Trials

STUDY PROTOCOL

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Effect of tirzepatide-induced weight loss on adipose tissue in obesity: rationale and design of the randomized placebo-controlled Tirzepatide Brown and Beige Adipose Tissue Activation (TABFAT) trial

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Abstract

Background Obesity is a complex disease marked by excessive, dysfunctional adipose tissue accumulation. Recent research underscores the pivotal role of brown adipose tissue (BAT) in metabolic health and its potential as a therapeutic target for obesity management. Emerging preclinical and clinical evidence suggests that second-generation anti-obesity drugs, especially dual agonists such as tirzepatide, may enhance BAT activity. Additionally, beige adipose tissue, derived from white adipose tissue (WAT), may contribute significantly to whole-body thermogenesis, yet its role remains underexplored.

Methods This investigator-initiated, randomized, placebo-controlled clinical trial aims to evaluate the effects of tirzepatide on BAT activity and WAT browning in premenopausal women with obesity. Thirty-four participants will be randomized 1:1 to receive either tirzepatide or a placebo for 24 weeks. Primary outcomes include changes in BAT volume and activity, assessed using 18F-FDG-PET/CT, MRI, and infrared thermography, as well as the induction of WAT browning, evaluated through changes in mRNA expression patterns and histomorphometric alterations in subcutaneous adipose tissue samples. Secondary outcomes will involve the assessment of whole-body composition, resting energy expenditure, and various metabolic health markers, correlated with thermogenic adipose tissue changes. Comparative analysis of BAT assessment methods will refine protocols for research and clinical use.

Discussion This study is the first to systematically explore the potential of pharmacological obesity management to enhance BAT activity and induce WAT browning. Results may establish thermogenic adipose tissue augmentation as a novel mechanism of action for second-generation anti-obesity medications.

Trial registration ClinicalTrials.gov NCT06893211. Registered on 2025 March 25.

Keywords Brown adipose tissue, Beige adipose tissue, Tirzepatide, Obesity

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Administrative information

Note: The numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Title {1}	Effect of Tirzepatide-Induced Weight Loss on Adipose Tissue in Obesity: Rationale and Design of the Randomized Placebo-Controlled Tirzepatide Brown and Beige Adipose Tissue Activation (TABFAT) Trial
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Introduction

Background and rationale {6a}

Obesity is a chronic, multifactorial disease characterized by excessive accumulation of dysfunctional adipose tissue, contributing to its recurrent and heterogeneous nature [1]. Adipose tissue, broadly classified into white (WAT), brown (BAT), and beige subtypes, extends beyond energy storage to function as a dynamic endocrine and immune organ, regulating energy balance, metabolism, and inflammation [2, 3]. Historically distinguished by morphology, these subtypes are now recognized for their distinct physiological roles, with growing evidence underscoring their relevance in obesity pathophysiology and treatment [4]. While ectopic and visceral fat accumulation is a well-established driver of adverse metabolic outcomes and a primary target for weight loss interventions, recent research highlights the association of BAT's activation with a favorable metabolic phenotype in obesity, prompting debate over its potential as a therapeutic target [5–10]. In addition to WAT and BAT, a third distinct form of adipose tissue has been identified: beige or brite ("brown in white") adipose tissue, arising from adipocyte progenitor induction or transdifferentiation of mature white adipocytes [11]. Beige adipocytes, intermediate between WAT and BAT, feature multiple lipid droplets, elevated mitochondrial content, and uncoupling protein 1 (UCP1) expression, suggesting a thermogenic capacity [11, 12].

Given BAT's emerging role in metabolic health, understanding its presence and activity in humans has become a priority. Historically, BAT was thought to be exclusive to small mammals, hibernating species, and human infants, where it facilitates non-shivering thermogenesis [13]. Advances in medical imaging techniques, particularly fluorine-18 fluorodeoxyglucose positron emission tomography combined with computed tomography (18F-FDG-PET/CT), in the early 2000s renewed scientific interest in this tissue by revealing symmetrical FDG uptake in the shoulder, neck, and thoracic spine regions—indicative of BAT activity in adults [14]. Subsequent studies confirmed BAT's presence and functionality across multiple anatomical sites in human adults, including the cervical, axillary, supraclavicular, mediastinal, paraspinal, and abdominal regions, constituting approximately 1 to 2% of total adipose mass [15–19]. Upon activation, BAT drives non-shivering thermogenesis by uncoupling proton conductance across the mitochondrial inner membrane via UCP1, independent of ATP synthesis, utilizing various substrates to increase energy expenditure [20]. Some authors estimate that BAT activation could contribute an additional one to several hundred kilocalories to daily energy expenditure [15–17, 21].

18F-FDG-PET/CT is the most commonly used and well-established method for visualizing activated BAT in humans [22]. However, it requires BAT activation by stimulators, exposes participants to ionizing radiation, and can be confounded by factors such as insulin resistance and certain medications [23, 24]. To address these limitations, magnetic resonance imaging (MRI) with water-fat separation algorithms has been explored to differentiate BAT from WAT based on BAT's higher water content and lower fat fraction, showing moderate success in correlating with 18F-FDG-PET/CT findings [25–28]. Infrared thermography (IRT) has also emerged as a non-invasive, cost-effective alternative for BAT detection, but its broader application is hindered by a lack of standardized protocols and clinical validation [29–31]. In contrast, techniques for assessing beige adipocytes remain underdeveloped. Histological and molecular techniques, such as immunohistochemistry and gene expression analysis, can identify beige adipocytes or evaluate WAT browning [32–34].

The emergence of second-generation anti-obesity medications marks a pivotal advancement in addressing the global obesity epidemic [35]. Limited yet consistent data from the STEP and SURMOUNT studies indicate beneficial changes in adipose tissue loss and distribution, with comparable or even lower lean mass loss compared to other weight loss interventions [36, 37]. However, weight loss is often accompanied by a decline in resting energy expenditure (REE), necessitating further exploration of thermogenic adipose tissue as a potential countermeasure [38]. BAT and beige adipocytes, with their capacity for non-shivering thermogenesis, may mitigate this decline. Some animal studies support the notion that central or peripheral GLP-1 receptor agonism induces weight loss not only by reducing food intake but also by increasing energy expenditure [39–44]. Clinical evidence remains limited and presents conflicting results, with small studies on liraglutide and exenatide showing inconsistencies [27, 28]. Data on the potential of new anti-obesity medications to induce the browning of subcutaneous adipocytes is even more scarce, with one positive result reported for the use of semaglutide in mice [45]. Dual GIP/GLP-1 receptor agonists like tirzepatide hold particular promise due to their superior reductions in body weight, glucose, and triglyceride levels compared to selective GLP-1 receptor agonists [46]. Unlike GLP-1 receptor, which is predominantly expressed in the pancreas and central nervous system, GIP receptors are expressed in adipose tissue, suggesting a direct mechanistic role in adipose function [46]. Preclinical studies indicate that GIP receptor agonism may uniquely enhance adipose tissue function by improving nutrient disposition and metabolic control, potentially contributing to tirzepatide's enhanced efficacy over monoagonists [46]. Preclinical data demonstrated that tirzepatide increases the catabolism of branched-chain amino acids and their keto acids in BAT mimicking changes traditionally seen in cold-induced BAT thermogenesis [47].

While tirzepatide has demonstrated a favorable safety profile in large-scale clinical trials, potential harms include gastrointestinal adverse events such as nausea, vomiting, diarrhea, and constipation, occurring primarily during dose escalation. Less frequent but serious risks may include pancreatitis, gallbladder-related events, and hypoglycemia, particularly in patients with concurrent antidiabetic therapies [37, 48–50]. In this trial, these risks are mitigated through careful participant selection, monthly safety monitoring, dose adjustments based on tolerability, and immediate access to the research team for adverse event reporting. The known and potential benefits are weighed against these risks, justifying the study in a controlled clinical setting with informed consent. Given tirzepatide's dual GIPR/GLP-1R agonism and its potential to target adipose tissue directly, the Tirzepatide Brown and Beige Adipose Tissue Activation (TABFAT) trial aims to investigate tirzepatide's potential to enhance thermogenic adipose tissue activity, offering new insights into its mechanisms and paving the way for optimized obesity management strategies.

Primary research question is as follows: In premenopausal women with obesity, does tirzepatide treatment, compared to placebo, increase BAT volume and activity, and induce WAT browning over 24 weeks, as assessed by multimodal imaging and molecular analyses?

Objectives {7}

TABFAT aims to achieve the following objectives:

- Objective 1: Assess whether tirzepatide treatment increases the volume and activity of brown adipose tissue (BAT).
- Objective 2: Determine whether tirzepatide induces morphological and molecular changes in subcutaneous white adipose tissue (WAT), with a particular focus on the promotion of browning.
- Objective 3: Compare different methods for assessing BAT in humans to establish optimized protocols for future clinical research.
- Objective 4: Evaluate the relationship between changes in resting energy expenditure (REE) and various other metabolic health markers and alterations in thermogenic adipose tissue following tirzepatide treatment.

Trial design {8}

The TABFAT study is a randomized, placebo-controlled, single-blind, parallel group exploratory trial designed to evaluate the effects of tirzepatide compared to placebo on thermogenic adipose tissue in women with obesity. Participants will be randomly assigned in a 1:1 ratio to receive either tirzepatide or placebo over a 24-week treatment period. The study is investigator-initiated, includes multimodal outcome assessment, and is structured to allow detailed phenotyping of thermogenic adipose tissue changes. Participants will be blinded to their allocation, while investigators will remain unblinded due to the need for dose titration and safety monitoring.

Methods: participants, interventions and outcomes

Study setting {9}

This single-center clinical trial will be conducted at the Department of Endocrinology, Diabetes and Metabolic Diseases, University Medical Centre Ljubljana, Slovenia. The site is a national referral center for obesity and metabolic disorders, with full access to clinical research infrastructure including nuclear medicine, radiology, endocrinology, and laboratory diagnostics. Participant recruitment, intervention administration, follow-up visits, imaging, and sample collection will all be carried out at this institution. The center provides integrated access to secure electronic health records, ensuring comprehensive and standardized data collection.

Eligibility criteria {10}

We aim to enroll 34 female participants who meet the criteria for pharmacological obesity intervention as defined by current international guidelines [51]. Inclusion will be restricted to women with a body mass index (BMI) of 30–40 kg/m^2, reflecting established variations in BAT quantity and activity across obesity categories [17]. The decision to focus solely on women stems from the limited sample size and evidence of sex-based differences in pharmacological responses to obesity treatment and BAT characteristics [52, 53]. Furthermore, targeting premenopausal women is justified by documented dynamic changes in BAT during menopause and the postmenopausal period [54]. We will exclude participants with secondary causes of obesity, such as thyroid disorders, autonomous glucocorticoid activity, or syndromic conditions. For participants who have not undergone a 1-mg dexamethasone suppression test or an assessment of pituitary-thyroid axis function within the past 2 years, these evaluations will be conducted prior to study enrollment.

Inclusion criteria

- Female sex
- Age between 18 and 50 years
- BMI between 30 and 40 kg/m^2 at pre-screening
- Prior comprehensive non-pharmacological and non-surgical management of obesity, including a history of at least 12 months of intensive lifestyle intervention with a maximum weight reduction of less than 5%
- Stable body weight within the 3 months preceding study enrollment (defined as weight fluctuations within 5%)
- No prior pharmacological or surgical interventions for obesity
- Euthyroid state
- Eumenorrhea or oligomenorrhea
- Ability to comprehend the study objectives and procedures
- Willingness to provide informed consent and to comply with the study protocol, including the use of highly effective contraception during the study period, with signed consent and agreement provided in duplicate
- Commitment to use highly reliable contraception and the absence of plans for pregnancy within the 8 months following enrollment

Exclusion criteria

- Pregnancy or lactation
- Postmenopausal
- Amenorea
- Type 2 diabetes
- Reliance on natural contraception methods
- Noncompliance with previous therapeutic regimens
- Personal history of malignancy
- Personal or family history of medullary thyroid carcinoma
- Personal history of pancreatitis
- Personal history of cholelithiasis
- Personal history of major depressive episodes or suicidal ideation
- Personal history of acute coronary events or hemodynamically significant coronary artery disease
- Psychiatric disorders
- Current treatment with sympathomimetics or sympatholytics
- Excessive alcohol consumption

All clinical procedures and assessments will be performed by personnel with appropriate qualifications

and relevant experience. Physicians administering the intervention and overseeing clinical assessments are board-certified specialists in endocrinology or internal medicine, with vast prior experience in clinical research and obesity treatment. Imaging will be performed by board-certified nuclear medicine specialists and radiologists. WAT biopsies will be conducted by trained physicians skilled in the proposed biopsy method. Laboratory analyses will be performed by trained biomedical laboratory technicians in accredited laboratories. All staff involved in the trial have completed good clinical practice (GCP) training.

Who will take informed consent? {26a}

The process begins with pre-screening, during which an investigator contacts patients listed in the hospital registry. All potentially eligible patients will receive written and oral information about the study including the potential adverse effects of the treatment, the procedures conducted, the reporting of these effects, and the recommended measures upon their occurrence. Given the use of low-dose radioactive exposure and a therapeutic agent with an uncertain safety profile during pregnancy, participants must also sign a statement committing to highly effective contraception throughout the study. Additionally, they will receive written details about anticipated radiation exposure, and informed consent acknowledging this risk will be obtained. Screening examinations will commence after participants provide written informed consent to the principal investigator.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

There are no plans for ancillary studies or additional use of participant data and biological specimens beyond the scope of this trial. Therefore, no additional consent provisions are required for such purposes.

Interventions

Explanation for the choice of comparators {6b}

A placebo comparator was selected to isolate the pharmacological effects of tirzepatide on brown and beige adipose tissue. This design enables a controlled evaluation of thermogenic and metabolic changes attributable solely to the investigational drug. Placebo injections will be administered using identical pens to maintain participant blinding and minimize bias in subjective outcome reporting.

Intervention description {11a}

Tirzepatide (Mounjaro, Eli Lilly) will be administered as a subcutaneous injection using prefilled pens, following a modified titration protocol. Participants in the intervention group will begin with a 2.5 mg weekly dose for 4 weeks, followed by potential increases of 2.5 mg every 4 weeks, based on clinical response and tolerability of adverse effects, up to a maximum dose of 15 mg. Participants in the placebo group will receive placebo via identical pens. At the study's outset, participants will receive education on self-administration and perform their first injection under supervision in clinic. Thereafter, participants will self-administer the treatment at home, attending monthly clinic visits for monitoring and dose adjustments.

Criteria for discontinuing or modifying allocated interventions {11b}

Participants may withdraw from the study at any time without stating a reason and without affecting their future treatment at our department. Reasons for dropout may include withdrawal of consent, adverse events, pregnancy detected during the trial, or noncompliance with the study medication or protocol. Participants who miss more than one injection or more than one scheduled visit will be excluded. Continuous monitoring will ensure that no participant meets exclusion criteria during the study; any who do will be promptly withdrawn. The tirzepatide dose will be increased by 2.5 mg every 4 weeks up to a maximum of 15 mg, based on clinical response and tolerability. If a participant experiences moderate adverse events (e.g., persistent nausea, vomiting, diarrhea, or significant fatigue) or if the participant achieves above 5% weight loss in one month, the dose will be maintained at the current level or reduced to the last tolerated dose. In cases of severe or treatment-limiting side effects, treatment will be paused and the participant evaluated.

Strategies to improve adherence to interventions {11c}

To promote and monitor adherence to the intervention protocol, participants will attend monthly clinic visits. During these visits, they will receive next month's injection pen, undergo assessment for adverse effects, and receive any necessary guidance or support regarding treatment. Adherence will be directly monitored through participant-maintained self-injection logs (reviewed at each visit) and counts of returned pens. Furthermore, participants will undergo interim laboratory testing (after months 1 and 3 for electrolytes, liver and renal function markers), which may indirectly reinforce adherence, while anthropometric measurements will provide indirect confirmation of treatment engagement. Participants will also have access to a dedicated direct communication channel with the research team, allowing them to report concerns, ask questions, and receive timely assistance. This ongoing engagement is intended to strengthen treatment adherence and ensure early identification and

management of any issues that might compromise protocol compliance.

Relevant concomitant care permitted or prohibited during the trial {11d}

Participants will not receive any structured or investigator-delivered lifestyle intervention (e.g., dietary counseling, physical activity programs) during the trial period, in order to isolate the pharmacological effects of tirzepatide on study outcomes. However, participants will continue to receive standard clinical care for comorbid conditions as deemed appropriate by their treating physicians. Adjustments to concomitant medications for chronic conditions are permitted, provided such changes are clinically indicated. All modifications to concomitant therapy will be systematically recorded and discussed during the monthly study visits to ensure transparency and allow for accurate attribution of effects during analysis. This pragmatic approach reflects real-world therapeutic dynamics while maintaining rigorous oversight of potential confounding variables.

Provisions for posttrial care {30}

All participants will continue to be followed up at our department after completion of the trial, ensuring continuity of care and appropriate clinical oversight. Any clinically relevant findings arising during the trial will be communicated to the participant and their primary care provider, with appropriate follow-up or referral arranged as needed. Participants who experience any harm related to trial participation will be managed according to standard clinical practice at our institution. No specific ancillary or additional posttrial interventions are planned beyond standard medical care, unless warranted by individual clinical circumstances.

Outcomes {12}

Primary outcomes

- Change in brown adipose tissue activity and volume (time frame: baseline to week 24)
    - Assessed using 18F-FDG-PET/CT, MRI, and infrared thermography. Outcome was measured as change in mean standardized uptake value (SUVmean), mean change in BAT volume (mL), mean change in proton density fat fraction (%), and mean change in maximum supraclavicular skin temperature (°C) in predefined anatomical regions (supraclavicular and cervical areas).

These measurements aim to quantify changes in BAT volume and activity, which are hypothesized to reflect increased thermogenic energy expenditure, a potential novel mechanism of action for tirzepatide.

- Change in molecular markers of browning in subcutaneous white adipose tissue (time frame: baseline to week 24)
    - Measured as mean fold-change in the expression levels of UCP1 and other browning-associated genes (via RT-PCR and RNA sequencing) in subcutaneous WAT biopsies. Additionally, it is assessed through histomorphometric analysis and aggregated as mean proportion (%) of multilocular adipocytes, mean immune cell infiltration score, and mean microvascular density to characterize tissue remodeling indicative of beige adipocyte induction.

These measures reflect molecular and structural evidence of WAT browning, which could contribute to sustained metabolic improvements beyond caloric restriction alone.

Secondary outcomes

- Correlation between different BAT assessment methods (time frame: baseline and week 24)
    - Comparative analysis between 18F-FDG-PET/CT, MRI, and infrared thermography for assessing BAT activity and volume analyzed using Spearman correlation coefficients and intraclass correlation coefficients.

This is clinically relevant for validating more clinically applicable BAT assessment methods to facilitate broader research in thermogenic adipose tissue.

- Change in resting energy expenditure (Time frame: baseline and week 24)
    - Measured by indirect calorimetry using a portable metabolic analyzer. Outcome was reported as absolute change in REE (kcal/day).

This will help evaluate whether thermogenic adipose tissue activation contributes to preservation or increase in REE.

- Association between changes in resting energy expenditure, metabolic health markers, and ther-

mogenic adipose tissue (time frame: baseline and week 24)

- Assessed by evaluating correlations between changes in resting energy expenditure (REE, measured by indirect calorimetry), metabolic health parameters (including glucose metabolism, insulin sensitivity indices, lipid profile, and hormonal markers: fasting glucose, 2-h OGTT glucose/insulin, glucose variability, HbA1c, HOMA-IR, total cholesterol, LDL-C, HDL-C, triglycerides, androstenedione, total/free testosterone, SHBG, TSH, hs-CRP, ESR, PINP/CTX), body composition changes (fat proportion, total fat mass, VAT mass, surface, and volume), and alterations in thermogenic adipose tissue activity and volume (as measured by 18F-FDG-PET/CT, MRI, and thermography). This outcome aims to determine the interrelationship between metabolic adaptations and thermogenic fat activation following tirzepatide treatment.

This outcome is clinically relevant for elucidating how tirzepatide-induced thermogenic changes relate to improved glucose/lipid metabolism and body composition in obesity.

- Association between resting energy expenditure, metabolic health markers, and thermogenic adipose tissue (time frame: baseline data)

  - Assessed by evaluating correlations between anthropometric data (BMI, waist circumference), resting energy expenditure (REE, measured by indirect calorimetry), metabolic health parameters (including glucose metabolism, insulin sensitivity indices, lipid profile, and hormonal markers: fasting glucose, 2-h OGTT glucose/insulin, glucose variability, HbA1c, HOMA-IR, total cholesterol, LDL-C, HDL-C, triglycerides, androstenedione, total/free testosterone, SHBG, TSH, hs-CRP, ESR, PINP/CTX), body composition (fat proportion, total fat mass, VAT mass, surface, volume), and thermogenic adipose tissue activity and volume (as measured by 18F-FDG-PET/CT, MRI, and thermography).

This outcome aims to determine variables that correlate with pre-treatment thermogenic adipose tissue quantity and activity.

Safety outcomes

Adverse events will be recorded at each monthly visit and categorized using CTCAE v5.0 grading. We will quantify the following:

- Incidence of gastrointestinal adverse events (e.g., nausea, vomiting, diarrhea)
- Discontinuation rate due to adverse events
- Occurrence of suspected pancreatitis (amylase/lipase elevation > 3 × ULN + symptoms)
- Gallbladder-related events (symptomatic or imaging-confirmed)
- Any unexpected serious adverse event (SAE), with documentation of causality and resolution

Laboratory safety markers (e.g., liver enzymes, creatinine, electrolytes) will be summarized (mean ± SD) and monitored longitudinally to detect any clinically significant changes.

Participant timeline {13}

The process begins with pre-screening, during which an investigator contacts patients listed in the hospital registry. Screening examinations will commence after participants provide written informed consent. Those who qualify will be enrolled, randomized, and followed for a 24-week study period. The inclusion frequency will be regularly evaluated.

Table 1 presents the planned visits and examinations for each visit. Standardized data collection forms, including case report forms (CRFs) for clinical measurements, adverse event reports, and questionnaires, as well as templates for imaging and laboratory data, will be used to ensure consistency. These forms are stored electronically in the REDCap system hosted at University Medical Centre Ljubljana and are available upon reasonable request from the corresponding author. The study will include two main sets of visits: an initial set before the start of intervention (K0) and a follow-up set after 24 weeks of intervention (Tx). To ensure comparable climatic conditions, these sets of visits are planned for the spring and autumn seasons. Throughout the treatment period, independent of the two main sets of visits (K0 and Tx), participants will attend monthly visits for monitoring and dose adjustment (K1–K5). Both main sets of visits (K0 and Tx) will be organized so that all examinations are completed within four sessions.

Sample size {14}

Sample size calculation was performed using power and sample size calculation version 3.0.43 for the most researched part of the primary outcome: BAT activity

Table 1 Schedule of enrollment, interventions and assessments

	Enrollment	Allocation	Post-allocation						Close-out
Timepoint	$-t_1$	0	*K0*	*K1*	*K2*	*K3*	*K4*	*K5*	t_x
Enrolment									
Eligibility screen	X								
Informed consent	X								
Clinical examination	X								
Allocation		X							
Intervention									
Tirzepatide			**Started with 2.5 mg with titration up to a maximum of 15 mg (weekly)**						
Placebo			**Placebo injections (weekly)**						
Assessments									
Clinical examination			X	X	X	X	X	X	X
Basic laboratory tests				X		X			
Extended hormonal and laboratory investigations and continuous glucose monitoring			X						X
Measurement of body composition, bone mineral density, and muscle strength			X						X
Determination of shivering threshold and thermography			X						X
Measurement of brown adipose tissue quantity and activity using 18F-FDG-PET/CT			X						X
Measurement of brown adipose tissue quantity and liver steatosis using MRI			X						X
Resting energy expenditure measurement			X						X
Physical activity, heart rate and sleep monitoring			X						X
Continuous ketone monitoring					X		X		
Subcutaneous WAT biopsies and analysis			X						X
Questionnaires			X						X

K0 refers to the first visit before the intervention, *K1–5* refers to the visits after months 1 to 5 of intervention, *Tx* refers to the last visit after 24 weeks of intervention

determined using 18F-FDG-PET/CT and expressed as mean standardized uptake value (SUVmean) [55]. In order to detect differences in the range of ± 0.4 for related samples (pre-/posttreatment changes) and ± 0.6 for independent samples (differences between placebo/intervention group) with 90% study power and alpha level of 0.05 based on standard deviation of about 0.5 in previous studies [56], each group has to consist of at least 15 patients. This sample size will also enable us to detect the following differences: ± 15.6 ml for related samples and ± 24.5 ml for independent samples for supraclavicular BAT volume (standard deviation in previous studies around 20 ml) [56] and ± 0.55 for related samples and ± 0.86 for independent samples for WAT UCP1 expression (standard deviation in previous studies around 0.7) [57]. To account for potential participant dropout, we plan to recruit 17 participants per group, ensuring sufficient power to address the primary outcomes despite possible attrition. However, this relatively small sample may be underpowered for detecting smaller but clinically relevant effects in secondary outcomes or subgroup differences and limits generalizability, precision of estimates, and the ability to identify subtle effects.

Recruitment {15}

We will identify patients referred to the first or follow-up endocrine checkup for obesity at the University Medical Centre Ljubljana, Slovenia. Since we are a national referral center for patients with obesity, the recruiting pool is expected to be sufficient. Active recruitment strategies include direct outreach by dedicated research staff, such as personal invitations during clinic visits, follow-up phone calls to registry-listed patients, and targeted informational mailings to eligible individuals. Interested patients will be offered detailed information sessions (in-person or virtual) to explain study procedures, risks, and benefits, aiming to improve understanding and enrollment rates. A dedicated research contact line (email and phone) will be available for additional questions. We aim to enroll participants over a period of approximately 3–4 months, with an expected recruitment rate of 3–5 participants per week. Recruitment progress will be monitored weekly. Contingency plans for delayed recruitment

include extending the period by up to 2 months or broadening referral sources to include collaborating regional clinics.

Assignment of interventions: allocation

Sequence generation {16a}

Participants will be randomized in a 1:1 ratio to receive either tirzepatide or placebo using a computer-generated randomization sequence generated through the REDCap randomization module. The sequence will not be stratified or blocked. To maintain allocation integrity, full details of the sequence generation are kept in a separate document, inaccessible to participants.

Concealment mechanism {16b}

Although study investigators will not be blinded to treatment allocation, participants will remain blinded throughout the trial. The allocation sequence will remain concealed from all individuals involved in the enrollment process until after the participant has been irrevocably enrolled and eligibility confirmed. A non-blinded study nurse will assign interventions based on a centralized REDCap allocation system and prepare visually identical pens labeled with unique identifiers to maintain participant blinding. The content of each pen will not be disclosed to the participants.

Implementation {16c}

The allocation sequence will be generated by an independent statistician unaffiliated with participant recruitment or clinical assessments. Participants will be enrolled by study investigators. A designated, unblinded study nurse will assign participants to their respective intervention arms and dispense the appropriately labeled pens at each visit, in accordance with the subject randomization list.

Assignment of interventions: blinding

Who will be blinded {17a}

This is a single-blind clinical trial in which participants will be blinded to the intervention assignment, while study investigators and clinical staff involved in intervention delivery will remain unblinded. However, all outcome assessors involved in the interpretation of imaging, histological analysis, and molecular data will be blinded to group allocation. Additionally, statistical analyses will be conducted on de-identified, group-coded datasets by analysts not involved in participant care or treatment allocation. Identical-appearing prefilled injection pens will be used for both the tirzepatide and placebo groups to maintain blinding at the participant level. Pens will be labeled with unique identification numbers without disclosing content. Blinding of participants is maintained to minimize expectation bias and subjective reporting influences, particularly in relation to appetite, energy levels, and gastrointestinal symptoms. The effectiveness of blinding will be reinforced by uniform procedures, instructions, and visit schedules for both treatment arms. Participants will not be informed of their group assignment during the study unless unblinding is clinically indicated.

Procedure for unblinding if needed {17b}

Unblinding of the participants will be permissible under the following circumstances: treatment of a participant in a medical emergency that requires knowledge of treatment allocation and treatment of a participant for an adverse event (AE), in the event of a suspected unexpected serious adverse reaction (SAR), and SAR is defined as any untoward medical occurrence that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly or birth defect.

The unblinding procedure will be initiated by the principal investigator or treating physician in consultation with the sponsor. Requests will be made via a secure, documented call or email to the unblinded study nurse, who will reveal the allocation through the centralized REDCap system. Unblinding will occur only after confirming the necessity, and details (e.g., reason, date, time, involved parties) will be documented in the participant's case report form and reported to the ethics committee within 24 h if related to a SAR. Unblinding will not occur for data analysis purposes, which will be performed on de-identified datasets by blinded statisticians.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Table 1 presents the planned visits and examinations for each visit. All study-specific data collection forms, including electronic case report forms (eCRFs) and visit checklists, are available upon reasonable request from the corresponding author.

Standard clinical examination

Upon enrollment in the study, demographic and clinical data of participants (age, comorbidities, regular medications, obesity development timeline, and prior obesity interventions) will be collected. Routine clinical measurements, including body height, weight, waist circumference, arterial blood pressure, and heart rate, will be performed using clinically validated instruments in accordance with standard protocols. Height will be measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body weight will be recorded to the nearest 1 kg

with a calibrated scale. Body mass index (BMI) will be calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Waist circumference will be measured using a 250-cm nonelastic tape measure. Blood pressure and resting heart rate will be measured in duplicate on the nondominant arm with a digital blood pressure monitor (Omron M3 Intellisense, HEM-75051-E, Omron Healthcare Europe B.V., The Netherlands) in a seated position after at least 5 min of rest.

Basic laboratory tests and clinical follow-up

Basic laboratory investigations will include an extended panel of electrolytes (sodium, potassium, chloride, magnesium, and calcium), liver function tests (ALT, AST, GGT, bilirubin, and albumin), nitrogen retention markers (urea and creatinine), pancreatic enzymes (lipase and amylase), and basic urine analysis. These tests will be performed using standardized, clinically validated laboratory methods routinely employed in the management of patients at our department. Blood samples will be collected after first and third month of treatment to ensure treatment safety. During monthly visits, clinical response to treatment will be assessed through changes in body weight, waist circumference, and BMI, alongside patient-reported outcomes on appetite and energy levels. Adverse effects, such as gastrointestinal symptoms, injection site reactions, or other tolerability concerns, will be systematically recorded using a standardized adverse event reporting form. Based on the observed clinical response and tolerability, the tirzepatide dose will be adjusted in increments of 2.5 mg every 4 weeks, up to a maximum of 15 mg, as per the study protocol. All findings will be documented in the participant's study record to ensure continuity of care and protocol adherence.

Extended hormonal and laboratory investigations

Participants must fast for at least 10 h prior to test days, abstaining from food, liquids (except plain water), and medications. Whenever feasible, the extended panel will be conducted during the ovulatory phase, ideally within days 3–5 of the menstrual cycle. The comprehensive laboratory and hormonal investigations will include an oral glucose tolerance test (OGTT) with glucose and insulin measurements, glycated hemoglobin (HbA1c), lipid profile, sex hormones (LH, FSH, androstenedione, DHEAS, total and free testosterone), sex hormone-binding globulin (SHBG), prolactin, thyroid-stimulating hormone (TSH), high-sensitivity C-reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), bone turnover markers (PINP and CTX), β-hydroxybutyrate, and 25-hydroxyvitamin D (25-OH-vitD), and basic laboratory tests (electrolytes, liver function tests, nitrogen retention markers, lipase and amylase). All analyses will be performed using standardized, clinically validated laboratory methods routinely employed in the clinical management of participants at our department.

Glucose levels will be measured using the standard glucose oxidase method (Beckman Coulter Glucose Analyzer, Beckman Coulter Inc., CA, USA). Insulin will be quantified with an immunoradiometric assay (Biosource Europe S.A., Nivelles, Belgium). HbA1c will be measured using an automated high-performance liquid chromatography (HPLC) method (Tosoh G8 HPLC Analyzer, Tosoh Bioscience, Tokyo, Japan). ESR will be determined using an automated sedimentation rate analyzer (Alifax Roller 20PN, Alifax S.r.l., Polverara, Italy). Bone turnover markers will be evaluated as follows: PINP using a chemiluminescent immunoassay (CLIA) and CTX using an electrochemiluminescence immunoassay (ECLIA), both on the Roche Elecsys platform (Roche Diagnostics, Basel, Switzerland). The lipid profile will be analyzed using the Advia 1800 Siemens analyzer (Siemens Healthcare, Erlangen, Germany). LH and FSH will be measured with an immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA). Androstenedione and DHEAS will be quantified using a double-antibody RIA with 125I-labeled hormones (Diagnostic Systems Laboratories, Webster, TX, USA). Total and free testosterone will be assessed via coated-tube RIA (DiaSorin S.p.A., Saluggia, Italy; Diagnostic Products Corporation, Los Angeles, CA, USA). SHBG will be determined using a chemiluminescence immunoassay (IMMULITE 2000 Analyzer, Siemens Healthcare, Erlangen, Germany). High-sensitivity CRP will be measured via a latex-enhanced immunochemical reaction (Atellica NEPH 630 Nephelometer Analyzer, Siemens Healthcare, Erlangen, Germany). Prolactin will be measured using an electrochemiluminescence immunoassay (ECLIA) on the Roche Elecsys platform. β-Hydroxybutyrate will be determined enzymatically using the AU5800 Analyzer (Beckman Coulter, Brea, CA, USA). 25-Hydroxyvitamin D will be measured using a chemiluminescent microparticle immunoassay (CMIA) on the Architect i2000SR system (Abbott Laboratories, IL, USA).

The OGTT will be conducted before 8:00 AM following an overnight fast, with the last meal consumed no later than 7:00 PM the previous day. Venous blood will be drawn at baseline (0 min) to measure glucose. Participants will then ingest 75 g of glucose dissolved in 250–300 ml of water within 5 min. After 120 min, venous blood will be drawn again to measure glucose and insulin levels. During the test, participants must refrain from eating, smoking, and engaging in vigorous physical activity and may only consume plain water. Insulin resistance will be calculated using the HOMA-IR, QUICKI, and Matsuda indices [58].

Measurement of body composition, bone mineral density and muscle strength

Whole-body composition will be evaluated using dual-energy X-ray absorptiometry (DXA) with the Discovery A system (Hologic, Waltham, MA, USA) and analyzed using the manufacturer's software (QDR for Windows, version 12.5). The DXA scan will provide measurements of whole-body fat mass, VAT indices, lean body mass, and bone mineral content, as well as regional data for specific body segments (arms, legs, and trunk). Bone mineral density (BMD) will be reported in grams per square centimeter (g/cm^2) and compared to age- and sex-matched reference populations, with Z-scores calculated to assess deviations from the norm. The trabecular bone score (TBS) will be derived from lumbar spine DXA scans and reported as a complementary measure to BMD. Hand grip strength will be measured using a calibrated Saehan hydraulic hand dynamometer SH5001 (Saehan Corporation, Seoul, South Korea). Participants will perform three trials per hand, with the highest value recorded in kilograms (kg) for each hand.

Determination of shivering threshold and thermography

Participants will change into standardized clothing (surgical tunic/pants and sandals) with a low CLO value (approximately 0.20) and will rest in a warm environment (22 °C, 30 min). Subsequently, they will don a water-cooled cooling vest (Cool Flow® Arctic Chiller Adjustable Vest System, Polar Products Inc., OH, USA) with an initial water temperature of 24 °C. The water temperature in the vest will then be reduced by 4 °C every 10 min until it reaches 16 °C. Thereafter, the temperature will be decreased by 2 °C at 10-min intervals until a final temperature of 5 °C is achieved. The temperature at which shivering begins will be recorded, and cooling will be stopped at that point. If no shivering is observed, cooling will continue for an additional 10 min at 5 °C and then stop at the maximal total time of 90 min. The participant will subjectively rate the degree of shivering from none, mild, to severe. During the determination of the shivering threshold, we will measure the amount and activation of brown adipose tissue using thermography. We will use a high-resolution thermal imaging camera (FLIR T650sc, FLIR Systems, OR, USA) for the study. The camera's nominal accuracy in the temperature range of 5 to 120 °C is ± 1% of reading or 1 °C at ambient temperatures from 10 to 35 °C. Before the study, the camera will be calibrated to confirm its accuracy. The analysis of thermographic images will be performed using ResearchIR Max software (FLIR Systems, OR, USA), which allows for the analysis of various regions of interest (ROI), such as different area shapes, individual points, automatic detection of hot/cold spots, temperature differences, isotherms, and line profiles. The camera will be mounted on an adjustable tripod, allowing for precise height and multi-axis rotation adjustments. It will be positioned at a distance of 1 m from the participant to ensure optimal focus and field of view of the face, neck, and upper thorax. To optimally measure the radiation from the supraclavicular (SCV) fossa, the camera will be placed along a line perpendicular to the SCV plane, which is externally and cranially rotated relative to the coronal plane. This positioning will focus on imaging one SCV fossa, prioritizing maximal detection of infrared radiation indicative of brown adipose tissue activity over symmetrical imaging of both fossae. While this approach may reduce reproducibility due to variable camera angles, it ensures a more accurate assessment of SCV temperature compared to a standardized frontal position. Besides measurement of absolute temperature values of regions of interest (ROIs), the differences will be observed because we expect much more comparable results between the differences than between the absolute values due to different thermoregulation of individuals and consequently different surface temperatures at their ROIs. The first measurement will be performed before the cooling begins, in a thermoneutral environment. Subsequent measurements will be taken every 5 min during the cooling with the final measurement at the time shivering begins or the cooling protocol ends. The measurements will be conducted under controlled environmental conditions (temperature, relative humidity, the absence of direct solar radiation, no exposure to nearby heat sources, proper assessment of the emissivity of the measured surface).

Measurement of brown adipose tissue quantity and activity using 18F-FDG-PET/CT

Participants will undergo PET/CT acquisition using a Siemens Biograph mCT Flow PET/CT scanner. During the PET/CT examination, the participant will first rest in a warm room for 30 min. Then, a cooling vest with water set at 4 °C above the shivering threshold (or at 5 °C if the threshold was not reached) will be applied for 60 min in a cold room. If shivering occurs during cooling, the water temperature in the vest will be increased by 1 °C, and the participant will be covered with a blanket for 2 min or until shivering subsides. After 1 h of cooling, a radiopharmaceutical ([18F]FDG, 0.8 MBq/kg body weight) will be administered, and the water temperature in the vest will be increased by 1 °C. After 60 min, imaging will be performed on the PET/CT device. The scan will be continuous from the base of the skull to the lower edge of the clavicle (Siemens Biograph mCT Flow, PET: 3D acquisition, field of view width 22.1 cm, movement speed 0.3 mm/s; CT: low radiation dose, 120 kEv, 25 mAs). The PET/CT scan will be uploaded in DICOM format for

processing in the Fiji/ImageJ software package with the PETCTViewer plugin.

Measurement of brown adipose tissue quantity and activity using MRI

The transformation of supraclavicular adipose tissue will be assessed using a 2-point DIXON pulse sequence on a 1.5 T MRI scanner (Siemens MAGNETOM Vida, Siemens Healthcare, Erlangen, Germany). Scans will be conducted in a thermoneutral environment (22 °C) without cold stimulation to ensure baseline BAT measurements. Using syngo.via (Siemens Healthcare, Erlangen, Germany) we will quantify fat fraction in the supraclavicular fat and assess liver steatosis. Also, the fat fraction of subcutaneous fat will be measured on the same slices as delineated supraclavicular regions to serve as a longitudinal point of reference. The MRI data will be analyzed by trained radiologists to ensure accuracy and consistency, with results reported in standardized units.

Resting energy expenditure measurement

REE will be measured using the Breezing Pro Metabolic Analyzer (Breezing, Tempe, AZ, USA), a portable indirect calorimetry device that calculates REE through breath-by-breath analysis of oxygen consumption (VO2) and carbon dioxide production (VCO2). Measurements will be conducted in a quiet, thermoneutral room (22 °C) following an overnight fast of at least 10 h, ensuring participants abstain from food, liquids (except plain water), caffeine, and nicotine. Participants will be instructed to avoid physical activity for 24 h prior to the test to minimize residual metabolic effects.

Physical activity level, heart rate and sleep monitoring

Participants will wear the Xiaomi Mi Band 9 Active (Xiaomi Inc., Beijing, China). The device will be used to monitor average daily step counts, resting heart rate, total sleep duration, and recorded physical activity. Data will be collected during three key time points: 1 month prior to treatment initiation, the first month of treatment, and the final month of treatment. All data will be synchronized via the Mi Fitness app, ensuring consistent and standardized tracking throughout the observation period.

Continuous glucose monitoring

We will utilize the Abbott FreeStyle Libre 2 Plus Continuous Glucose Monitoring (CGM) System to monitor glucose levels. The FreeStyle Libre 2 Plus is FDA-cleared sensor-based wearable device that will be worn on the upper arm for 15-day periods, providing real-time interstitial glucose readings every minute. Data will be continuously streamed and recorded via the LibreLink app, allowing detailed analysis of glycemic patterns and variability.

Continuous ketone monitoring

We will utilize the Sibionics KS1 Continuous Ketone Monitoring (CKM) System to monitor ketone levels. The KS1 CKM, a CE-certified wearable biosensor, will be worn on the upper arm for 14-day periods, providing real-time interstitial fluid ketone readings every 5 min. Data will be streamed via the Sibionics CKM app. The sensor will be applied after the first and third month of treatment to assess ketone dynamics during different phases of therapy.

Subcutaneous WAT collection and analysis

Subcutaneous fat tissue samples will be obtained using a true-cut biopsy technique with the BD Magnus biopsy system (Cardio Medical, Ljubljana, Slovenia), which includes a guide and a biopsy needle to ensure precise and consistent sampling. The procedure will be performed under local anesthesia by first injecting 3 mL of 2% lidocaine solution into the subcutaneous fat layer of the lower abdomen, a region selected for its accessibility and adequate fat deposition in participants with obesity. After achieving sufficient anesthesia (confirmed by a pinprick test), a guide needle will be inserted to direct the biopsy needle accurately. Using the Magnus gun system, three consecutive biopsies will be taken from the same site to collect sufficient tissue for downstream analyses, including RNA sequencing, RT-PCR, and histological evaluation. The samples will be collected in syringes containing an RNAse inhibitor to preserve RNA integrity, transferred to vials, and then frozen in liquid nitrogen. Total RNA will be extracted, and the quality and integrity of the isolated RNA will be assessed using Agilent biochip analysis, with only samples having an RNA integrity number (RIN) $\geq$ 7.0 being utilized for further experiments. In the initial phase, whole transcriptome sequencing (RNAseq) will be performed on pooled RNA samples from patients receiving tirzepatide therapy and placebo. This pooling strategy will optimize the cost and statistical power for identifying candidate differentially expressed genes associated with the treatment. We anticipate pooling 5 RNA samples per group, resulting in 3 to 4 replicates of pooled samples in both the treatment and placebo groups. Bioinformatic analysis of the RNA-seq data will be conducted to identify differentially expressed genes and perform functional enrichment analysis, including Gene Ontology (GO) annotation, pathway enrichment (e.g., KEGG, Reactome, GSEA), and gene set analysis to determine biological processes, molecular functions, and cellular components affected by the intervention. Additionally, co-expression network

analysis (e.g., WGCNA) may be performed to identify gene modules associated with specific phenotypic traits. In the subsequent phase, RT-PCR will be used to validate the differentially expressed genes identified in the RNAseq experiment across all individual RNA samples. Genes confirmed as differentially expressed will be validated by RT-PCR in individual samples. The tissue samples for histological analysis will be fixed in buffered formalin (4%). The samples will be embayed in full so that one paraffin block will be obtained. Sections of tissue, thickened to 5 μm, will be stained with hematoxylin and eosin. The slides will be analyzed microscopically with an Eclipse 80i microscope (Nikon, Japan). Microscopic pictures will be captured by a DS-Fi2 (Nikon, Japan) digital camera and analyzed by an image analysis system. Univacuolar and plurivacuolar adipocytes, as well as immune cell infiltration and the distribution of small blood vessels, will be analyzed.

Questionnaires

Participants will complete standardized questionnaires in a quiet, private setting, with responses recorded electronically and analyzed according to validated scoring manuals. The questionnaires will assess various domains including eating behavior (Dutch Eating Behavior Questionnaire, DEBQ; 33 items, measuring emotional, external, and restrained eating), physical activity (International Physical Activity Questionnaire—Short Form, IPAQ-SF; 7 items, reported in MET minutes/week), psychological state (Hospital Anxiety and Depression Scale, HADS; 14 items, separate scores for anxiety and depression, each ranging 0–21), obesity-related psychosocial burden (Obesity-related Problems Scale, OP-scale; 8 items, scored 0–100), sexual function (Female Sexual Function Index, FSFI; 19 items, score range 2–36), dietary habits (Short Food Frequency Questionnaire, S-FFQ; assessing frequency of intake across major food groups), and cognitive and behavioral aspects of eating (Three-Factor Eating Questionnaire—Revised 18-item version, TFEQ-R18; assessing cognitive restraint, uncontrolled eating, and emotional eating).

Plans to promote participant retention and complete follow-up {18b}

To promote participant retention and ensure complete follow-up, all participants will attend monthly in-person visits throughout the 24-week intervention period, during which they will receive the next month's injection pen, undergo safety evaluations, and review any adverse events or concerns with the study team. These regular touchpoints are designed to reinforce adherence, maintain engagement, and ensure timely clinical oversight. Participants will also have direct access to the research staff via a dedicated phone line or secure email, providing immediate support and clarification as needed. Interim laboratory testing will be performed to monitor safety and treatment response, reinforcing the clinical value of ongoing participation. If a participant is unable to attend a scheduled visit, flexible rescheduling options will be offered to accommodate individual needs. For participants who discontinue treatment prematurely but remain willing to undergo final assessments, we will strongly encourage completion of all remaining outcome assessments at the end-of-treatment visit. These data will be analyzed separately from the per-protocol population and may be included in modified intention-to-treat or sensitivity analyses. For those who deviate from protocols (e.g., missed doses or visits but continue in the study), we will collect all scheduled outcomes where possible. The extent and nature of protocol deviations will be recorded in REDCap and considered during statistical analysis. In cases of complete withdrawal from follow-up, only baseline data will be included for secondary outcome analyses.

Data management {19}

Participants will be identified by a unique study ID, which will be used consistently across all collected data to ensure confidentiality. Study data will be collected and managed using the REDCap application, which is hosted on a secure server at the University Medical Center Ljubljana (UKCL). The data from outpatient examinations and laboratory tests will also be stored in the Hipokrat information system, while PET/CT and MRI scan results will be archived in the Impax system, and DXA results will be stored in the Hologic Discovery system. All these platforms are accessible only to registered users with individual accounts and passwords on the secure UKCL server. Access to these data will be granted exclusively to healthcare personnel involved in the research and patient care. Access permissions will be managed by authorized IT personnel employed at our institution. Laboratory data, including those from clinical analyses, will be electronically transferred from the performing laboratory and securely archived on encrypted hard drives with backup. Additionally, all biological materials, including blood and adipose tissue samples obtained from study participants, will be preserved in a research biobank. Each sample will be labeled with the corresponding study ID to maintain anonymity and traceability. The biobank's infrastructure ensures that all samples are analyzed concurrently to minimize instrumental variations, thereby enhancing the reliability of the results. Data derived from whole transcriptome sequencing and differentially expressed gene analyses will be digitally stored on the server of the Biotechnical Faculty, University of Ljubljana. As these

data involve confidential genetic information, they will be stored in an anonymized form to protect participant identity. Given that the data collected and analyzed in this study include personal and sensitive information, they cannot be made publicly available as they cannot be anonymized to a degree that would completely eliminate the risk of identifying individual participants. Furthermore, certain critical details might be lost if randomization is applied too stringently. Therefore, storage in a public repository is deemed inappropriate. Instead, the data will be preserved long term on the REDCap platform and within the Hipokrat information system. Access to these data will not be time-restricted, thus ensuring transparency, the possibility of re-evaluation, and potential future use by individuals with authorized access. This trial adheres to the Data Protection Act, which mandates that data be anonymized as soon as practicable to protect participant privacy.

To ensure data quality in electronic entry via REDCap, built-in validation rules will be implemented, including automated range checks, logic checks, and branching logic. For critical fields (e.g., primary outcomes like BAT SUVmean), double data entry will be performed by independent staff, with discrepancies resolved by the principal investigator. All entries will be audited periodically for completeness and accuracy. All changes will be audit-tracked within REDCap. Data exports for statistical analysis will occur only after database lock, following final data validation.

Confidentiality {27}

All personal information collected during the trial will be handled in compliance with applicable data protection regulations and institutional policies to ensure participant confidentiality. Each participant will be assigned a unique study identification number at enrollment, which will be used to label all clinical data, biological samples, and imaging records. No personally identifiable information (e.g., name, date of birth, national identification number) will be included in any datasets used for analysis or publication. Data will be collected and managed using REDCap, a secure, access-controlled electronic data capture system hosted on institutional servers at the University Medical Centre Ljubljana. Access to identifiable data will be restricted to authorized study personnel directly involved in patient care or data collection, all of whom will be bound by confidentiality agreements. Imaging, laboratory, and questionnaire data will be stored in institutional platforms (e.g., Hipokrat, Impax, and Hologic Discovery systems) that are protected by individual logins and institutional firewalls. Biological samples will be labeled only with the participant's study ID and stored in a secure research biobank. Genetic and transcriptomic data derived from these samples will be anonymized prior to storage on secured servers at the Biotechnical Faculty. Data will not be transferred to external parties in identifiable form and will not be made publicly available due to the risk of reidentification. All data will be preserved according to institutional archiving policies for future research use, with access restricted to authorized investigators.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

Biological specimens include venous blood samples and subcutaneous white adipose tissue biopsies. All biological materials will be labeled with the participant's unique study identification number and stored in a secure institutional biobank under controlled conditions. Samples will be preserved in accordance with best practices. Storage and analysis of specimens will be conducted exclusively at certified facilities affiliated with the University Medical Centre Ljubljana and the Biotechnical Faculty, University of Ljubljana. Samples may be used for future ancillary studies related to obesity, adipose tissue biology, or pharmacological interventions, contingent upon additional ethical approval and patient consent. No genetic material will be used for purposes unrelated to the objectives of this trial without explicit participant consent. All analyses will be performed on anonymized specimens, and results will be linked to clinical data solely through coded identifiers to preserve participant confidentiality.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Descriptive statistics will summarize variables—normally distributed continuous data as mean ± standard deviation, non-normally distributed data as median with interquartile range (25th–75th percentiles), and categorical data (e.g., adverse events) as frequencies and percentages. Fisher's exact test will be used to compare dichotomous categorical data. Independent samples *t*-tests or Mann–Whitney U-/Kruskal–Wallis tests will assess between-group differences (tirzepatide vs. placebo), Wilcoxon signed-rank tests will evaluate within-group changes, repeated measures ANOVA (or Friedman test) will analyze longitudinal data, Spearman's rho (ρ) with partial correlations will explore relationships, and BAT measurements from 18F-FDG PET/CT (SUV), MRI (volume, PDFF), and IRT (SCV temperature) will be compared using Bland–Altman plots and intraclass correlation coefficients (ICC) for agreement, *t*-tests/Mann–Whitney U for group differences, Wilcoxon tests for pre-post changes, Spearman's rho

for correlations with clinical outcomes, and repeated measures ANOVA for IRT temperature dynamics. Additionally, RNAseq data will be analyzed using edgeR and DESeq2 with FDR correction, focusing on thermogenesis and browning pathways, compiling differentially expressed genes with ≥ twofold change, followed by functional enrichment (GO, KEGG, Reactome, GSEA). All analyses will be conducted in IBM SPSS Statistics, version 29.0 (IBM Corporation, Armonk, NY, USA), using $p < 0.05$ for statistical significance.

Interim analyses {21b}

No formal interim analyses are planned for this study. Given the relatively short duration of the intervention (24 weeks) and the modest sample size, the risk–benefit profile of the intervention will be continuously monitored through scheduled safety assessments rather than predefined interim efficacy analyses. Participant safety will be reviewed on an ongoing basis by the principal investigator and designated clinical staff. If a serious safety concern arises—such as a suspected unexpected serious adverse reaction (SUSAR), treatment-related hospitalization, or a cluster of significant adverse events—an ad hoc safety review will be conducted to assess the need for study modification or early termination. The decision to pause or terminate the trial prematurely will be made by the sponsor in consultation with the institutional ethics committee. All adverse events will be recorded, evaluated for severity and causality, and reported according to regulatory requirements. Any protocol amendments resulting from safety concerns will be submitted for prior approval by the ethics committee before implementation.

Methods for additional analyses (e.g. subgroup analyses) {20b}

Not applicable.

Methods in analysis to handle protocol nonadherence and any statistical methods to handle missing data {20c}

The primary analysis will be conducted on the per-protocol population, defined as all participants who complete the full 24-week intervention period with adequate adherence (defined as no more than one missed injection and no more than one missed visit) and who undergo both baseline and final outcome assessments. This approach is justified for this exploratory trial, as it focuses on evaluating tirzepatide's effects under conditions of high protocol adherence to provide precise insights into novel mechanisms, where nonadherence could obscure subtle changes. Participants who deviate substantially from the study protocol, including those who prematurely discontinue treatment or fail to attend key assessments, will be excluded from the primary outcomes analysis. To mitigate potential bias and align with best practices, a sensitivity analysis will be performed on the intention-to-treat (ITT) population, including all randomized participants analyzed according to their allocated group.

Missing data will not be imputed in the primary analysis to avoid assumptions in this small sample; instead, complete-case analysis will be used. The reasons for missing data will be documented in detail, and patterns of missingness will be examined to identify any systematic bias (e.g., via descriptive summaries or logistic regression). Sensitivity analyses will be performed where appropriate to assess the impact of missing data on secondary outcomes.

Plans to give access to the full protocol, participant level-data and statistical code {31c}

The full study protocol will be made available upon reasonable request to the corresponding author or preferably published in a peer-reviewed journal. De-identified participant-level datasets will not be made publicly accessible due to the inclusion of sensitive data that cannot be sufficiently anonymized without compromising data integrity or violating participant confidentiality. However, qualified researchers with methodologically sound proposals may request access to specific datasets and analysis scripts for non-commercial, ethically approved research purposes. Such requests will be reviewed by the study investigators and the institutional ethics board, and data sharing will be governed by a formal data use agreement that ensures compliance with data protection regulations and institutional policy. Summarized trial results, including primary and secondary outcome data, will be published in peer-reviewed journals and presented at scientific conferences to ensure transparency and scientific dissemination.

Oversight and monitoring

Composition of the coordinating center and trial steering committee {5d}

The study is coordinated by the Department of Endocrinology, Diabetes and Metabolic Diseases at the University Medical Centre Ljubljana, which serves as the sole trial site and coordinating center. The coordinating team is responsible for the day-to-day management of the study, including patient recruitment, data collection, safety monitoring, regulatory compliance, and overall protocol execution. The principal investigator will oversee all aspects of trial conduct, ensure adherence to good clinical practice (GCP) guidelines, and act as the

primary liaison with the sponsor and regulatory authorities. A designated clinical research coordinator will manage logistical aspects of participant follow-up, data entry, and documentation. Given the single-center design and relatively small sample size, a formal Trial Steering Committee (TSC) has not been established. However, regular internal meetings will be held among investigators, clinical staff, and the biostatistics team to review recruitment progress, protocol adherence, and safety data. Any protocol amendments or safety concerns will be discussed collectively and, if necessary, referred to the institutional ethics committee for approval.

Composition of the data monitoring committee and its role and reporting structure {21a}

Given the single-center design, limited sample size, short study duration, and low anticipated risk associated with the intervention, a formal independent Data Monitoring Committee (DMC) has not been established for this trial. Instead, safety monitoring and oversight responsibilities will be carried out by the principal investigator and core clinical research team at the Department of Endocrinology, University Medical Centre Ljubljana. Adverse events will be reviewed continuously during monthly visits, and any serious or unexpected events will be evaluated by the research team in real time. Where appropriate, such events will be reported to the institutional ethics committee according to national regulatory requirements. Should any safety concerns arise that might warrant study modification or early termination, these will be escalated to the sponsor and the National Medical Ethics Committee for independent review.

Adverse event reporting and harms {22}

Adverse events (AEs) and serious adverse events (SAEs) will be actively monitored, documented, and managed throughout the study period in accordance with good clinical practice (GCP) and applicable national regulations. Participants will be evaluated at monthly clinic visits, where they will be systematically questioned about potential adverse effects using a standardized AE reporting form. Participants will also have direct access to the research staff between visits to report any emerging symptoms or concerns. All reported events will be assessed by the study investigators for severity, expectedness, and causality in relation to the investigational product. Particular attention will be given to known adverse effects of tirzepatide, including gastrointestinal symptoms, injection site reactions, hypoglycemia, and signs of pancreatitis or gallbladder disease. Laboratory safety monitoring will be conducted after the first and third month of treatment, and any clinically significant findings will be further evaluated. All SAEs—including events resulting in death, life-threatening conditions, hospitalization, persistent or significant disability, or congenital anomaly—will be reported to the sponsor and the Slovenian National Medical Ethics Committee within the required timelines. Suspected unexpected serious adverse reactions (SUSARs) will be reported immediately. Appropriate medical care will be provided for all participants experiencing adverse events. In case of severe or intolerable side effects, treatment discontinuation will be considered, and participants will continue to be followed per protocol if feasible. All adverse events and management actions will be recorded in the study database (REDCap) and reviewed periodically by the clinical research team to identify any safety trends.

Frequency and plans for auditing trial conduct {23}

No independent auditing is planned for this study. Internal monitoring will be conducted by the study team to ensure adherence to the protocol, regulatory compliance, and data integrity. Any deviations or safety concerns will be reviewed and addressed promptly.

Plans for communicating important protocol amendments to relevant parties (e.g., trial participants and ethical committees) {25}

Any significant protocol modifications—such as changes to eligibility criteria, study procedures, outcomes, or analysis methods—will be submitted for approval to the Slovenian National Medical Ethics Committee prior to implementation. Approved amendments will be communicated to all investigators, trial personnel, and relevant regulatory bodies and will be documented in the trial registry (ClinicalTrials.gov).

Dissemination plans {31a}

The results of this trial will be submitted for publication in peer-reviewed scientific journals and presented at national and international conferences. Authorship will follow ICMJE guidelines. Participants will be informed of the overall study findings upon request, but no individual-level results will be disclosed. Trial registration details and key findings will also be updated on ClinicalTrials.gov following study completion.

Discussion

The Tirzepatide Brown and Beige Adipose Tissue Activation (TABFAT) study is designed to explore the effects of tirzepatide, a second-generation anti-obesity medication, on brown adipose tissue (BAT) quantity, activity, and the potential induction of white adipose tissue (WAT) browning in humans. By focusing on tirzepatide,

this study leverages the drug's unique molecular profile, which preclinical evidence suggests may directly enhance adipose tissue function also through GIP receptor-mediated effects [38]. This trial is the first to systematically explore these effects in humans, addressing a critical gap in clinical research where data on BAT activation and WAT browning with second-generation anti-obesity medications remain scarce, with only limited and inconsistent findings from studies on GLP-1 monoagonists like liraglutide and exenatide [27, 28].

A key strength of TABFAT lies in its multimodal approach to BAT assessment, integrating 18F-FDG-PET/CT—the gold standard for BAT evaluation—with emerging techniques like MRI and IRT. By comparing these methods, the study not only aims to validate noninvasive, radiation-free alternatives like IRT, which could enable large-scale BAT research across diverse populations due to its cost-effectiveness and accessibility [29], but also seeks to establish standardized protocols for future clinical and research applications. By evaluating these tools side by side, TABFAT aims to contribute practical insights into their reliability and applicability, which could help refine BAT assessment techniques for future studies.

Additionally, the focus on beige adipocyte induction through molecular analyses of subcutaneous WAT biopsies offers a novel perspective on thermogenic potential, addressing the understudied role of WAT browning in human obesity. This is particularly relevant given preclinical data suggesting that tirzepatide may enhance browning processes, potentially amplifying energy expenditure beyond BAT activation alone [45, 47]. If confirmed in humans, this could point to an additional mechanism by which tirzepatide supports weight loss and metabolic improvement, beyond its well-documented effects on appetite suppression and glucose regulation [46]. Furthermore, the study's findings could inform the development of novel obesity management strategies that prioritize energy expenditure alongside traditional approaches like appetite suppression, potentially addressing unmet needs in patients where REE decline hinders sustained weight loss [38].

Beyond its primary focus on BAT and WAT browning, TABFAT will also examine how changes in thermogenic adipose tissue relate to broader metabolic outcomes, such as resting energy expenditure (REE), body composition, and markers of glucose and lipid metabolism. Weight loss often leads to a reduction in REE, which can hinder long-term success in obesity management [38]. If tirzepatide enhances BAT activity or WAT browning, it might help offset this decline, offering a complementary approach to existing strategies. That said, the clinical relevance of BAT activation in humans is still debated—some studies estimate its contribution to energy expenditure as modest [15–17, 21], while others highlight its association with improved metabolic profiles [5–10]. TABFAT's results could add clarity to this discussion, though we recognize that a sample of 34 participants may not fully resolve these broader questions.

While the small sample size presents a limitation, this will be mitigated by employing a homogeneous sample. The decision to focus on premenopausal women with obesity helps control for variables like sex and age, which are known to influence BAT activity and pharmacological responses [52–54]. By including only participants with a BMI of 30–40 kg/m^2, the study benefits from examining a narrow spectrum of obesity, which enhances consistency in baseline BAT characteristics and treatment responses, strengthening the trial's internal validity. This homogeneity allows for a more focused analysis of tirzepatide's effects within a specific obesity range. However, this same restriction also limits the study's applicability to other populations, such as men or postmenopausal women, where BAT dynamics may differ, as well as to individuals with more severe obesity (e.g., BMI > 40 kg/m^2), where BAT activity and therapeutic outcomes might vary. Additionally, while tirzepatide's dual agonism offers a promising avenue for research, disentangling the specific contributions of GIP versus GLP-1 receptor activation will be difficult within this trial's design.

This study also has practical implications for obesity treatment. If tirzepatide is shown to enhance thermogenic adipose tissue activity, it could highlight a mechanism that sets second-generation anti-obesity drugs apart from earlier options. This might encourage further exploration of energy expenditure-focused therapies, complementing traditional approaches like caloric restriction. However, we remain cautious in our expectations—while preclinical data are encouraging, human studies on thermogenic adipose tissue activation have often yielded inconsistent results. TABFAT aims to provide a step forward, but its findings will need to be interpreted in the context of these broader uncertainties.

In summary, TABFAT offers a detailed look at how tirzepatide might affect BAT and WAT browning in humans, addressing a gap in the clinical understanding of second-generation anti-obesity medications. By combining advanced imaging, molecular analyses, and metabolic assessments, the study seeks to shed light on these processes in a controlled clinical setting. While the results may point to thermogenic adipose tissue as a contributing factor in tirzepatide's efficacy, we anticipate that they will also raise new questions for future research. Ultimately, this trial aims to contribute to the evolving field of obesity management by providing data that could

inform both clinical practice and the development of next-generation therapies.

Trial status

This protocol corresponds to version 1.0, dated 15 March 2025. Recruitment for the trial (ClinicalTrials.gov identifier: NCT06893211) began on 10 January 2025 and was completed on 18 April 2025. The first participant will begin measurements on 21 April 2025.

Abbreviations

AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BAT	Brown adipose tissue
BMD	Bone mineral density
BMI	Body mass index
CGM	Continuous glucose monitoring
CKM	Continuous ketone monitoring
CLIA	Chemiluminescent immunoassay
CT	Computed tomography
CTX	C-terminal telopeptide of type I collagen
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
DXA	Dual-energy X-ray absorptiometry
ECLIA	Electrochemiluminescence immunoassay
ESR	Erythrocyte sedimentation rate
FDG	Fluorodeoxyglucose
FSFI	Female Sexual Function Index
FSH	Follicle-stimulating hormone
GCP	Good clinical practice
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GO	Gene Ontology
HbA1c	Hemoglobin A1c
HADS	Hospital Anxiety and Depression Scale
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
ICC	Intraclass correlation coefficient
IRT	Infrared thermography
ITT	Intention to treat
LH	Luteinizing hormone
MRI	Magnetic resonance imaging
OGTT	Oral glucose tolerance test
PDFF	Proton density fat fraction
PET	Positron emission tomography
PINP	Procollagen type I N-terminal propeptide
RIN	RNA integrity number
RNA-seq	RNA sequencing
SAE	Serious adverse event
SAR	Serious adverse reaction
SHBG	Sex hormone-binding globulin
SUV	Standardized uptake value
TFEQ-R18	Three-Factor Eating Questionnaire, Revised 18-item Version
UCP1	Uncoupling protein 1
WAT	White adipose tissue

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Not applicable.

Authors' contributions {31b}

The study concept and protocol design were primarily developed by RH, AJ, and MJ. RH prepared the initial draft of the protocol. AJ and MJ critically reviewed and revised the manuscript. The [^{18}F]FDG-PET/CT protocol was developed by LL, the infrared thermography protocol by IP, VM, and LH, and the MRI protocol by ZS and LP. Statistical methodology and analysis planning were provided by KG. RNA sequencing and adipose tissue biopsy procedures were coordinated by SH, while histological processing and analysis plans were performed by AC. All authors reviewed and approved the final version of the manuscript.

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Data availability

Access to the final trial dataset will be restricted to the principal investigator and designated members of the research team directly involved in data analysis. All data will be stored on secure institutional servers and managed within the REDCap system under individual access controls. External collaborators will not have direct access to the full dataset unless specifically authorized through a data-sharing agreement, subject to ethical and regulatory approvals. There are no contractual limitations on the investigators' access to the final dataset.

Declarations

Ethics approval and consent to participate {24}

The study protocol has been reviewed and approved by the Slovenian National Medical Ethics Committee (reference number: 0120–56/2024–2711-7). All study procedures will be conducted in accordance with the Declaration of Helsinki and GCP guidelines. Written informed consent will be obtained from all participants prior to enrollment.

Consent for publication {32}

Not applicable. This protocol does not include any individual person's data in any form (including images, videos, or case details). Participants will not be identified in any publication arising from the trial.

Competing interests {28}

The authors declare that they have no competing interests.

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