Clinical Trial Protocol

Joule 3: Mechanisms of Weight Loss with Semaglutide and Tirzepatide

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1.0 Background

Weight gain is simplistically an imbalance of energy intake (EI) and energy expenditure (EE). Imbalances as small as 10-30 kcal/day favouring EI lead to weight gain[1]. Obesity treatments have variable effect[6], and all are hampered by compensatory metabolic mechanisms whose purpose is to preserve body fat mass in the face of weight loss[7-9]. These energy balance mechanisms described below need to be targeted for more effective sustained weight loss (Figure 1).

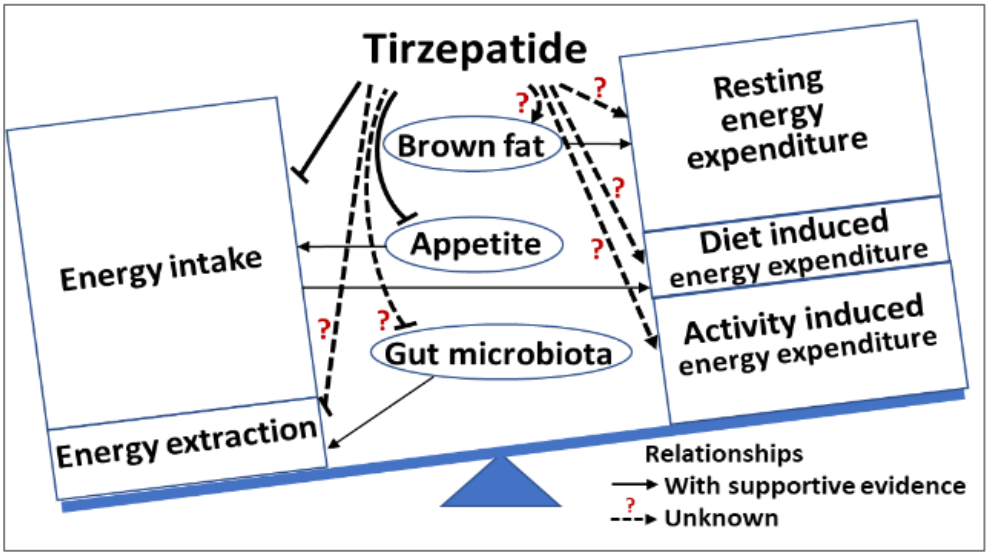


Figure 1. Putative effects of tirzepatide on energy balance & related mechanisms

Weight-preserving changes in energy expenditure

EE in response to weight loss is reduced in many ways. Resting EE (which accounts for 60-70% of EE) declines[7-9]. Resting EE is linked to the basal metabolism of muscle and organs, which comprise the fat free body mass. Thus, resting EE is highly correlated with fat free mass. Although typical weight loss is (fortunately) mostly fat tissue, about 25% of weight loss is fat free mass, thereby reducing resting EE. However, the reduction in resting EE is beyond that expected by fat free mass reduction, a phenomenon called metabolic adaptation or adaptive thermogenesis. One of the main drivers of adaptive thermogenesis is brown adipose tissue (BAT), which contributes to resting EE by uncoupling mitochondrial oxidation from ATP formation, particularly in response to cold and sympathetic stimuli[10]. Low BAT activity may play a central role in developing and maintaining obesity[11,12].

In addition to reductions in resting EE, when food intake is reduced to lose weight, the energy expended to digest, absorb, process and store that smaller amount of food (diet-induced EE) is proportionately reduced. Furthermore, physical activity-induced EE is also reduced in order to conserve energy, and this applies to both exercise and non-exercise activity[7-9].

Weight-preserving changes in energy input

On the other side of the energy balance equation, appetite is stimulated in order to increase EI so that fat stores can be re-established. Several orexigenic and anorexigenic hormones from fat tissue and the gastrointestinal tract including, among others, glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide (GIP) have been implicated and are targets for drug therapy[7].

Downstream of EI is energy extraction. Human small bowel typically absorbs all fats, ~85% of carbo-hydrates and 65–95% of proteins ingested. The colon receives 20-60g of undigestible polysaccharides

available to gut microbiota for fermentation and colonic absorption[15]. Gut microbiota composition has been linked to obesity via activation of energy-regulating centres of the brain, and energy extraction from food[16]. Weight loss by diet is followed by a compensatory shift to microbiota associated with greater energy harvest[20]. A 20% shift in microbiota composition has been associated with up to 150 kcal/d difference in energy extraction[19].

Obesity treatments and energy balance

Drug treatments[23] have had minimal effect (2.9-6.8% weight loss)[24] until the advent of GLP-1 receptor agonists (GLP-1 RA), analogs of an endogenous gut hormone that affects insulin secretion (an incretin), gastric emptying and appetite centres in the brain[25]. One of these drugs, semaglutide causes 15% weight loss[26]. GLP-1 RA do not increase EE in randomized trials[27-31]. The main effect of GLP-1 RA is to reduce appetite and EI [27-31]; semaglutide reduces EI by 725 kcal/day vs placebo[31]. Energy extraction in response to GLP-1 RA has not been described.

Tirzepatide, a new unimolecular dual receptor agonist of the incretins GLP-1 and GIP[32] is even more effective at causing weight loss than semaglutide – an unprecedented 20%[36]. It is not known why it is so much more effective. Despite the presence of GIP receptors in the appetite centres of the brain, GIP RA alone do not consistently affect weight in animals[32,38,39], and short-term GIP infusion has no effect on EE or EI in humans[40]. Thus, tirzepatide has an effect on weight that is above and beyond the additive effects of GLP1 RA and GIP RA when given alone. One recent trial showed no significant difference in EI reduction between tirzepatide and semaglutide[41]. This finding is suggestive that the additional ~5% weight loss from tirzepatide is through novel effects on energy extraction or EE components of the energy balance equation. In animal studies, tirzepatide leads to both reduced EI and increased EE[42]. Furthermore, tirzepatide induces expression of thermogenic genes in BAT[43]. If these findings extend to humans, tirzepatide would be the first available weight loss drug to address the EE side of the equation. While several other gut peptides are known to affect gut microbiota, data on GIP or GLP-1/GIP RA effects on intestinal energy extraction and gut microbiota are lacking[44]. To date, all we know from human studies is that tirzepatide causes reduced appetite[42,45] and EI no differently than semaglutide[41]. This makes formal evaluation of tirzepatide's effect on comprehensive energy balance in humans critically important.

To understand the dramatic weight-loss effect of tirzepatide in comparison to semaglutide (and gain insights into targets for new obesity treatments), we must understand how it affects the weight-preserving compensations in EE, EI and energy extraction, as well as underlying mechanisms including BAT activity, appetite and gut microbiota composition (Figure 1).

Results from feasibility study

A feasibility study was conducted prior to this study to determine the feasibility of completing procedures for evaluating energy expenditure, energy intake, and energy extraction in adults. The primary objective was to determine the proportion of participants that can adhere to all elements of the protocol (screening, 3-day diet, stool collection, and 24-hour room calorimetry including meals, questionnaires, and exercise). The secondary objectives were to assess participants' acceptability of elements of the protocol, as mentioned above, and to assess data collection completeness and burden.

16 of 16 enrolled participants completed any 3-day diet with 15 of 16 completing the 3-day diet completely. Fifteen of 16 enrolled participants completed any stool collection; the one individual who did not complete the stool collection is the same individual who did not complete the 3-day diet completely. All 16 participants completed all elements of 24-hour room calorimetry (including all

meals, questionnaires, and full exercise protocol). When assessing acceptability, the elements with the lowest rated scores included lack of food preferences in 3-day diet and during the 24-hour room calorimetry. Overall, 11 of 16 of participants indicated that they would, if given the opportunity, repeat the procedures again in 3 months' time.

The above findings have indicated that adherence and acceptability to the 3-day diet, stool collection, and elements of the 24-hour room calorimetry visit is high and that providing foods (for the 3-day diet and the 24-hour visit) that are more in line with participants' food preferences would improve the acceptability of these elements. Based on this information, the current study is indeed feasible.

2.0 Research Questions

2.1 Primary Research Question

Among adults with obesity and without known diabetes, what is the effect of 12 weeks of treatment with tirzepatide compared to semaglutide on 24-hour EE?

2.2 Secondary Research Questions

Does 12 weeks of tirzepatide versus semaglutide affect the following:

1. Other energy balance outcomes:
    a. Components of EE (resting EE, sleep EE, diet induced EE, activity induced EE, and work efficiency)
    b. EI
    c. Energy extraction from food
2. Mechanistic outcomes:
    a. EE-related: activity of thermogenic BAT
    b. EI-related: self-reported appetite and satiety
    c. Energy extraction from food-related: gut microbiota composition
3. Body composition

2.3 Hypotheses

We hypothesize that tirzepatide will increase EE, particularly resting EE vs semaglutide, and this will explain most of the weight loss difference between the two drugs. We hypothesize tirzepatide will have smaller but significant incremental effects on energy extraction vs semaglutide.

3.0 Research Design

A single-centre open-label randomized controlled parallel-group trial will be conducted with 1:1 assignment of 80 participants to tirzepatide or semaglutide for 12 weeks to assess energy dynamics and mechanisms at baseline and at 12 weeks.

3.1 Randomization

Research staff will activate a centralized web-based system (REDCap) to randomly allocate consenting, eligible individuals 1:1 to tirzepatide or semaglutide in a concealed fashion using a computer-generated random sequence with stratification by age (≤40; >40 years), BMI (≤40; >40 kg/m^2) and sex. Neither sex will comprise more than 60% of participants to ensure balance.

4.0 Trial Population

Consenting adults with obesity will be studied. The study physician will make an assessment of each participant to ensure entry criteria including lab results below are met prior to randomization.

4.1 Inclusion criteria

1. Age 18-60 years
2. BMI >30 kg/m^2
3. Stable body weight (<5kg change in the 3 months prior to screening)

4.2 Exclusion criteria

1. History of type 1 or type 2 diabetes or current diagnosis
2. Glucose-lowering or weight loss drugs in the last 3 months or planned in the next 3 months
3. Prior or planned bariatric surgery or planned low calorie meal program in the next 3 months
4. Known clinically significant gastric emptying abnormality (for example, severe gastroparesis or gastric outlet obstruction) or chronic GI symptoms (nausea, vomiting, diarrhea, constipation, bloating or unexplained abdominal pain) or chronically take drugs that directly affect GI motility
5. Known liver disease other than non-alcoholic fatty liver disease, or ALT >3x ULN
6. Intake of standard alcoholic drinks more than 21/week for men, or 14/week for women
7. Estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2
8. History of pancreatitis
9. Personal or family history of medullary thyroid cancer or multiple endocrine neoplasia 2
10. Currently pregnant/breastfeeding, previous pregnancy within the last 6 months, or unwillingness to use contraception for 3 months
11. Other medications, allergies or comorbidities that, in the opinion of the investigator could interfere with study medication or study measures (e.g., catecholamine medications) or current medication use that has been unstable in the past 8 weeks or expected to change within the next 3 months
12. Claustrophobia precluding a 24 hour stay in a whole room calorimeter or MRI scan
13. Inability to undergo MRI (weight >250 kg, incompatible implanted device, e.g., pacemaker)
14. Use of cannabis or its related products (d-9-THC, CBD, d-8-THC, and cannabinol) in the past three months
15. Use of nicotine/tobacco that has been unstable in the past 8 weeks or expected to change within the next 3 months (exclusion from imaging visit ONLY)
16. Known monogenic, syndromic hypothalamic causes of obesity
17. Not able to communicate in English
18. History of cardiac conditions that precludes exercise
19. Participation in any intervention clinical study within 90 days before screening
20. History of eating disorder within the last year

4.3 Recruitment

Potential participants will be recruited using research recruitment posters and flyers made available to the public at the different locations in the community, around the Hamilton Health Sciences network, McMaster University, local clinics and doctor's offices and using print, online, and radio ads. Additionally, participants from our previous studies who consented to recontact will be approached. Those expressing interest in the study will reach out to the research team via phone call or email.

5.0 Study Interventions

Tirzepatide and semaglutide are self-injected subcutaneously once weekly. There are 6 tirzepatide dose levels (2.5 mg, 5 mg, 7.5 mg, 10 mg, 12.5 mg, 15 mg) and 5 semaglutide dose levels (0.25 mg, 0.5 mg, 1 mg, 1.7 mg, 2.4 mg).

As the focus of the trial is to assess effects of the drugs and not weight loss, *per se*, participants will not be instructed to change their diet or physical activity patterns. Participants will be instructed to not start any other new weight loss strategies during the 12 weeks of the trial.

5.1 Tirzepatide group

Tirzepatide will be taken once per week for 12 doses. Participants will be started at 2.5 mg of tirzepatide and instructed to increase by one dose level every 4 weeks if tolerated (allowing up to 3 dose levels over 12 weeks), to a maximum dose of 7.5 mg of tirzepatide. Tolerance to the study medication and adverse events will be assessed throughout the 12 weeks. In the presence of adverse effects, participants will be given the option to maintain (as gastrointestinal side effects improve with time) or reduce the dose, with the opportunity to increase the dose at a later time when symptoms improve, based on discussions with the study physician.

5.2 Semaglutide group

Semaglutide will be taken once per week for 12 doses. Participants will be started at 0.25 mg of semaglutide and instructed to increase by one dose level every 4 weeks if tolerated (allowing up to 3 dose levels over 12 weeks), to a maximum dose of 1 mg of semaglutide. Tolerance to the study medication and adverse events will be assessed throughout the 12 weeks. In the presence of adverse effects, participants will be given the option to maintain (as gastrointestinal side effects improve with time) or reduce the dose, with the opportunity to increase the dose at a later time when symptoms improve, based on discussions with the study physician.

6.0 Study Schedule and Measurements

From screening to final assessment, each participant will have 5 in-person visits (plus 2 additional visits to pick up study supplies) and approximately 6 phone calls over approximately 14 weeks. Assessments and their timing are outlined in Appendix 1 - Visit schedule & schema.

6.1 24-hour energy expenditure and its components

Indirect whole room calorimetry: (Baseline, week 12) We will follow the Room Indirect Calorimetry Operating and Reporting Standards (RICORS 1.0)[51] for this study. Participants will enter an airtight respiratory chamber (Promethion High-Definition Room Calorimetry System, Sable Systems International, NV, USA) equipped with a toilet, chair, bed and exercise step/exercise bike at approximately 8am and remain for approximately 23 hours wearing an Actigraph GT3X accelerometer at the hip or thigh, and a heart rate monitor on the chest. They will eat all meals and snacks in the chamber. Prior to arrival, participants will be asked to avoid vigorous exercise for 48 hours and caffeine for 12 hours. Components of energy expenditure described below will be calculated using standard methods[51,52] (see Figure 2):

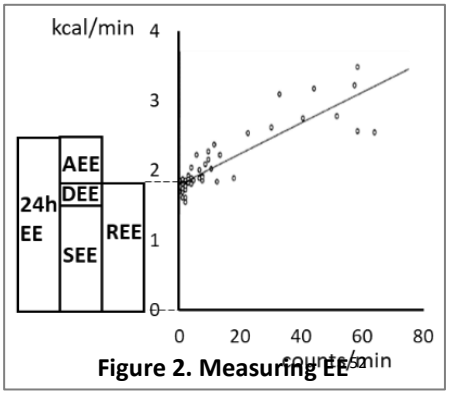


Figure 2. Measuring EE

- ***24-hour EE*** (24h EE) will be assessed in the respiratory chamber. Metabolic rate in kcal/day will be estimated using the Weir formula, from inspired oxygen and expired carbon dioxide measured every minute by software in the Promethium System, summed over 23 hours and extrapolated to 24 hours by multiplying by 24/23.
- ***Resting EE*** (REE) is the intercept of the regression line of EE on activity counts per minute (cpm) determined by accelerometry (i.e., EE at 0 cpm).
- ***Sleep EE*** (SEE) better approximates basal metabolic rate than REE, as REE also includes EE of arousal (and in the way it is measured here, diet induced EE). SEE is defined as the lowest average EE over 3 hours of sleep from 0000h to 0700h and multiplied by 8 to be expressed as kcal/day.
- ***Diet induced EE*** (DEE) is calculated a) as the difference between REE and SEE (this value also includes the energy expended to be awake) and b) as the additional energy expended over 4 hours after breakfast above EE measured at rest first thing in the morning.
- ***Activity induced EE*** (AEE) will be calculated a) as the difference between 24h EE and REE, and b) as the energy expended during a standardized exercise bout in the afternoon. After confirming safety and fitness to participate in exercise using the CSEP Get Active Questionnaire, participants will sit on the cycle ergometer for approximately 6 minutes before exercising. Participants will maintain a cadence of approximately 60 rpm for three 6-minute segments at increasing power: 25W, 50W and 75W. At completion of each segment, participants will be asked how hard they think they are pushing themselves during the exercise, using the Borg Rating of Perceived Exertion (RPE) scale. Heart rate will also be monitored continuously throughout the test.

Macronutrient utilization will be calculated for fat oxidation and carbohydrate oxidation by software in the Promethium System.

6.2 Energy intake

Meal test: (Baseline, week 12) In the whole room calorimeter, a standardized breakfast with energy content corresponding to 40% of expected REE based on the Mifflin equation [53] will be served. ***EI under controlled ad libitum conditions*** will be assessed by measuring the caloric content of an *ad libitum* lunch of standardized macronutrient composition served 4 hours after breakfast.

Free-living meal plan: (Week -1, week 10.5) Participants will be given 3 days-worth of meals and snacks with known calorie counts that they select from a menu. They will receive these identical items two times: during the week before the baseline and 12 Week visits. They will be instructed to eat all they want from only these food items for 3 days, and to return all food waste in a plastic container. Energy in homogenized food waste will be accurately measured using established procedures[54] in an isoperibol oxygen combustion (bomb) calorimeter (Isoperibol Calorimeter, Parr Model 6200) and subtracted from energy in the provided food to determine ***EI over 3 days of free living***.

6.3 Energy extraction from food

Stool assessment: (Week -1, week 10.5) To calculate the ***energy extraction from food*** passing through the gastrointestinal tract, pills containing nonabsorbable dyes will be ingested at the beginning and end of the 3-day free-living meal plan. Participants will collect all feces from appearance of the first dye until appearance of the second dye. Energy in homogenized feces will be accurately measured in the bomb calorimeter using established procedures[54]. Energy extraction will be calculated as difference between EI (described above) and calories in the feces and will be expressed as kcal/day and a percentage of EI.

6.4 Brown adipose tissue

MRI assessment: (Baseline, week 12) ***BAT activity*** will be measured using a protocol developed by our team at McMaster[13,14,55]. Participants will arrive after at least an 8-hour fast and acclimate to room temperature for 30 minutes. To evaluate cold-stimulated BAT activity, MRI scans to quantify proton density fat fraction (PDFF) and T2* in the supraclavicular region of the neck between the levels of C2/C3 disc and the T4/T5 disc will be performed before and after cold exposure using a full-body water-perfused suit, at 18°C for 1 hour[55]. ***Hepatic fat*** will also be assessed with MRI at the T4/T5 disc.

6.5 Appetite and satiety

Meal test: (Baseline, week 12) ***Appetite, hunger, fullness and satiety*** will be assessed using validated visual scales[56] scored 0-100 before breakfast, 30 minutes later and hourly for 4 hours.
Questionnaires: (Baseline, week 12) ***Control of eating and degree of food cravings*** over the past 7 days will be assessed by the validated 16-item Control of Eating Questionnaire[57].

6.6 Stool microbiota composition

Stool collection: (Baseline, week 12) Stool samples will be collected at home and stored in a home freezer in biohazard Ziploc bags. ***Stool microbiota*** analysis completed using 16S rRNA primers will include α-diversity (amplicon sequence variants (ASVs) richness and evenness within each sample) and β-diversity (between samples), and predicted functional metagenomics using PICRUSt or similar software.

6.7 Body composition and physical activity

Anthropometrics: (Screening, baseline, week 12) Assessments will include ***height*** using a wall-mounted stadiometer, ***body weight*** using an electronic scale, and calculated ***BMI***. ***Waist circumference*** will be measured using a non-stretchable standard tape measure attached to a spring balance exerting a force of 750 gm. ***Blood pressure*** *and* ***heart rate*** using an oscillometric device.
Body composition: (Baseline, week 12) ***Fat mass*** and ***fat-free mass*** will be assessed using DXA scanner and bioelectrical impedance analysis (BIA). Fat free mass will also be used to adjust analyses of resting EE. DXA evaluation of body composition is related to evaluations using a four-component model, Evaluation of total body fat and lean mass will be ascertained from the analysis.
Muscle mass: (Baseline, week 12) ***Muscle mass*** will be estimated using D3-Cr (a stable isotopically (deuterium) labelled creatine). This method requires participants to orally ingest a small dose (~20-30mg) of encapsulated deuterium (D)-labelled creatine (D3-creatine; D3-Cr), and 48–96h after dosing, provide a single spot, fasted-state urine sample. This method provides a safe, non-invasive, and highly accurate measure of skeletal muscle mass.
Physical activity: (Week -1, week 10.5) ***Physical activity*** will be measured using a waist-worn accelerometer for approximately one week prior to the baseline and week 12 visits.

6.8 Blood draw

Blood collection: (Screening, week 12) Blood draws will be conducted at screening and at week 12. Blood taken at screening will be used to determine eligibility. Extra blood and blood drawn at week 12 will be stored for biomarker assessment (serum and plasma). If the participant has agreed to the genetic portion of the study, a sample for genetic testing will be obtained at the screening and week 12 visit.

Proteomic analysis will be performed in the Paré laboratory (PHRI) using the Olink platform which is able to simultaneously measure up to 3,072 circulating protein concentrations from plasma samples. The Olink platform uses proximity extension assays method to accurately and specifically measured

protein concentration (https://www.olink.com/products/). This comprehensive coverage provides a unique opportunity to discover novel biomarkers of pathophysiological relevance, with an emphasis on biomarkers related to cardiovascular diseases, insulin-related pathways, inflammation, adipose tissue, digestive-released peptides, metabolism, and lipids, among many others. The Olink platform provides log2 normalized protein expression (NPX) values with relative quantification. Quality controls, and quantile normalization the biomarker measures before statistical analyses will be performed following a protocol that we previously described (PMID: 33010842). Genetic analyses will be conducted in the Dr Paré lab. These will include genome-wide genotyping (PMRA-Thermofisher) for common variants analyses and polygenic risk scores, as well as epigenetic markers pre- and post-intervention.

7.0 Study Outcomes

1) The primary outcome is 24-hour energy expenditure (EE)/kg fat free mass
2) Secondary outcomes:
    a. Components of 24h EE (resting EE, sleep EE, diet induced EE, activity induced EE) per kg fat free mass
    b. Energy intake: under controlled *ad libitum* conditions, and over 3 days of free living
    c. Energy extraction from food
    d. BAT: supraclavicular brown fat and cold-stimulated brown fat activity
    e. Appetite and satiety
    f. Stool microbiota composition
    g. Body composition: body weight, BMI, fat mass, fat-free mass

8.0 Safety Measurements

The study physician will assess adverse events throughout the duration of the trial.

8.1 Gastrointestinal side effects

Gastrointestinal symptoms of nausea, vomiting, constipation and diarrhea will be assessed as absent, mild, moderate or severe at screening, baseline, each titration visit and the Week 12 visit.

If pancreatitis is suspected (characteristic abdominal pain with or without nausea, vomiting, fever), lipase should be measured and consideration for referral for ultrasound, CT or MRI should be made. If pancreatitis is suspected, study drug should be temporarily stopped, and if confirmed, should be permanently stopped.

8.2 Adverse events

An adverse event is any untoward medical sign, symptom or disease that occurs during observation in a clinical study, regardless of causation by any treatment as part of the study. Adverse events including their severity (mild, moderate or severe) will be documented on case report forms in this trial if they lead to an unexpected change in dose or frequency (including temporary withholding) of study medication by the participant or investigator.

Every attempt will be made to describe an adverse event in terms of a single diagnosis. If a clear diagnosis has been made, individual signs and symptoms will not be recorded. If a clear diagnosis cannot be established, each sign and symptom will be recorded individually. Note that a surgical procedure is not an adverse event, but the condition necessitating the surgery is.

8.3 Serious adverse events

A serious adverse event (SAE) is an AE that fulfills at least one of the following criteria:

a) results in death;
b) is life-threatening (i.e. participant was at immediate risk of death at the time of the serious adverse event; it does not refer to an adverse event that hypothetically might have caused death if it were more severe);
c) requires inpatient hospitalization or prolongation of existing hospitalization;
    a. Complications that occur during hospitalization are AEs and if they prolong the hospitalization or fulfill any seriousness criteria, the event is serious
    b. Hospitalization for elective treatment planned before study inclusion or of a condition that was present prior to the timepoint which AEs are collected and did not worsen are not considered an AE
d) results in persistent or significant disability or incapacity (i.e. there is a substantial disruption of a person's ability to carry out normal life functions);
    a. Minor events such as headache nausea vomiting diarrhea influenza and accidental trauma should not be included in this definition
e) causes a congenital anomaly or birth defect;
f) is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above

If an AE is judged by an investigator to be an SAE, this will be documented on an SAE case report form within one working day and will include the investigator's assessment of causality. SAEs will be reported to the REB as required. SAEs that are thought to be possibly or probably causally related to study drug and unexpected (based on the product monographs) will be subject to expedited reporting to the manufacturer of the assigned drug as required. Causality will be classified by the investigator as probable (i.e. sufficient documentation to assume a causal relationship), possible (i.e. a causal relationship cannot be dismissed) and unlikely (i.e. likely related to something other than the therapy studied).

8.4 Pregnancy

Any pregnancies occurring during the trial will be documented and followed to term. Upon knowledge of pregnancy, study drug will be stopped and referral will be made for pregnancy care. Follow-up forms will be completed documenting pregnancy outcomes. All pregnancies will be reported to the manufacturer of the assigned drug. Pregnancy will be tested via a urine sample or blood test at screening.

9.0 Sample Size and Statistical Power

95 individuals will be screened and 80 randomized, to achieve the required 72 completers to detect an effect of treatment on the primary outcome, based on the following rationale. In one head-to-head trial of tirzepatide and semaglutide, weight loss at 8 weeks was 3.5 kg (with tirzepatide titrated to 5mg weekly) and 2.3 kg (with semaglutide titrated to 0.5mg weekly), a difference of 1.2 kg[49], equivalent to an incremental energy deficit of 165 kcal/d[58]. It would be clinically important if ⅔ of the incremental energy deficit due to tirzepatide (110 kcal/d) were due to a reduction in 24h EE (as opposed to EI or energy extraction). In order to detect a 110 kcal/d difference in change in 24h EE from baseline to 8 weeks with 80% power and 5% risk of type 1 error, and assuming a standard deviation (SD) of change in 24h EE of 167 kcal/d (the upper end of variability seen in similar GLP-1 RA studies[27,29]), 36

participants per group (72 total) are needed to complete the trial. To achieve this number, 80 individuals will be randomized, expecting 10% post-randomization loss to follow-up. Approximately 95 people will be screened, to account for 15% dropping out after experiencing the full gamut of investigations, but before randomization (screen failures). As each participant's involvement in the trial is short compared to the recruitment period, recruitment can be increased to ensure sufficient completers. This sample size will provide 80% power to detect these differences in change from baseline: 160 kcal/d post-meal EI assuming SD 240, 2.7 kg body weight (SD 4.0) and 1.8 kg body fat mass (SD 2.6).

10.0 Statistical Analyses

A detailed statistical analysis plan will be finalized before viewing any treatment group-specific outcome data. Baseline characteristics will be presented by treatment group. The primary outcome, between-group difference in change in 24h EE/kg fat free mass from baseline to 12 weeks will be assessed using the intention-to-treat principle by ANCOVA adjusted for stratification variables (age, sex, BMI). The analysis will be repeated without dividing by current fat-free mass. Between-group differences in change in all secondary outcomes (except β-diversity) at 12 weeks will be assessed using ANCOVA adjusted for age, sex, BMI (and divided by current fat free mass for EE outcomes). For β-diversity, PERMANOVA will be used. As the protocol allows individualized increases of study medication to maximal tolerated dose, dose-related variability will be addressed by a supplementary analysis, adjusted for the achieved dose level of study medication. Only complete data will be used, with no imputation of missing data. In addition, mediation analysis by bootstrapping[63] will be used to explore mechanisms of weight loss with tirzepatide.

Differential effects of tirzepatide by age (≤40; >40 years), BMI (≤40; >40 kg/m^2) and sex will be explored by including interaction terms in the statistical models described above. As these are exploratory analyses, no adjustment for multiple testing will be made.

No interim analyses are planned.

11.0 Ethical and Regulatory Requirements

The trial will be conducted according to Good Clinical Practice Guidelines. As medications are being administered for approved indications at approved doses according to the product monographs in this phase 4 trial, Health Canada approval is not required. Local Research Ethics Board approval will be obtained before starting the trial. The trial will be registered in a clinical trials registry before starting. Study records will be retained for 15 years.

12.0 Study Coordination and Management

Dr. Zubin Punthakee and Dr. Katherine Morrison will oversee execution of this trial and Dr. Punthakee will be the site principal investigator. The trial will be coordinated through the McMaster Metabolism, Obesity, and Diabetes Research (MODR) Centre. A senior research coordinator at MODR and post-doc will manage the trial, coordinate concealed computerized randomization and electronic data capture and data management and quality control (in a blinded manner) using REDCAP, and manage

biospecimens. Dr. Punthakee will meet weekly with the research coordinator, and other project staff for day-to-day running of the trial.

12.1 Steering committee and safety monitoring

The steering committee will meet quarterly to review progress, challenges and solutions, and new relevant published evidence. Safety issues will be discussed during scheduled meetings, but if urgent safety matters arise, Z Punthakee can convene the Steering Committee for an urgent meeting. It will also advise on financial issues and approve plans for data analysis. There will be no DSMB as longer term studies of tirzepatide and semaglutide including those for regulatory approval have established their safety, and new signals are unlikely to be identified in our small study.

13.0 Dissemination of Results

Findings of this trial will be presented in abstract form at international scientific meetings, and published in a high impact factor peer-reviewed metabolism journal. Additional substudies and subanalyses may be undertaken on approval of the steering committee.

References

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List of abbreviations and acronyms

24h EE	24 hour energy expenditure
AEE	activity-induced energy expenditure
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
ASV	amplicon sequence variants
ATP	adenosine triphosphate
BAT	brown adipose tissue
BMI	body mass index
C2/C3	cervical vertebra 2/cervical vertebra 3
cpm	counts per minute
DEE	diet-induced energy expenditure
DSMB	data and safety monitoring board
DXA	dual-energy x-ray absorptiometry
EE	energy expenditure
eGFR	estimated glomerular filtration rate
EI	energy intake
GI	gastrointestinal
GIP	glucose-dependent insulinotropic peptide
GLP-1	glucagon-like peptide 1
kcal/d	kilocalories per day
MODR	Metabolism, Obesity, and Diabetes Research Centre
MRI	magnetic resonance image
NV	Nevada
PDFF	proton density fat fraction
PERMANOVA	permutational multivariate analysis of variance
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
r	Pearson's correlation coefficient
RA	receptor agonist
REB	research ethics board
REE	resting energy expenditure
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
SD	standard deviation
SEE	sleep energy expenditure
T2*	"T2 star" - effective transverse relaxation time
T4/T5	thoracic vertebra 4/thoracic vertebra 5
ULN	upper limit of normal
USA	United States of America

Appendix 1. Visit schedule and schema

Visit schedule

	-1.5W	0W	1,2,4,5, 8,9W*	10.5W	12W
Eligibility & Consent	X				
Randomization		X			
Dispense study drug		X	X		
Titrate study drug (at 4-week intervals)			X		
Collect study drug					X
Reinforce drug adherence		X	X	X	
Collect adverse events			X	X	X
Eligibility bloodwork (eGFR, ALT, for females HCG)	X				
Anthropometrics	X	X			X
Provide food, stool collection kits	X**			X	
Collect food waste, stool collection kits		X			X
Whole room indirect calorimetry		X			X
Meal test		X			X
Appetite Questionnaire (0,0.5,1,2,3,4h)		X			X
Body composition		X			X
MRI for BAT		X			X
MRI post cold for BAT activity		X			X

* Phone calls.
** Provision will occur after eligibility is confirmed

Visit schema

Applies at Baseline and Week 12. DXA – dual energy x-ray absorptiometry; MRI – magnetic resonance imaging. Timings and days are approximate.

