11.1 Description of Statistical Methods

Participants recruited to the study will be compared within and by treatment group. For the latter, each active treatment arm, whether medication, medication and energy restriction diet or placebo and energy restriction diet, will be compared against the placebo only arm. Baseline variables will be presented by arm using N (%) for categorical variables and mean (standard deviation) or median (interquartile range) as appropriate, for continuous variables.

The primary outcome is change in appetite hormones from baseline to week 24 within and between treatment groups. AUC values for appetite hormone measurements at baseline and 24 weeks will be calculated using the trapezoidal rule. Paired t-tests or equivalent non-parametric tests will be used to analyse the change in appetite hormones within individual treatment groups. One way analysis of variance will be used to calculate mean changes in AUC from baseline to 24 weeks by treatment group. Adjusted treatment effects will be calculated via linear regression analysis or similarly appropriate methods, to compare each treatment against placebo. Models will include a categorical treatment variable and will be adjusted for stratification factors; age and BMI as well as baseline AUC value. Changes in appetite hormones from baseline to weeks 2, 6 and 12 will also be analysed using the same methods; however will represent hypothesis generating analyses only. Similarly, overall changes in appetite hormones over time will be analysed using multilevel models, adjusted for stratification factors.

The analysis for the primary outcome will be repeated for secondary endpoints; changes in resting energy expenditure, weight and body composition, physical activity and biochemical parameters from baseline.

All analyses of outcome data will be carried out on a complete case basis, so only those patients with complete data will be included in the analysis. Intention to treat and per protocol analyses will be carried out as sensitivity analyses, using multiple imputation and including only those who remain on their treatment regimen for the duration of the study, respectively.

11.2 The Number of Participants

The power calculation is based on the primary outcome of change in appetite hormones and a standard deviation of AUC of 96.2pg/ml of total PYY based on a study looking at the influence of resistance and aerobic exercise on hunger and circulating levels of total PYY in healthy males (Broom 2009). To detect a minimum clinically significant difference of 120pg/ml in AUC of total PYY between groups, we will require 15 participants in each of the four arms with 80% power and 2-sided alpha of 1.7%. This will allow three comparisons between arms. To account for up to 20% dropout, 19 participants will be required in each arm; therefore 76 participants will be recruited in total.

11.3 The Level of Statistical Significance

Statistical significance will be taken at the level of $p<0.05$.

11.4 Criteria for the Termination of the Trial

No formal criteria for termination of the trial will be set. A Data Safety Monitoring Committee (DSMC) will oversee the trial in terms of safety and efficacy. The DSMC will be made up of two clinicians and a statistician, all of whom will be independent of the trial. The DSMC will assess adverse and serious adverse events unblinded to treatment allocation. The DSMC can make a recommendation to the TSC to stop the trial on both safety and overt efficacy grounds. There are no plans for a formal interim analysis.

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

Data will be entered into a database with real-time validation, which will limit spurious data. Before analysis begins all data will be checked and any anomalies will be checked against the source data.

During data collection we will attempt to minimise missing data items. Participants with missing data will be excluded on a case-wise basis, i.e. only from those analyses which required the missing items. The primary and main secondary outcomes will be analysed on both a complete case basis and using multiple imputation to estimate the effect of missing data.

11.6 Procedures for Reporting any Deviation(s) from the Original Statistical Plan

A statistical analysis plan will be written and agreed by all investigators prior to database lock. Any deviations from the statistical analysis plan will be stated in the trial publication with the reasons and justification for the deviation.

11.7 Inclusion in Analysis

All analyses of outcome data will be carried out on a complete case basis according to randomised group. Therefore those with missing outcome data will be excluded from this analysis. As a sensitivity analysis, for the primary and main secondary outcome, multiple imputation will be used to perform an intention to treat analysis, and to assess the impact of missing outcome data. We will also conduct a per protocol analysis excluding those who report no longer taking their randomised treatment at the four follow ups.

12. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures (SOPs).

Regular monitoring will be performed according to ICH GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

As part of the quality management process, a monitoring plan will be developed by the Sponsor in accordance with Sponsor SOPs. The monitoring plan will be based on a study risk assessment which is determined by the level of risk within the study to participant safety, integrity of the trial and trial data validity. All trial monitoring will be conducted in accordance with the monitoring plan and will be undertaken by the trial Sponsor. All monitoring will be performed by staff who are ICH/GCP trained and are competent in monitoring to GCP standards. A documented monitoring log and audit trail will be maintained throughout the lifetime of the study.

The trial manager will also undertake internal audit check to ensure compliance with protocol, GCP and regulatory requirements.

All source data, study documents, and participant notes will be made available for monitoring, audits and inspections by the Ethics Committee, the Sponsor and the Regulatory Authority.

14. CODES OF PRACTICE AND REGULATIONS

14.1 Ethics

Participants will be free to withdraw at any time from the study without giving a reason and without their legal rights being affected. All study procedures including risks involved will be explained clearly to the participant at the Screening Visit and subsequently before each procedure is performed.

The overall care and comfort of the participant will be considered paramount at all times during the study.

14.2 Sponsor Standard Operating Procedures

All relevant Sponsor SOPs will be followed to ensure that this study complies with all relevant legislation and guidelines.

14.3 Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004).

14.4 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

14.5 Approvals

The trial will not commence until Sponsor green light is given. Once Sponsor (University of Leicester) authorisation has been confirmed, the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), regulatory authorities (MHRA in the UK), and host institution's research and development department for written approval.

Once Sponsor authorisation has been confirmed, the Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

14.6 Participant Confidentiality

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

All research data will be kept in a secure office environment within Leicester Diabetes Centre, Leicester General Hospital during the active phase of the study and until the data have been analysed. It will then be archived in line with University of Leicester policy.

14.7 Other Ethical Considerations

Placebo study drug will be received by 50% of the study participants. The reason for this will be clearly explained to the participant at the screening visit and described in the participant information leaflet.

15. DATA HANDLING AND RECORD KEEPING

The participants will be identified by a study specific participant number and/or code in any database. The name and any other identifying detail will not be included in any study data electronic file.

Each participant will be assigned a unique identification number upon recruitment. Patient contact details will be held on an independent database and used to arrange data collection visits and send out follow-up questionnaires. The database will be password protected and only researchers collecting data will have access. All data collected during the study will be stored anonymously on a separate database on NHS computers. Again access will be password protected and restricted to relevant members of the research team.

Paper copies of the CRFs and questionnaires will be stored in a secure office environment in the Leicester Diabetes Centre, Leicester General Hospital. Neither hard copies nor electronic files containing personal information will be removed from the research office or stored in a non-secure manner electronically. The study research team will comply with the Data Protection Policy of the University of Leicester and the local NHS Trusts.

The Leicester Clinical Trials Unit (CTU) is a UK Clinical Research Collaboration fully registered CTUs and has been involved in this application from the beginning and will be involved throughout the project. The CTU has a well-established IT infrastructure and will be providing support for this study through database development and data management. They will use a Clinical Data Management System called InferMed Macro v4 to set up a tailored data capture method that meets the needs of the study. This is a secure and validated database solution, with quality control mechanisms to ensure that the data collected are complete and accurate. The CTU has a robust quality management system (QMS). All staff working on this project will work within this QMS framework ensuring that the relevant staff are adequately trained, fully supported and working to common standard operating procedures. The CTU will ensure compliance with the appropriate governance, stipulations and trial protocol. The database solutions that the CTU implement using MACRO are designed to meet the requirements of International Conference on Harmonisation GCP and the Medicines for Human Use (Clinical Trials) Regulations 2004. The installation of InferMed Macro and the hosting environment is fully validated is configured using an automatic backup and failover architecture. The hosting agreement sets out a target of 99.95% availability excluding scheduled downtime for maintenance.

All study data will be entered on a customised database developed by the CTU. The electronic data entry system will be validated and SOPs maintained.

16. STUDY GOVERNANCE

The study will be conducted in accordance with the UK Policy Framework for Health and Social Care Research, ICH GCP and the Data Protection Act. The Sponsor responsible for checking research governance arrangements will be the University of Leicester.

16.1 Trial Steering Committee (TSC)

TSC will comprise an independent chair, at least one other independent clinician with expertise in the management of diabetes, the chief investigator and key co-investigators. The trial statisticians may attend as needed. This committee will be responsible for the overall management and oversight of the trial and will meet once prior to start up and then every 6 months, normally within 2 months of the Data Safety Monitoring Committee (DSMC) (although additional meetings may be called by the CI or TSC or DSMC chair) to review and approve protocol amendments and any sub-study proposals, review recruitment rates, protocol adherence, retention, compliance, safety issues, planned analyses and reports and act on recommendations of the DSMC. Minutes from the TSC will be copied to the Sponsor.

16.2 Data Safety Monitoring Committee (DSMC)

The DSMC charter will be prepared as per Sponsor SOP. DSMC will comprise an independent clinician, an independent chair and independent statistician. The chief investigator or trial statistician may be invited to attend to provide specific input by the DSMC Chair. The DSMC will be responsible for the interests and safety of the participants and its main role will be to make advisory recommendations to the TSC. To this end, the DSMC will undertake safety data reviews every six months after recruitment begins, unless otherwise deemed necessary. In addition, the DSMC will review analysis plans.

16.3 Trial Management Group (TMG)

TMG will report to the trial steering committee and will include the chief investigator, other senior investigators and the day-to-day project management team. The group will meet monthly or bi-monthly depending on need, either face-to-face or by teleconference, to discuss the details and logistics of recruitment, retention and follow-up data collection.

17. PUBLICATION POLICY

It is envisaged that the results of the study will be published in the relevant Diabetes Journals and disseminated at national and international conferences and meetings.

Acknowledgement of any supporting organisations, including funders, the University of Leicester, Loughborough University, Leicester Clinical Trials Unit, and Leicester-Loughborough Lifestyle BRU will be included.

18. REFERENCES

1. Bailey CJ. Renal glucose reabsorption inhibitors to treat diabetes. Trends Pharmacol Sci. 2011;32(2):63-71. doi: 10.1016/j.tips.2010.11.011 [doi].

2. Bailey CJ. SGLT2 inhibitors:Glucuretic treatment for type 2 diabetes. British Journal of Diabetes and Vascular Disease. 2010;10:193-199.

3. Hasan FM, Alsahli M, Gerich JE. SGLT2 inhibitors in the treatment of type 2 diabetes. Diabetes Res Clin Pract. 2014;104(3):297-322. doi: 10.1016/j.diabres.2014.02.014 [doi].

4. Bolinder J, Ljunggren O, Kullberg J, et al. Effects of dapagliflozin on body weight, total fat mass, and regional adipose tissue distribution in patients with type 2 diabetes mellitus with inadequate glycemic control on metformin. J Clin Endocrinol Metab. 2012;97(3):1020-1031. doi: 10.1210/jc.2011-2260 [doi].

5. Broom DR, Batterham RL, King JA, Stensel DJ. Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. Am J Physiol Regul Integr Comp Physiol. 2009; 296(1):R29-35.

6. Ferrannini E, Seman L, Seewaldt-Becker E, Hantel S, Pinnetti S, Woerle HJ. A phase IIb, randomized, placebo-controlled study of the SGLT2 inhibitor empagliflozin in patients with type 2 diabetes. Diabetes Obes Metab. 2013;15(8):721-728. doi: 10.1111/dom.12081 [doi].

7. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest. 2014;124(2):499-508. doi: 10.1172/JCI72227 [doi].

8. Ferrannini G, Hach T, Crowe S et al. Energy balance after sodium glucose cotransporter 2 (SGLT2) inhibition. Diab Care 2015. DOI 10.2337/dc15-0355.

9. Haring H-U, Merker L, Seewaldt-Becker E et al. Empagliflozin as add-on to metformin plus sulphonylurea in patients with type 2 diabetes: a 24-week, randomized, double-blind, placebo-controlled trial. Diabetes Care 2013;36:3396-3404.

10. Haring H-U, Merker L, Seewaldt-Becker E et al. Empagliflozin as add-on to metformin in patients with type 2 diabetes: a 24-week, randomized, double-blind, placebo-controlled trial. Diabetes Care 2014;37:1650-1659.

11. Kovacs CS, Seshiah V, Swallow R et al. Empagliflozin improves glycaemic and weight control as add-on therapy to pioglitazone or pioglitazone plus metformin in patients with type 2 diabetes: a 24-week, randomized, placebo-controlled trial. Diab Ob Metab 2014;16:147-158.

12. Madsbad S. The role of glucagon-like peptide-1 impairment in obesity and potential therapeutic implications. Diab Obes Metab 2014;16:9-21.

13. Merovci A, Solis-Herrera C, Daniele G, et al. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. J Clin Invest. 2014;124(2):509-514. doi: 10.1172/JCI70704 [doi].

14. Mifflin MD, St Jeor ST, Hill LA et al. A new predictive equation for resting energy expenditure in healthy individuals. Am J Clin Nutr 1990;51:241-7.

15. Rahmoune H, Thompson PW, Ward JM, Smith CD, Hong G, Brown J. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. Diabetes. 2005;54(12):3427-3434. doi: 54/12/3427 [pii].

16. Riderstrale M, Andersen KR, Zeller C et al. Comparison of empagliflozin and glimepiride as add-on to metformin in patients with type 2 diabetes: a 104-week randomised, active-controlled, double-blind, phase 3 trial. Lancet Diab Endorcrinol 2014;2:691-700.

17. Roden M, Weng J, Eilbracht J et al. Empagliflozin monotherapy with sitagliptin as an active comparator I patients with type 2 diabetes: a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Diab Endocrinol 2013:208-19.

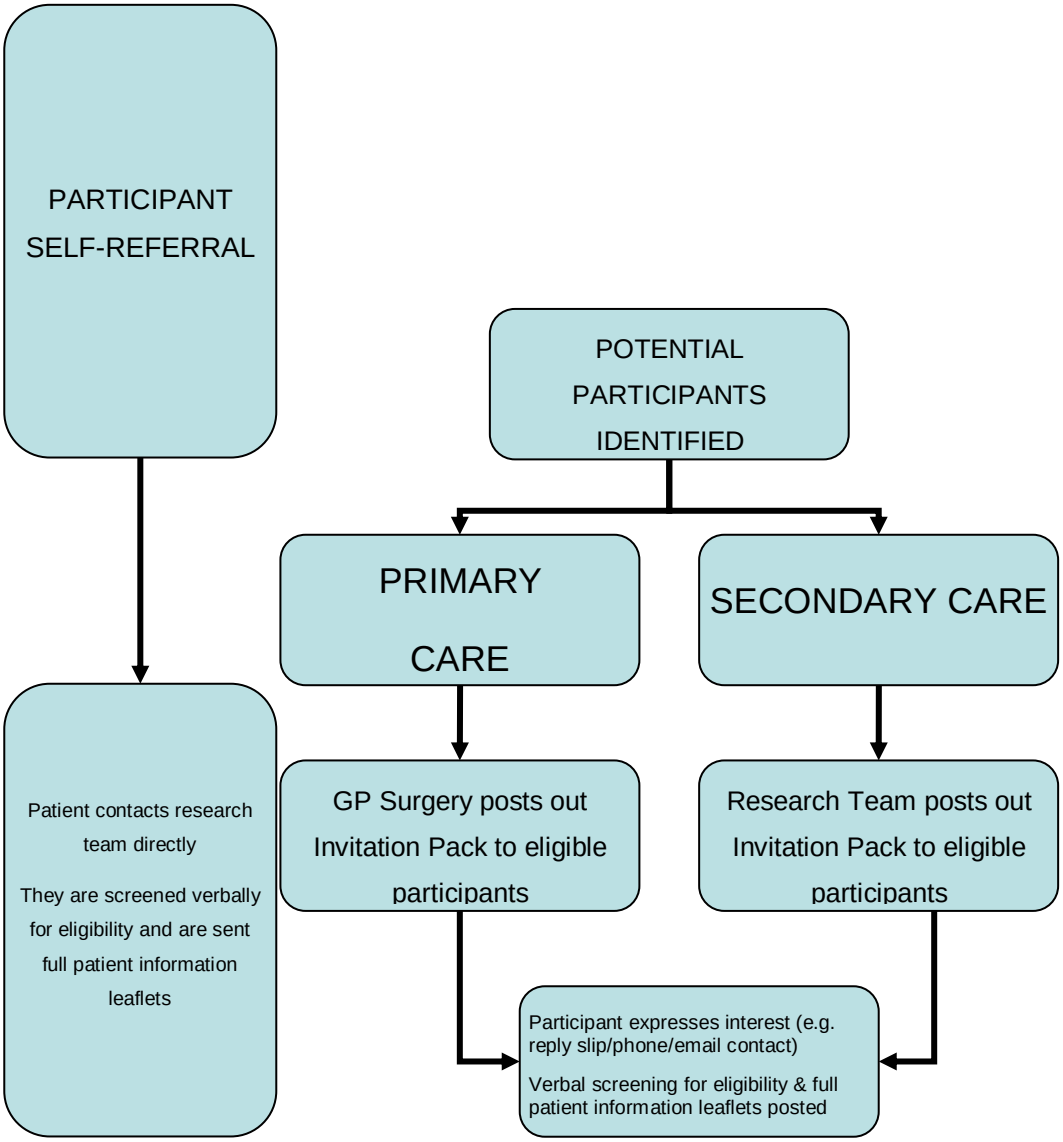
18. Rosenstock J, Jelaska A, Frappin G et al. Improved glucose control with weight loss, lower insulin doses and no increased hypoglycaemia with empagliflozin added to titrated multiple daily injections of insulin in obese inadequately controlled type 2 diabetes. Diabetes Care 2014;37:1815-1823.

19. Sumithran P, Prendergast LA, Delbridge E, et al. Long-term persistence of hormonal adaptations to weight loss. N Engl J Med. 2011;365(17):1597-1604. doi: 10.1056/NEJMoa1105816 [doi].

20. Tahrani AA, Barnett AH, Bailey CJ. SGLT inhibitors in management of diabetes. Lancet Diabetes Endocrinol. 2013;1(2):140-151. doi: 10.1016/S2213-8587(13)70050-0 [doi].

21. Vasilakou D, Karagiannis T, Athanasiadou E, et al. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: A systematic review and meta-analysis. Ann Intern Med. 2013;159(4):262-274. doi: 10.7326/0003-4819-159-4-201308200-00007 [doi].

APPENDIX 1 – Participant Recruitment Pathway



SEESAW – Visual Analogue Scale

Visual Analogue Scale

Time: Pre-breakfast (baseline)

Researcher to measure from left to right in mm to give a score between 0-100.
Write this score above where participant marks **X**

Place a mark on the horizontal lines below after considering the following questions:

How hungry do you feel?

I am not hungry at all ——— I have never been more hungry

How satisfied do you feel?

I am completely empty ——— I cannot eat another bite

How full do you feel?

Not at all full ——— Totally full

How much do you think you can eat?

Nothing at all ——— A lot

How nauseous do you feel?

Not at all nauseous ——— Very nauseous

APPENDIX: THREE-FACTOR EATING QUESTIONNAIRE

One point is given for each item in Part I and for each item (numbered question) in Part II. The correct answer for the true/false items is underlined and beside it is the number of the factor that it measures. The direction of the question in Part II is determined by splitting the responses at the middle. If the item is labelled '+', those responses above the middle are given a zero. Vice versa for those with a '−'. For example, anyone scoring 3 or 4 on the first item in Part II (item No. 37) would receive one point. Anyone scoring 1 or 2 would receive a zero.

Part I

				Factor Number
1.	When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.	T	F	2
2.	I usually eat too much at social occasions, like parties and picnics.	T	F	2
3.	I am usually so hungry that I eat more than three times a day.	T	F	3
4.	When I have eaten my quota of calories, I am usually good about not eating any more.	T	F	1
5.	Dieting is so hard for me because I just get too hungry.	T	F	3
6.	I deliberately take small helpings as a means of controlling my weight.	T	F	1
7.	Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	T	F	2
8.	Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.	T	F	3
9.	When I feel anxious, I find myself eating.	T	F	2
10.	Life is too short to worry about dieting.	T	F	1
11.	Since my weight goes up and down, I have gone on reducing diets more than once.	T	F	2
12.	I often feel so hungry that I just have to eat something.	T	F	3
13.	When I am with someone who is overeating, I usually overeat too.	T	F	2
14.	I have a pretty good idea of the number of calories in common food.	T	F	1
15.	Sometimes when I start eating, I just can't seem to stop.	T	F	2
16.	It is not difficult for me to leave something on my plate.	T	F	2
17.	At certain times of the day, I get hungry because I have gotten used to eating then.	T	F	3
18.	While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.	T	F	1
19.	Being with someone who is eating often makes me hungry enough to eat also.	T	F	3
20.	When I feel blue, I often overeat.	T	F	2
21.	I enjoy eating too much to spoil it by counting calories or watching my weight.	T	F	1
22.	When I see a real delicacy, I often get so hungry that I have to eat right away.	T	F	3
23.	I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.	T	F	1
24.	I get so hungry that my stomach often seems like a bottomless pit.	T	F	3
25.	My weight has hardly changed at all in the last ten years.	T	F	2
26.	I am always hungry so it is hard for me to stop eating before I finish the food on my plate.	T	F	3
27.	When I feel lonely, I console myself by eating.	T	F	2
28.	I consciously hold back at meals in order not to gain weight.	T	F	1
29.	I sometimes get very hungry late in the evening or at night.	T	F	3

30.	I eat anything I want, any time I want.	T	F	1
31.	Without even thinking about it, I take a long time to eat.	T	F	2
32.	I count calories as a conscious means of controlling my weight.	T	F	1
33.	I do not eat some foods because they make me fat.	T	F	1
34.	I am always hungry enough to eat at any time.	T	F	3
35.	I pay a great deal of attention to changes in my figure.	T	F	1
36.	While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.	T	F	2

Part II

Directions: Please answer the following questions by circling the number above the response that is appropriate to you.

37. How often are you dieting in a conscious effort to control your weight?

1	2	3	4	
rarely	sometimes	usually	always	+ 1

38. Would a weight fluctuation of 5 lbs affect the way you live your life?

1	2	3	4	
not at all	slightly	moderately	very much	+ 1

39. How often do you feel hungry?

1	2	3	4	
only at mealtimes	sometimes between meals	often between meals	almost always	+ 3

40. Do your feelings of guilt about overeating help you to control your food intake?

1	2	3	4	
never	rarely	often	always	+ 1

41. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?

1	2	3	4	
easy	slightly difficult	moderately difficult	very difficult	+ 3

42. How conscious are you of what you are eating?

1	2	3	4	
not at all	slightly	moderately	extremely	+ 1

43. How frequently do you avoid 'stocking up' on tempting foods?

1	2	3	4	
almost never	seldom	usually	almost always	+ 1

44. How likely are you to shop for low calorie foods?

1	2	3	4	
unlikely	slightly unlikely	moderately likely	very likely	+ 1

45. Do you eat sensibly in front of others and splurge alone?

1	2	3	4	
never	rarely	often	always	+ 2

46. How likely are you to consciously eat slowly in order to cut down on how much you eat?

1	2	3	4	
unlikely	slightly likely	moderately likely	very likely	+ 1

47. How frequently do you skip dessert because you are no longer hungry?

1	2	3	4	
almost never	seldom	at least once a week	almost every day	− 3

48. How likely are you to consciously eat less than you want?

1	2	3	4	
unlikely	slightly likely	moderately likely	very likely	+ 1

49. Do you go on eating binges though you are not hungry?

1	2	3	4	
never	rarely	sometimes	at least once a week	+ 2

50. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?

0
eat whatever you want, whenever you want it + 1

1
usually eat whatever you want, whenever you want it

2
often eat whatever you want, whenever you want it

3
often limit food intake, but often 'give in'

4
usually limit food intake, rarely 'give in'

5
constantly limiting food intake, never 'giving in'

51. To what extent does this statement describe your eating behavior? 'I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'

1	2	3	4	
not like me	little like me	pretty good description of me	describes me perfectly	+ 2

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

Name..... **Sex (F/M)** **Age**.....**yrs**

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives.

The questions will ask you about the time you spent being physically active in the last 7 days.

Please answer each question even if you do not consider yourself to be an active person.

To describe the intensity of the physical activity, two terms (Moderate and Vigorous) are used:

Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal.

Thank you for participating!

1. The first question is about the time you spent sitting during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

During the last 7 days, how much time did you spend sitting during a day?

_____ hours ____ minutes

---

2 Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

_____ Days ⇒ How much time did you usually spend walking on one of those days?

or

☐ No day _____ hours ____ minutes

---

3. During the last 7 days, on how many days did you do moderate physical activities like gardening, cleaning, bicycling at a regular pace, swimming or other fitness activities.

Think *only* about those physical activities that you did for at least 10 minutes at a time. Do not include walking.

_____ Days ⇒ How much time did you usually spend doing moderate physical activities on one of those days?

or

☐ No day _____ hours ____ minutes

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4. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, heavier garden or construction work, chopping woods, aerobics, jogging/running or fast bicycling?

Think *only* about those physical activities that you did for at least 10 minutes at a time.

_____ Days ⇒ How much time did you usually spend doing vigorous physical activities on one of those days?

or

☐ No day _____ hours ____ minutes

IPAQ-E (English version)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____**days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____**hours per day**

   _____**minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____**days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____**hours per day**

_____**minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____**days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____**hours per day**

_____**minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____**hours per day**

_____**minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.