Aintree University Hospital Research & Development

ECAL Clinical Protocol

A Randomized Controlled Trial to Evaluate Impact of Energy Expenditure Information from Indirect Calorimeter on the Outcome of Weight Loss during a Standardised Multicomponent Weight Management Intervention in Non-diabetic & Pre-diabetic Obese Subjects (ECAL)

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Confidentiality Statement

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Study Summary

Title: A Randomized Controlled Trial to Evaluate Impact of Energy Expenditure Information from Indirect Calorimeter on the Outcome of Weight Loss during a Standardised Multicomponent Weight Management Intervention in Non-diabetic & Pre-diabetic Obese Subjects (ECAL)

Background: Weight loss is important to improve overall health and reduce risk of obesity-related comorbidities such as diabetes. Most effective weight loss methods require a reduction in calorie intake. Some individuals with obesity find it more difficult to lose weight than others, due to inter-individual variation of resting metabolic rate and physical inactivity. Furthermore, individuals with predisposed genetic propensity to obesity who are deemed to be metabolically 'thrifty', require further structured intensification of lifestyle changes. Resting metabolic rate and respiratory quotient have proven to be effective predictors of weight loss.

Aims: This study aims to investigate whether providing energy expenditure information to patients and clinicians during a standardized multicomponent weight management intervention would influence the outcome of weight loss.

Methods: Participants recruited from the University Hospital Aintree weight management clinics, will be subsequently randomised to receive either 1) standard care (SC) - standard multicomponent weight management intervention (diet, exercise, behaviour modification therapy) or 2) intervention group (ECAL) – SC plus use of energy expenditure information to both clinicians and participants to influence dietary advice provided over a 6-month period. Primary outcome measure is the magnitude of weight loss. 7-day food diary used to establish compliance and MET average physical activity scores will be recorded.

This study will adhere to the principles outlined in the UK Policy Framework for Health and Social Care Research (v3.2 10th October 2017). It will be conducted in compliance with the protocol, the Data Protection Act, and other regulatory requirements as appropriate.

GLOSSARY OF ABBREVIATIONS

AE	Adverse Events
BMI	Body Mass Index
BP	Blood Pressure
BPI-SF	Brief Pain Inventory short form
CRF	Case Report Form
CI	Chief Investigator
DN4	Douleur Neuropathique 4
ECAL	*Intervention* Group
eGFR	Estimated glomerular filtration rate
EQ-5D	European Quality of Life – 5 Dimensions
FBC	Full blood count
HbA1c	Glycated haemoglobin
HDL	High Density Lipoprotein
HRA	Health Research Authority
IC	Indirect calorimetry
IWQoL-Lite	Impact of Weight on Quality of Life - Lite
KOSC	King's Obesity Staging Criteria
LFT	Liver Function Test
LDL	Low Density Lipoprotein
mTCNS	modified Toronto Clinical Neuropathy score
MT	Meal test
NEFA	Non-Esterified Fatty Acids
OSA	Obstructive sleep apnoea
PI	Principal Investigator
QoL	Quality of life
REC	Research Ethics Committee
REE	Resting energy expenditure
RMR	Resting metabolic rate
RQ	Respiratory quotient
SAE	Serious adverse event
SAR	Serious adverse reaction
SC	*Standard care group*
SOP	Standard operating procedure
SUSAR	Serious unexpected suspected adverse reaction
TFT	Thyroid function test
T2DM	Type 2 Diabetes Mellitus
U+Es	Urea, creatinine and electrolytes
UAR	Unexpected adverse reaction
VAS	Visual analogue scale for pain
WL	Weight loss

KEYWORDS

Weight management; Obesity; Complex Obesity; Diet; Structured dietary intervention; Calorie restriction; Indirect Calorimetry; Resting Energy Expenditure; Resting Metabolic Rate; Respiratory Quotient; Substrate oxidation; Macronutrient composition; Weight loss predictor; Peripheral neuropathy

SYNOPSIS

Study Title	A Randomized, Controlled Trial to Evaluate Impact of Energy Expenditure Information from ECAL Indirect Calorimeter on the Outcome of Weight Loss during a Standardised Multicomponent Weight Management Intervention in Non-diabetic & Pre-diabetic Obese Subjects (ECAL study)
Study Setting	The study will be co-ordinated by the appointed research & development team based at University Hospital Aintree. The Liverpool Clinical Trials unit will provide trial management, statistical input, and database management for this trial. The study will include patients attending Weight Management Clinics at University Hospital Aintree.
Trial Design	A 24-week randomised controlled trial for subjects with obesity referred to a specialist weight management service. Participants will be randomised to receive 1) standard care (SC) control group receiving multicomponent weight management intervention (diet, exercise, and behaviour modification therapy), or 2) intervention group (ECAL) energy expenditure information via indirect calorimetry to guide the multicomponent weight management intervention. The aim of this study is to establish whether providing energy expenditure information to both clinician and patient from indirect calorimetry would influence the outcome of weight loss.
Trial Participants	All eligible patients currently attending specialist weight management services at University Hospital Aintree.

1. Introduction

Recent figures in 2016 demonstrated that over a 1 in 4 adults in England were obese, with a body mass index (BMI) of 30 kg/m^2 or higher(1). A further 41% of men and 31% of women were overweight, with a BMI of at least 25 but less than 30 kg/m^2(1). Obesity is a multifactorial condition which results from chronic accumulation of fat in adipose tissue, which may stem from excessive energy intake, and or changes in body energy expenditure, resulting in a positive energy balance. The total amount of daily energy required to maintain normal bodily function including growth, repair of tissues and metabolic function is known as the basal metabolic rate (BMR). The BMR is the amount of calories (kcal or kJ) required to perform the body's basic metabolic function, most accurately measured in the lab settings. In contrast, the resting metabolic rate (RMR) is measured in a resting state, in the morning before any food, without any physical activity and after several hours of restful sleep overnight. Although the terms BMR and RMR are used interchangeably, the term resting metabolic rate is more commonly used. The RMR is calculated through application of the first law of thermodynamics and the Weir's formula. Using the principles from the first law of thermodynamics and Weir's formula, the simplified Weir formula(2) states that:

Resting Metabolic Rate (kcal per day) = 1.44 x (3.94 VO2 + 1.106 VCO2) – 2.17 UN

Where VO2 is oxygen consumption in millilitres per minute and VCO2 is the rate of carbon dioxide production in millilitres per minute. Indirect calorimetry allows capture of the exhaled gas using a face mask or mouthpiece connected to oxygen and carbon dioxide analysers.

The total daily energy expenditure (TDEE) is an estimation of how much calories an individual burn per day, taking into account the amount of calories burned through physical activity. TDEE is measured by multiplying BMR with the factor of physical activity level (PAL). The physical activity level is estimated using a sum of all physical activities (sum of Δ PAL) within a 24-hour period, hence: TDEE = BMR x factor of physical activity level (PAL). The role of physical activity is equally important as energy restriction in inducing and maintaining weight loss (3).

Accurate determination of BMR is possible using validated techniques in clinical trials in the research setting (4). Furthermore, indirect calorimetry has also been validated through comparison with BMR calculated based on predictive equations like Harris-Benedict equation (5).

1.1 Rationale for study

Das et al. (2004) prospectively measured total energy expenditure (TEE) and resting energy expenditure (REE) in extremely obese individuals and established that these individuals have high absolute values for TEE and REE, indicating that excess energy intake contributes to the maintenance of excess weight (6). However, Melo et al. (2008) found that obese individuals are economical, from the metabolic point of view (7). Therefore, the energy expenditure (EE) per kilogram of body weight at the given time is lower in obese individuals. Lam and Ravussin (2017) went on further to demonstrate complexity of the energy expenditure equation suggesting the variations between individuals and underlined the hypothesised concept of the 'thrifty' versus 'spendthrift' phenotypes(8). In theory, the thrifty individuals are more metabolically energy-conserving and thus are prone to weight gain.

1.2.1 Current Research on Energy Expenditure

TDEE calculated via predictive equations in obese hospitalised patients were only accurate in about half of patients, and tend to overestimate EE (9, 10). Nutrition and energy advice on critically ill (11), hospitalised patients or those in a hypocaloric state (12) are increasingly reliant on the accuracy of indirect calorimetry. Limited experience and technical variabilities of indirect calorimetry use in obese and severely obese individuals require further development and exploration (13, 14). Measurement of EE serves as an important component of comprehensive clinical nutrition assessment in institutions providing weight management interventions for obese patients. The hypothesis in this study is to examine whether utilising an indirect calorimeter to provide RMR and RQ would help obese patients to lose and maintain their weight loss.

1.2.2 Current Research on Respiratory Quotient

A prospective study of morbidly obese subjects with a BMI $\geq$ 35kg/m^2 with a predisposition to weight gain subsequent to the cessation of a conventional rapid-weight-loss-diet or a very low-calorie diet were assessed during a 12-month follow up (15). A significant predictor for maintenance of weight loss in morbidly obese individuals was the observed differences in their resting RQ. The individuals who demonstrated an RQ in the lower range (<0.72) were more able to maintain the weight-loss achieved on a caloric restriction and avoid a weight loss rebound as compared to those with RQ in the higher range (>0.75)(16). This suggested that RQ could prove useful in clinical practice as a prognostic marker for long-term effectiveness of low- and very-low-calorie diets used to induce rapid weight loss. The Baltimore Longitudinal Study on Aging shared a similar finding that fasting RQ or respiratory exchange ratio (RER) adjusted for age, BMI, FFM was positively related to weight change(17).(18)

1.2.3 Current Research on Resting Metabolic Rate

Fat free mass (FFM) which represents numerous metabolically active tissues (skin, bone, muscle) and organs (heart, liver, kidney, brain) is thought to be a major determinant of energy expenditure and fat utilization in lean healthy individuals. Resting metabolic rate (RMR) adjusted for fat free mass (FFM) was a predictor for change in body weight, with studies demonstrating RMR is inversely related to rate of weight gain(19). Two further studies by Piaggi et al. (2013, 2015) supports that 24-hour EE was inversely related to rate of weight gain, and further demonstrated that a higher 24-hour RQ and EE, but not FFM, were independent predictors of ad libitum food intake(15, 20). Similarly, a study on ethnic Pima Indians with high prevalence of obesity demonstrated a low RMR was a significant risk factor for weight gain(21). Further evaluation of this suggestion of a “Thrifty genotype” amongst this population, with obesity prevalence that exceeds 75%, led to the conclusion that a low RMR is predictive of overall weight gain(21). Another study comparing the RMR of 10-year old Pima Children with Caucasian cohorts found that RMR adjusted for body composition and FFM was not significantly different(22). However, other studies report no significant association was found between RMR and weight gain in obese individuals(17, 23).

1.3.1 Definition of Prediabetes

Prediabetes is diagnosed when:

- **Glycated haemoglobin (HbA1c)** 42 - 47 mmol/mol (24)
- **Impaired Fasting Glucose** - Fasting blood glucose level of (5.5 mmol/L to 6.9 mmol/L) and / or
- **Impaired Glucose Regulation** - Two-hour glucose tolerance test after ingesting the standardized 75 g glucose meal to the blood sugar level of 7.8 – 11.0 mmol/L and / or

We defined prediabetes among individuals without previously diagnosed diabetes using HbA1c cut-offs as specified by the NICE(24) . This cut-off has been shown in a meta-analysis to be predictive of progression to diabetes. Patients who had a previous diagnosis of diabetes were excluded because the current glycaemic status of those patients may represent diabetes control. The levels above these readings would be a diagnosis for diabetes.

Prevalence of prediabetes is on the sharp increase from 11.6% to 35.3% from comparative surveys in 2003 to 2011(25), while about 1 in 10 individuals with prediabetes progress to develop diabetes every year (26).

1.3.3 Peripheral neuropathy and Prediabetes

A prospective Canadian study demonstrated that individuals with prediabetes carries a similar risk of peripheral neuropathy as new-onset type 2 diabetes in people around 50 years of age(27). Patients with at least one or more risk factors of developing type 2 diabetes were monitored over a 3-year period. Results of PROMISE trial showed that over the course of the 3-year monitoring period, 50% of participants had peripheral neuropathy(27). Of those that developed prediabetes (either impaired fasting glycaemia or impaired glucose tolerance), 49% also had peripheral neuropathy. By comparison, of those participants that maintained healthy blood glucose levels, 29% had peripheral neuropathy.

The results outline that people with prediabetes are at a higher risk of peripheral neuropathy than the general population and that monitoring for signs of nerve damage should be carried out to reduce the risk of foot damage and potential consequences that can result.

1.4 Substrate oxidation

Obesity is associated with impaired glucose tolerance and impaired utilisation of fat as a fuel during post-absorptive conditions(28). The proposed mechanism of impaired glucose tolerance, which is due in part to increased availability of plasma free fatty acids (FFA) and reduced capacity of skeletal muscle fat utilization (28, 29), leading to accumulation of intracellular fatty acid metabolites that interfere with insulin signalling pathways(30, 31). In lean metabolically healthy individuals, measurement of the RQ reveals a higher reliance on fat oxidation (lower RQ) during fasting conditions. Insulin infusion in these individuals readily suppresses this preference of fat oxidation as muscle shifts to higher reliance upon glucose oxidation (higher RQ). Thus metabolically healthy skeletal muscle is characterised by the ability to switch easily between glucose and fat oxidation in response to homeostatic signals. In comparison, the skeletal muscles of obese individuals and type 2 DM demonstrate a great reduction in this metabolic flexibility. RQ values measured in a fasted state are elevated in obese and type 2 DM individuals (32), yet the stimulation of glucose oxidation in response to insulin is blunted. This blunted capacity and inefficient response to both to the stimulus of fasting to enhanced fat oxidation and to the stimulus of glucose oxidation in response to insulin, is termed “metabolic inflexibility”.

1.5 Proposed research

Indirect calorimetry has been extensively used in both research and clinical setting in lean and healthy individuals, with lack of congruent results on individuals with complex obesity. This study aims to explore the use of indirect calorimetry to provide energy expenditure information to clinicians and patients during a 6-month standardised multicomponent weight management intervention.

Research Question:

- Does providing energy expenditure information to both clinicians and patients during weight intervention influence the outcome of weight loss?

2 Study objectives

2.1 Primary Objective

- To determine whether providing energy expenditure information from indirect calorimetry to specialist clinicians and patients influences the outcome of weight loss in patients with obesity and severe obesity.

2.2 Secondary Objectives

1. To establish the acceptability and reproducibility of using indirect calorimetry to measure energy expenditure as compared to use of standard predictive energy equations.
2. To determine whether metabolic parameters of REE or RQ are potential surrogate biomarkers to predict weight loss.

3 Study design

Type of study: Randomized-controlled trial

Primary endpoint

Reduction in body weight from baseline to end-of treatment (24 weeks)

4. Study population

4.1 Recruitment

Study information will be given to all adult patients attending weight management service at Aintree University Hospital. Eligible participants will be identified and provided with patient invitation leaflet after their clinic appointment. The researcher will verbally explain the study to the patient, confirm eligibility and hand out the patient information leaflet. Individuals who are interested in taking part will be invited back for a screening visit, in the Clinical Sciences

Centre, Aintree University Hospital. The study will be explained again at the screening visit where any questions can be answered and informed consent can be obtained. A letter will be written to the participant, their GP and their secondary care diabetes clinician at Aintree University Hospital detailing brief overview of the trial.

4.2 Inclusion criteria

- Man or woman, 18 to 70 years of age, inclusive
- BMI $\geq$30 kg/ m^2 to $\leq$ 60 kg/m^2 at screening visit
- Stable weight (ie, change of < 5% within 12 weeks before screening based on medical history)
- Subjects are in the investigators opinion, well-motivated, capable, and willing to learn how to undergo indirect calorimetry testing, as required for study
- Willing and able to adhere to the prohibitions and restrictions specified within this protocol

4.3 Exclusion criteria

- Taking weight loss medication within 12 weeks prior to randomisation
- Previous or planned bariatric surgery
- History of Type 1, Type 2 diabetes mellitus, DKA or diabetes secondary to pancreatitis
- Has a HbA1c of $\geq$ 6.5% (or $\geq$ 48 mmol/mol)
- History of obesity with a known secondary cause (e.g. Cushing's disease / syndrome)
- Oral corticosteroid use (except in the short term use of a 7-10 day course)
- Ongoing, inadequately controlled thyroid disorder defined as thyroid-stimulating hormone >6mIU/litre or <0.4 mIU/litre
- History of malignancy within 3 years before screening (or diagnosis of malignancy within this period)
- eGFR $\leq$30ml/min/1.73m^2 on serum testing
- Alanine aminotransferase level is >2.0 times the upper limit of normal or total bilirubin is >1.5 times the upper limit of normal at screening
- Other major illness likely to preclude participation in the trial
- History of glucagonoma

- An MI, unstable angina, revascularization procedure (stent or bypass graft surgery) or cerebrovascular accident within 12 weeks before screening
- Heart Failure NYHA Class III-IV
- End-stage Chronic Obstructive Pulmonary Disease (COPD)

4.4 Sample size

Previous studies in this population from Aintree LOSS(33) community-based, multidisciplinary weight management programme for patients with severe and complex obesity, demographic data of patients who attended had an average weight loss of 1.29 kg (+/- SD 4.88) and mean BMI of 45.6 (+/- SD 6.8) kg/m^2. The study will have 80% power to detect additional weight loss of 3 kg between the SC and ECAL group. This difference is taken from cornerstone studies like the Diabetes Prevention Programme, which suggests a weight loss of 3-5% of initial body weight would lead to risk reduction in progression of diabetes. Using a power calculator with SD above, the sample size is 42 patients per group; hence n = 84 patients (primary outcome). Allowing for attrition rate of 20%, the sample size required would be 84 / 0.80 = 105 patients.

4.5 Withdrawal criteria

Participants may withdraw consent from the study before study completion if they decide to do so, at any time and for any reason. If a participant decides to withdraw from the study, this will be recorded in the study records. They will be sent a letter thanking them for their participation, and informing them that the data collected up to the time point they withdrew will be included in the study analysis and that they will not be contacted again with regards to this study. They will not be asked to attend further visits. Furthermore, we will emphasise that their standard care will not be affected by their withdrawal from this study and that they will return to standard care.

Participants will be withdrawn from this study by the research team as agreed by the PI if:

- They are diagnosed with a terminal illness
- The PI, sponsor or study clinician deem it unsafe for continuation in the study for medical, safety, regulatory or other reasons consistent with applicable laws, regulations, or Good Clinical Practice (GCP)
- They are considered to be lost to follow-up as deemed by the clinician.
- Loss of capacity during participation in research
- Treatment with medications which the PI determines to require permanent withdrawal from the study (e.g. systemic corticosteroids)

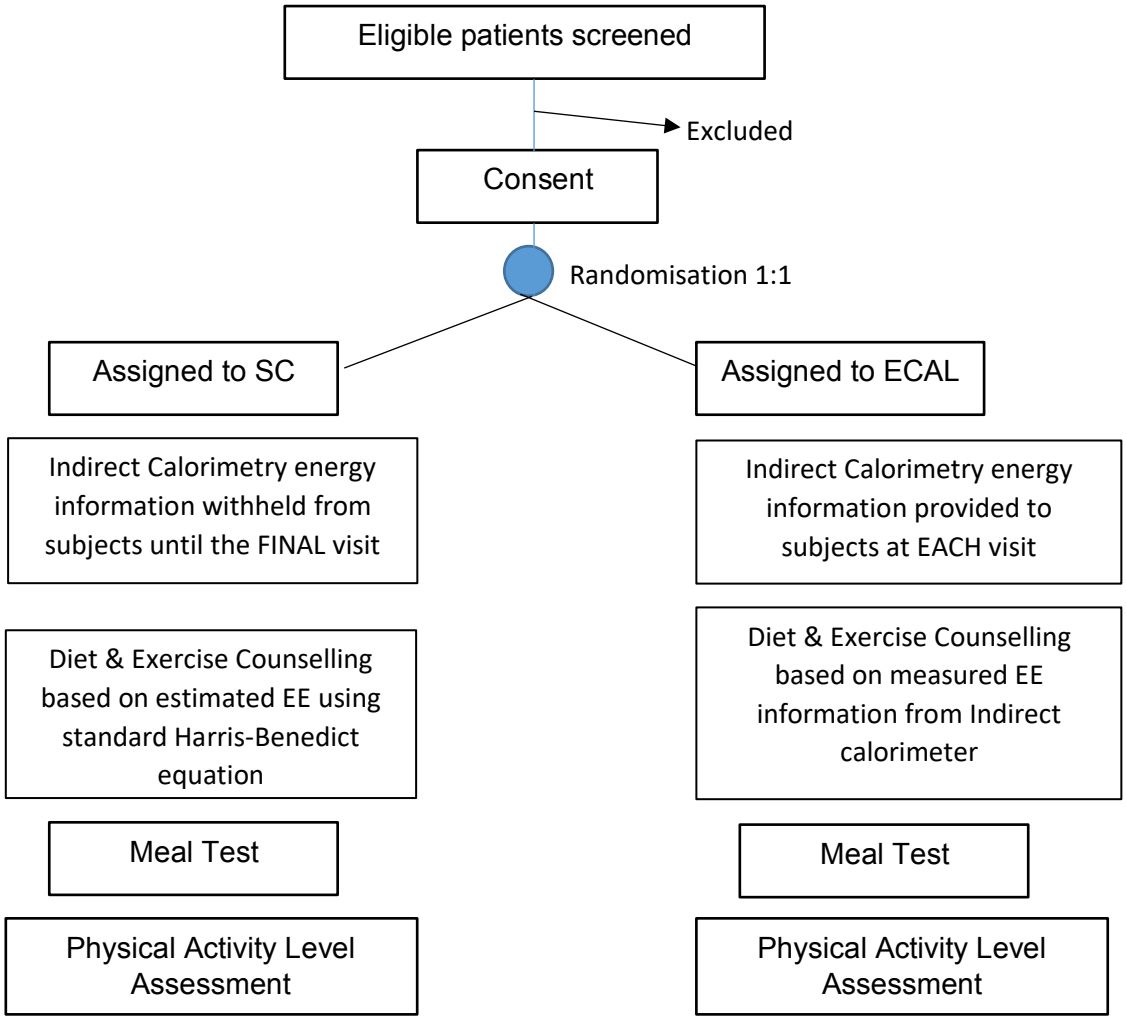
- Female participants with a positive pregnancy test

4.6 Prohibitions and Restrictions

Potential participants must be willing and able to adhere to the following prohibitions and restrictions during the study to be eligible for participation:

- Willing to have an overnight fast of at least 8 hours or greater before the IC measurement.
- Willing to avoid stimulants and stressors such as caffeine, nicotine, and tobacco for at least 8 hours prior to the test. The allowed beverages are water only for the duration of pre-testing phase.
- Willing to avoid strenuous physical activity for 12 hours prior to the measurement.

Figure 1: Recruitment, consent, and randomisation of trial participants.



5. Experimental protocol

5.1 Screening Visit

The Screening Visit is performed at least one day and up to 2 weeks prior to the Randomisation Visit.

Patients will attend an appointment with a healthcare professional at the study site. The healthcare professional will confirm and check their eligibility to participate and explain in detail what the study involves and allow for any questions regarding the trial. Full written informed consent to participate will then be taken. The person obtaining consent will also have been named in the delegation log of staff as undertaking this duty and approved as study personnel by the relevant governance procedures.

After consent has been obtained, a member of the team will perform measurement of anthropometrics (height, weight, waist circumference, blood pressure and pulse rate) for all the patients in line with study Standard Operating Procedures (SOPs) and arrange for standard laboratory testing (listed blood tests – HbA1c, FBC, U+E, Lipids, LFTs, TFTs) and urine pregnancy test (female participants of childbearing potential only).

Each participant will have a copy of the consent form and patient information leaflet. A copy will be placed in their hospital medical records and the original copy held in the site Masterfile.

5.2 Baseline visit & Randomisation

Eligible participants will be able to proceed to the Baseline Visit (Week 0). Weight, blood pressure and pulse rate will be recorded for all patients in line with study SOPs. The patient will also complete questionnaires to provide demographic and medical history details and additional data will be collected. Participants will be counselled about the study visits and provided with a food diary to record their food intake during the week. Participants will each receive support from the dieticians and weight management practitioners and/or clinicians with regards to maintaining reduced calorie intake according to their respective groups. Participants may contact their study team or GP at any time.

Eligible participants will then be randomly assigned to one of two groups in a 1:1 ratio:

1) The *control group* (SC) – standard care including diet, exercise and behaviour modification therapy. Energy expenditure information (RMR, RQ) measured from IC will be **withheld** from participants until the final visit. Participants will receive a standard nutritional energy goal based on the Harris-Benedict equation, the standard predictive equation used at Tier 3 weight management service at Aintree University

Hospital. Both participants and the study clinician providing advice and care will be "blinded" to the client and practitioner report until the completion of the final visit (week 24). To maintain the "blinding" of both participant and clinician in the SC group, the IC measurement is performed and an electronic copy of this is stored in the database, without showing the report to the participant or clinician. Upon completion of the final visit, SC group participants will be able to request a copy of the relevant client reports, should they wish to do so.

2) The *intervention group* (ECAL) – diet, exercise, and behaviour modification therapy, with the addition of a personalised nutrition energy goal based on the RMR measured from the IC at every visit. ECAL group participants will receive a client report which is a layperson summary of their personalised RMR, fat burning (%) and glucose availability (%) in a client report printout. The client report is generated directly from the ECAL indirect calorimeter using the ECHealth software contained within. Layperson summary is simplified, easy to understand and has graphic representations of their personalised measurements. The participants will be given one copy of this client printout after each IC measurement. On the other hand, the clinician will receive a practitioner report, a different version to the client printout, also generated from the ECHealth software, which contains the clinical results. The clinician will make recommendations of dietary modification and physical activity counselling based on the clinical results of measured RMR and RQ.

All participants will receive diet and physical activity counselling and provided with a food diary to record their food intake (at least 4 days during the week). Support will be offered from the dieticians and weight management practitioners and clinicians with regards to maintaining a low-calorie intake. Compliance and adherence to dietary plan will be assessed by the dietician or weight management practitioner or clinician review during appropriate study visits with diet and physical activity counselling. A discussion about patient's food intake will be carried out and documented alongside self-reported food intakes based on their food diary completion.

5.3 Randomisation

During the baseline visit (week 0), randomisation will be conducted through a validated online system provided through the Clinical Trials Unit. Eligible participants will be randomly assigned in a 1:1 ratio to:

1) SC (control group) – standard care including diet, exercise and behaviour modification therapy, but energy expenditure information is **withheld** from both the clinicians and participants until the final visit.
2) ECAL (intervention group) – in addition to diet, exercise and behaviour modification therapy, participants will receive verbal feedback on nutrition energy goal based on their REE measurement. The REE information will then be used to guide dietary and lifestyle recommendations. The measured energy information will be presented to the participant as a layperson summary at respective visits. This includes RMR, RQ and any other metabolic measurements obtained from IC tests.

Participants will be informed of their randomization assignment during the Baseline Visit. A letter will be sent to the participant's GP notifying them of their patient's participation in the study.

5.4 Standard Care Group Clinical Visits

(weeks 1, 2, *4, 8, 12, 16, 20 and 24)*

Standard Care (SC) group participants will receive IC testing at baseline (week 0), week 1, 2, 4, 8, 12, 16, 20 and week 24, but energy expenditure information is **withheld** from the patient until the final visit. Participants would receive a standard nutritional energy goal based on standard predictive equations. At the final visit (week 24), a summary of their energy expenditure (RMR, RQ) information will be provided and presented as a layperson print out.

SC group participants will continue to receive diet, exercise and behavior modification therapy as a standard multicomponent weight management intervention as part of their weight management service.

5.5 - ECAL Group Clinical Visits

(*week 1, 2, 4, 8, 12, 16, 20 and 24)*

In addition to the multicomponent weight management intervention (diet, exercise and behavior modification therapy), ECAL group participants will receive energy expenditure information measured from IC.

ECAL group participants will receive IC testing at baseline (week 0), week 1, 2, 4, 8, 12, 16, 20 and week 24. RMR measured from IC test will is proven to be more accurate and less variable than standard predictive equations. REE will provide the basis of specific caloric restriction necessary for the individual to maintain their target weight loss. RQ will also be generated from the ratio of VCO2/ VO2 which provides information about substrate oxidation, more specifically measured fat utilization over the past 24 hours. REE and RQ

information will be used to guide clinicians in recommending the appropriate caloric restriction and lifestyle changes to help promote active weight loss. Participants will receive verbal feedback and a layperson summary of the recommendations. The indirect calorimetry can also be used to demonstrate and compare the results of REE and RQ from previous weeks.

5.6 Control & Intervention Group

1) *Control group* – SC Group. Standard care including multicomponent weight management intervention (diet, exercise and behaviour modification therapy) **but** energy expenditure information is **withheld** from both the clinicians and participants until the final visit.
2) *Intervention group* - ECAL Group. In addition to standard care, participants will receive verbal feedback on nutrition energy goal based on their REE measurement. The REE information will then be used to guide dietary and lifestyle changes. The measured energy information will be presented as a layperson summary at respective visits. This includes RMR, RQ and any other metabolic measurements obtained from IC tests.

5.7 Neuropathy screening questionnaires

(weeks 0, 4, 12 and 24)

All the participants will be offered 3 neuropathy screening questionnaires DN4, mTCNS, BPI-SF and a visual analogue scale (VAS) of neuropathic pain at week 0 (baseline), 4, 12 and week 24. These questionnaires are anticipated to take no longer than 10 minutes to complete.

5.8 Vibration Perception Threshold & Neurothesiometry

(weeks 0, 4, 12 and 24)

For measuring quantitative vibration perception threshold (VPT) a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford and Nottingham, UK) will be used.

Procedure for Neurothesiometer

The patient should be in a semi-recumbent position, with heels resting on the bed. Prior to the testing, patient will be familiarized with the sensation of vibration at the wrist. The probe is then balanced on the tip of the great toe (right and left). The amplitude of the vibration for the probe should be increased from zero, by turning the Neurothesiometer dial slowly, during which patient's eyes are shut. Participants will be instructed to say "yes" when he/she first

detects the slightest “buzzing” or vibration sensation, and this is recorded as the vibration perception threshold which is repeated three times on each test site. The unit of VPT is in volts and has a range between 0-50 volts. A grading scale which can be used is: no neuropathy (VPT < 15V), mild neuropathy (VPT < 25V), moderate neuropathy (25-35V), severe neuropathy (VPT > 35V).

5.9 Indirect Calorimetry

(Week 0, 1, 2, 4, 8, 12, 16, 20 and 24 weeks)

Preparing for the Test

Participants will be advised to abstain from food and alcohol for 7 hours or greater before the IC measurement. Participants will be advised to avoid stimulants and stressors such as caffeine, nicotine, and tobacco for at least 4 hours prior to the test. Beverages allowed include water, caffeine-free and calorie-free drinks. A standard operating procedure for ECAL indirect calorimetry will be adhered to as attached in Appendix.

Steady State Testing

After measuring their height, weight, and other anthropometric data, the participant will lie supine with head elevated 30 degrees, extended limbs in a silent room. In line with safety measures for current COVID19 pandemic, patients will be asked to sit behind a transportable Perspex screen. Using the screen will prevent large droplet dispersal and protect the staff member and surrounding area. Whilst lying in a physically comfortable position, the participant will be asked to maintain a regular inspiratory pattern, avoid moving and speaking during the test. Participant will be advised to remain rested for at least 10 minutes. The mouthpiece will be held with either hand, and participant will be required to breathe through the mouthpiece, with a single-use nose clip applied over the nostrils. The mouthpiece has a two-way filter and will be single-use only. Upon completion it will be disposed of in the clinical waste bin.

REE will be measured using the ECal Indirect calorimetry (Metabolic Health Solutions, Australia) at participant’s bedside. Gas and volume calibration of the device will be performed before each measurement. A mouthpiece will be used to measure inspired oxygen and expired carbon dioxide for up to 6-10 minutes, with the first 2 minutes of data discarded. REE (kcal/day) is calculated by the ECal device. Patients were considered to have reached a steady-state REE if a minimum of 5 consecutive minutes with less than 10% coefficient of variation in VO2 and VCO2 was achieved; otherwise repeat testing was indicated.

Accordingly, one measure would be sufficient to measure the REE. However, if the operator cannot guarantee the stability of the readings, the combination of two or three repetitions would increase the precision of the measurements. In such instances, the average of the two best readings will be utilized. A designated and trained healthcare professional will be responsible to operate and check the accuracy of data collection from IC.

Energy expenditure (EE) information obtained from indirect calorimetry includes resting Energy Expenditure (REE) and respiratory quotient (RQ).

Measures to ensure safety relating to the current COVID-19 pandemic will be introduced as per government guidance at the time of the participant visit. At the time of writing, the following measures will be instigated: social distancing, use of protective face masks, and any appropriate personal protective equipment when social distancing not possible. One-way flow of participants and an assessment of any possible COVID-19 symptoms the day prior to attendance and day of attendance. If there are any symptoms, advice will be provided, and the visit rescheduled for a later date and advised to contact local healthcare service and undertake COVID-19 testing. Temperature will be assessed on arrival and if ≥ 37.8°C then the visit will be rescheduled. Medical advice / testing will be performed if applicable.

5.10 Patient and staff acceptability surveys

Following REE measurements, patients completed a short survey of 7 questions examining acceptability of the procedure (open-ended; yes/no; 5-point Likert scale). Questions focused on the timing and duration of the IC, comfort, and willingness to repeat the procedure. Nursing and medical staff were also invited to complete a 10-question survey (open-ended; yes/no; 5-point Likert scale). Questions focused on the impact of the IC on ward activities, staff role in the procedure, and perceived barriers to implementation of IC into usual care. Completion of staff surveys was voluntary and anonymous.

5.11 Food Intake Assessment

Participants are expected and encouraged to adhere to their prescribed dietary intake as advised by their clinicians throughout the study period.

A food diary for every patient will be provided to assist more accurate measure of food consumption. Diary will include all the meals consumed during any two (2) consecutive weekdays and one (1) weekend day before the visit. The assessment will include energy intake, carbohydrate intake, fat intake, protein intake, and fiber intake. The food diary should

be reviewed with the participant in detail for accuracy and completeness by the clinician or the dietician.

5.12 Physical Activity Level

The International Physical Activity Level questionnaire will be administered **at** four intervals during the study at baseline, week 8, 16 and 24. The hours spent in sleep and light, moderate, and hard activities were multiplied by respective metabolic equivalent tasks (METs), summed and finally expressed as total MET-h/d.

5.13 Quality of Life Assessment

Assessment of Quality of Life will be conducted via the IWQoL-Lite & EQ-5D-5L questionnaires. Participants will be asked to complete these questionnaires at the baseline visit and at the final visit.

6 Data storage and analysis

6.1 Source data

The CRF and study questionnaires are the primary data collection instruments. All data requested on the CRF will be recorded. All missing data will be explained. If the item is not applicable, then N/A will be written. All entries will be printed legibly in black ink. If any entry error has been made, to correct the error, a single line will be drawn through the incorrect entry and the correct data entered above it. All such changes will be initialled and dated. For clarification of illegible or uncertain entries, the clarification will be printed above, and this will be initialled and dated.

A copy of the patient consent form and the information sheet will be placed in the hospital notes of all participants and in the Investigator Site File. A sticker will be placed on the cover of the notes (or inside cover) detailing the study title, contact details of the PI, participant ID and the fact that the notes should not be destroyed. All study visits and adverse events will be recorded in the hospital notes.

The PI and his / her staff must ensure that these documents are pseudonymised. Past medical history within the CRF required for inclusion in the study will be verified by participant hospital notes and/or GP medical history.

6.2 Statistical Analysis

Patient characteristics and clinical data will be analysed using SPSS software and STATS Direct. Normally distributed data will be expressed as mean +/- standard deviation (SD). Categorical variables will be analysed by using Chi-squared testing. Continuous variables between groups will be evaluated with unpaired student's t-test (if normal distribution) or Mann-Whitney U test (if not normally distributed). P values < 0.05 will be considered significant.

6.3 Storage, archiving and destruction of data

Research data will be stored in an anonymous format. Patients will be given a study number which will then be used to link information without identifying individual patient details. All information and test results will be fed into a secure password protected computer on the 3rd floor of the Clinical Sciences Centre, Aintree University Hospital, and all data and results will be coded and securely stored. The Clinical Sciences Centre has secure facilities including locked doors requiring ID access, ID badges, 24-hour security and alarm system.

The study will end once the last participant has completed the last visit and data filed and collected. After the study end personal data will not be retained and will be destroyed by confidential means. Anonymised data will be kept for 10 years in a locked cupboard on the 3rd floor of the Clinical Sciences Centre, University Hospital Aintree and will then be destroyed by confidential means.

7 Adverse Events

7.1 Definitions

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- Results in death
- Is life-threatening – refers to an event in which the subject 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 hospitalisation, or prolongation of existing inpatients' hospitalisation
- Results in persistent or significant disability or incapacity

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

7.2 Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

7.2.1 Non-Serious AEs

All such events, whether expected or not, should be recorded.

7.2.2 Serious AEs

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to <condition>, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the <name of REC> where in the opinion of the Chief Investigator, the event was:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting AEs

Please send SAE forms to:
Research and Development,
Third Floor Clinical Sciences Building,
University Hospital Aintree,
Longmoor Lane,
Liverpool
L9 7AL
Tel: 0151 529 5885 / 0151 529 5917
Fax: 0151 529 5888

8 Assessment and Follow-up

All participants should currently be registered with a weight management service. Should they require follow up after the study, they will return to their registered weight management service provider for any follow-ups.

9 Regulatory issues

9.1 Ethics approval

Approval will be obtained from a Research Ethics Committee and submitted for Site Specific Assessment. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

9.2 Consent

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered, and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases, the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

9.3 Confidentiality

The Chief Investigator will preserve the confidentiality of participants taking part in the study and will abide by the Data Protection Act. All identifiable personal information collected for the study will remain strictly confidential accessible only to the research team. Once enrolled participants will be given a study number which will be used to identify their data and anonymise them.
Electronic and hard copy data will be stored in a room, accessible only by electronic ID access on 3rd Floor, Clinical Sciences Building, University Hospital of Aintree, Lower Lane, Liverpool, L9 7AL.

9.4 Indemnity

The University of Liverpool holds Indemnity and insurance cover

9.5 Sponsor

The University of Liverpool will act as the main Sponsor for this study. Institute of Ageing and Chronic Disease will sponsor database (REDCAP), statistical support up to £10k, salary for Clinical Research Fellow and patient travelling expenses.

Equipment for study will be provided by Medtronic (CGMS) and Metabolic Health Solutions (ECAL indirect calorimeter).

9.6 Audits

The study may be subject to inspection and audit by the University of Liverpool under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research.

10 Study Management

The day-to-day management of the study will be coordinated through Research & Development Centre, Third Floor Clinical Sciences Building, University Hospital Aintree NHS Trust.

11 End of Study

End of study is defined as the completion of data collection and data analysis.

12 Archiving

Data and all appropriate documentation should be stored for a minimum of 5 years after the completion of the study, including the follow-up period, unless otherwise directed by the funder/sponsor/regulatory bodies.

13 Publication policy

The use of study methods, protocol, anonymised data, and statistical analysis will contribute toward the attainment of a PhD and relevant journal articles as deemed fit by the funder/sponsor/regulatory bodies.

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