Research protocol

Section 1: Protocol identity

1.1 Title
1.1.1 Title *It should be consistent with the main research question. Abbreviations and unnecessary punctuation are to be avoided.* *Wordlimit:* ***35***
Comparative effects of tirzepatide versus liraglutide on body composition and bone turnover markers in individuals with class III obesity. A multicenter prospective cohort study.

Section 2: Summary

2.1 Summary
Sections: Scientific basis, Purpose, Type of study, Material, Protocol, Intervention (if applicable), Outcome results, Sample size.
Wordlimit: ***500***

Scientific Basis:

Obesity represents a critical global health challenge and is closely associated with metabolic derangements and musculoskeletal complications, including significant bone loss and increased susceptibility to fragility fractures. While intentional weight reduction improves cardiometabolic health, it is concurrently associated with decreased bone mineral density (BMD) and altered bone turnover markers (BTMs), raising concerns regarding long-term skeletal health. Pharmacotherapies such as **liraglutide**, a GLP-1 receptor agonist, and **tirzepatide**, a dual GLP-1/GIP receptor agonist, have demonstrated significant efficacy in promoting weight loss and metabolic control. However, their potential differential impact on bone metabolism and body composition due to their different mechanism of action, remains inadequately explored. This study seeks to elucidate the comparative effects of these agents on bone health, offering crucial insights into optimizing obesity pharmacotherapy.

Objectives

- **Primary** To compare the effects of tirzepatide versus liraglutide on bone turnover markers.
- **Secondary**
    - To assess changes in BMD at the lumbar spine and hips and trabecular bone score (TBS) at 6 and 12 months.
    - To analyze body composition parameters (lean mass %, total fat mass, and relative skeletal muscle index [RSMI]) for sarcopenia evaluation at 6 and 12 months.
    - To adjust comparisons for the magnitude of weight loss, ensuring accurate interpretation of bone-related outcomes.

Study Design

Type: Open-label, multicenter, observational cohort study.

Study sample

- Adults aged ≥30 years
- BMI ≥40 kg/m²

Study Protocol

All eligible participants will be asked to provide an informed consent.

Baseline Assessments:

- Full medical history and physical exam
- Anthropometry: height, weight, BMI, waist-to-hip ratio(WHR), waist-to-height ratio(WHtR)
- Bioelectrical impedance analysis (BIA)
- Resting metabolic rate (RMR)
- Sarcopenia evaluation: grip strength, chair stand test, gait speed
- Dual-energy X-ray absorptiometry (DXA): lumbar spine, bilateral hips, TBS, and whole-body scan (WBS)
- Fasting blood samples: CTX, P1NP, TRAP5b, and relevant biochemical markers

Follow-Up Schedule:

- At 3, 6, and 12 months: DXA (only at 6 and 12 months) and BTMs reassessment
- Monthly: weight, BMI, BIA, WHR, WHtR, dietary adherence, and physical activity monitoring

Participants will receive either:

- **Liraglutide**: Initiated at 0.6 mg/day, titrated up to 3 mg/day
- **Tirzepatide**: Initiated at 2.5 mg/week, titrated up to 15 mg/week Treatment allocation will be based on clinical judgment, patient preference, and tolerability.

Outcome Results:

Primary Endpoint:

- Change in serum CTX levels at 3 months between tirzepatide and liraglutide groups.

Secondary Endpoints:
i. Changes in P1NP and TRAP5b levels at 3 and 6 months
ii. CTX changes at 6 months
iii. Group comparisons of BTM changes at 3 and 6 months, adjusted for weight loss
iv. Longitudinal assessment of BMD and body composition at 6 and 12 months
v. Between-group differences in BMD/body composition changes, adjusted for weight loss

Sample Size: Based on the detection of clinically meaningful differences in serum CTX levels following intervention, a sample size of **32 participants per group** is required to achieve **80% statistical power** at a **5% significance level (two-tailed)**. To account for a potential **10% dropout rate**, the study will enroll **36 participants per group** (**total 72 participants**).

Section 3: Research hypothesis and scientific basis

3.1 Hypothesis(es) tobetested

Clear formulation of the research hypothesis which the research project is expected to answer [preferably in the form of null (H_0) and alternative (H_1) hypothesis]. Numbering of primary and secondary research hypotheses. The wording should be such that ultimately the case can be answered as "accepted" or "rejected"

We hypothesize that treatment with tirzepatide versus liraglutide will yield distinct effects on markers of bone turnover and body composition, independent of the magnitude of weight loss due to their different molecular mechanisms of action.

3.3 Scientific basis of the proposed research project

3.2.1 Analysis of concepts related to the case to be tested

The description must be composed in such a manner as to be fully understood by scientists and researchers knowledgeable within the relevant scientific field, although a particularly in-depth probe into the specific topic of the proposed research project is not considered necessary.
What is already known ("state of the art" knowledge along with the corresponding bibliographic documentation) is to be stated.
Word count range: ***500 – 1000***

Obesity is a critical global health concern, intricately linked to a range of metabolic and systemic disorders, including hypertension, type 2 diabetes mellitus, metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), obstructive sleep apnea, and increased cardiovascular risk. Beyond these well-recognized associations, obesity also exerts complex effects on skeletal health. While increased mechanical loading from excess body weight may offer some protective benefits to bone mass, emerging evidence reveals that obesity contributes to alterations in bone metabolism, resulting in an elevated risk of osteoporosis and fragility fractures.

The relationship between obesity and bone health is multifactorial and influenced by numerous variables, including fat distribution, adipose tissue-derived cytokines and adipokines, nutrient imbalances (e.g., calcium and vitamin D), and systemic inflammation. These factors collectively disrupt bone remodeling homeostasis and promote bone loss.

Conversely, rapid and substantial weight loss—achieved via caloric restriction, pharmacotherapy, or bariatric surgery—has been consistently associated with reductions in bone mineral density (BMD), increased bone turnover, and higher fracture risk. This paradoxical outcome underscores the need for careful monitoring of bone health in the context of obesity treatment.

Bone turnover can be non-invasively assessed using circulating bone turnover markers (BTMs), which reflect dynamic changes in skeletal remodeling. KeyBTMsinclude:

- **Resorption markers**: C-terminal telopeptide of type I collagen (CTX), tartrate-resistant acid phosphatase 5b (TRAP5b)
- **Formation markers**: Procollagen type 1 N-terminal propeptide (P1NP), osteocalcin (OC), alkaline phosphatase (ALP), and osteoprotegerin (OPG)

Multiple studies have shown that diet-induced weight loss can lead to adverse skeletal changes, evidenced by increased bone resorption and decreased BMD as measured by dual-energy X-ray absorptiometry (DXA). Similarly, bariatric surgery—particularly procedures involving malabsorption—has been strongly associated with accelerated bone loss and a substantially increased fracture risk, particularly within the first two years

postoperatively.

Pharmacologic agents for weight management, such as **liraglutide** (a GLP-1 receptor agonist) and **tirzepatide** (a dual GLP-1/GIP receptor agonist), have demonstrated considerable efficacy in promoting weight loss. However, their effects on bone health remain incompletely characterized.

In diabetic and glucocorticoid-induced osteoporosis (GIOP) mouse models, **liraglutide** has been shown to improve bone microarchitecture and reduce bone resorption by modulating key BTMs (e.g., CTX, TRAP5b, ALP, OC, OPG, and P1NP).

In clinical trials, results have been inconsistent. A randomized, placebo-controlled study in individuals with type 2 diabetes (n = 56, mean age 63) treated with liraglutide (1.8 mg/day) reported no significant changes in BTMs or BMD. Conversely, another randomized trial involving women with obesity (n = 37, mean age 46, BMI 34 kg/m²) following a hypocaloric diet showed that liraglutide (1.2 mg/day) led to a 16% increase in P1NP levels, suggesting a potential anabolic effect on bone during weight loss.

Data regarding **tirzepatide's** effects on bone metabolism are limited. Preclinical evidence suggests that, despite inducing significant weight loss, tirzepatide may exert a neutral effect on bone mass and architecture. However, robust clinical data in humans are currently lacking. Given tirzepatide's superior weight-loss efficacy relative to GLP-1 agonists alone, it is plausible that its dual agonist activity may engage distinct mechanisms influencing bone remodeling and skeletal integrity.

This gap in knowledge highlights a critical need for comparative clinical studies to delineate the bone-related effects of liraglutide and tirzepatide in the context of obesity management. Understanding these effects is essential to optimize therapeutic strategies that balance metabolic benefits with skeletal safety.

3.2.2 Necessity for the research project – Originality

Why is it necessary to conduct the protocol? What is its originality? What knowledge gap is it seeking to fill? Is this the first study carried out on this topic? If not, how do the researchers plan to make its design and/or methodology distinct from that of preexisting studies?
Word count range: ***100 – 250***

Human studies investigating the effects of incretin-based therapies on bone metabolism in populations with obesity and type 2 diabetes remain limited. Existing trials assessing liraglutide have predominantly utilized doses up to 1.8 mg/day, primarily targeting glycemic control rather than weight loss, and have yielded inconclusive or minimal effects on bone metabolism. Importantly, there are currently no human studies evaluating the impact of tirzepatide—a dual GIP/GLP-1 receptor agonist—on bone turnover markers or bone health outcomes.

The necessity and originality of this study stem from its focus on the comparative effects of liraglutide (at the approved weight loss dose of 3 mg/day) and tirzepatide (at dose up to 15 mg/week) on both body composition and bone metabolism in individuals with obesity. This is the first study to directly investigate the interplay between substantial pharmacologically induced weight loss, differential incretin receptor activation, and skeletal remodeling processes.

The study aims to address a critical gap in current knowledge by elucidating whether the distinct mechanisms of GLP-1 mono-agonism versus dual GIP/GLP-1 receptor agonism result in differential effects on bone turnover markers. It will also explore how these effects may be modulated by the magnitude and composition of weight loss, contributing novel insights into the metabolic-skeletal interface in obesity pharmacotherapy.

3.2.3 Significance

What is the significance of rejecting or accepting the research hypothesis (references, if required)? How does the knowledge gained from the completion of the thesis promote existing knowledge, andalso future research in the specific area?
Wordcountrange: ***100 – 250***

The acceptance or rejection of the research hypothesis will have important implications for understanding the impact of GLP-1 receptor agonists and dual GIP/GLP-1 receptor agonists on bone health in individuals with obesity. Should the hypothesis be accepted—demonstrating differential effects of liraglutide and tirzepatide on bone turnover markers and body composition—it would support the concept that distinct incretin pathways influence skeletal metabolism independently of weight loss alone. This would introduce a new paradigm in obesity pharmacotherapy by highlighting bone health as a critical factor in treatment choice.

Conversely, if no significant differences are observed, this would suggest that weight loss, rather than the specific mechanism of action, may be the predominant driver of changes in bone metabolism. In either case, the study will fill a critical gap in the literature, as current human data on tirzepatide's skeletal effects are lacking, and most liraglutide studies have been conducted at submaximal doses not approved for obesity treatment.

The findings will contribute to the refinement of treatment strategies in obesity, informing both clinical decision-making and patient counseling regarding potential musculoskeletal effects. Additionally, this research will pave the way for future studies on long-term bone health outcomes and fracture risk associated with incretin-based therapies.

Section 4: Methodology

4.1 Type of study
(e.g., observation, intervention, control patients). See: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2689572/.
A different type of study may be required for each research question. If necessary, an equivalence needs to be identified between the research hypotheses and the types of studies that will be applied to investigate them.

This is an open label- observational cohort study

4.2 Selection criteria of the population to be studied
Classification of study groups (e.g., patients, controls). Detailed and precise inclusion and exclusion criteria for each of the study groups. Corresponding listing of inclusion criteria if animal, cell line, or meta-research studies.

Eligibility criteria:

Adults aged over 30 years with BMI ≥40 kg/m²

Exclusion criteria:

1. Type 2 Diabetes Mellitus (T2DM) and type 1 Diabetes Mellitus (T1DM)
2. Chronic kidney disease
3. Liver failure
4. Heart failure
5. Malignancy coexistence
6. Previous bariatric or gastrointestinal surgery involving intestinal bypass
7. Uncontrolled hypo/hyperthyroidism
8. Uncontrolled hypo/hyperparathyroidism
9. Pregnancy and lactation
10. Recent fracture (within 2 years)
11. Rare Metabolic Bone Diseases (e.g., Paget's disease of bone, fibrous dysplasia, osteopetrosis)
12. Inflammatory arthritis
13. Medications which can affect bone markers: bone-anabolic agents, antiresorptive agents, antiandrogenic agents, vitamin K antagonists, antipsychotic agents, contraceptives, glucocorticoids (oral), methotrexate, thiazides, aromatase inhibitors etc)
14. History of Hemolytic anemias

4.3 Research protocol (flow chart)
Brief description of the stages of the conduct the research segment of the study. In addition to the description, a concise presentation of the research protocol with a diagram is recommended.

The research flow chart of the study includes the following steps.

All eligible participants will provide their informed consent, after thorough explanation of the treatment protocol.

Visit 1

At this visit the following parameters will be recorded or will be retrieved from the relevant patient's history databases.

1. Past and current Medical history referring to concomitant diseases and medications

2. Documentation and measurement of anthropometric parameters (Height, Weight, Body Mass Index (BMI), Waist-to-Hip Ratio, waist to height ratio).

3.Assessment of SARC-F questionnaire (for the evaluation of sarcopenia) Short-Form 36 Health Survey (for assessment of quality of life) and Perceived Stress Scale - 14 item (PSS-14) (for assessment of chronic stress), all validated for the Greek population.

4. Bioelectrical impedance analysis (BIA)

5. Resting Metabolic Rate (RMR)

6. Grip strength,

7. Chair stand test and Gait speed.

8. DXA at three skeletal sites (lumbar spine and both hips), calculation of Trabecular Bone Score (TBS) and Whole-Body Scan.

9. Fasting Blood sampling.

After all tests and questionaries have been complete yypocaloric diet designed by the same dietary team, providing an energy intake 20-30% below the Total Energy Expenditure, as estimated from the Resting Metabolic Rate and exercise recommendations will be provided for all participants. Patients then will be prescribed liraglutide or tirzepatide based on their discretion and their treating physician's choice.

Weekly estimation of each participant from Group A - liraglutide Group to assess the potential for a dosage increase starting from an initial dose of 0.6 mg daily, with incremental increases of 0.6 mg, up to the maximum tolerated dose, with a maximum target dose of 3 mg per day.

Monthly estimation of each patient from Group B - tirzepatide Group to assess the potential for a dosage increase, starting from an initial dose of 2.5 mg weekly, up to the maximum tolerated dose, with a maximum target dose of 15 mg per week.

Visit 2-Follow up in 3rd month

The following parameters will be recorded or will be retrieved from the relevant patient’s history databases.

1. Medical history

2. Measurement of anthropometric parameters (Height, Weight, BMI, Waist-to-Hip Ratio, waist to height ratio).

3. Assessment of SARC-F questionnaire, Short-Form 36 (SF-36) Health Survey and Perceived Stress Scale - 14 item (PSS-14) (validated for the Greek population).

4. BIA.

5. RMR.

6. Grip strength, Chair stand test and Gait speed.

7. Blood sampling after overnight fast.

Patients continue to receive liraglutide or tirzepatide.

Monitoring the adherence to the diet and exercise program based on the patient's reports.

Visit 3-Follow up in 6th month

The following parameters will be recorded or will be retrieved from the relevant patient's history databases.

1. Medical history documentation and measurement of anthropometric parameters (Height, Weight, BMI, Waist-to-Hip Ratio, waist to height ratio).
2. Assessment of SARC-F questionnaire, Short-Form 36 (SF-36) Health Survey and Perceived Stress Scale - 14 item (PSS-14) (validated for the Greek population).
3. Bioelectrical impedance analysis (BIA).
4. Resting Metabolic Rate (RMR).
5. Grip strength, Chair stand test and Gait speed.
6. DXA at three skeletal sites (lumbar spine and both hips), calculation of Trabecular Bone Score (TBS) and Whole-Body Scan.
7. Blood sampling.

Patients continue to receive liraglutide or tirzepatide.

Monitoring the adherence to the diet and exercise program based on the patient's reports.

Visit 4-Follow up in 12th month

The following parameters will be recorded or will be retrieved from the relevant patient's history databases.

1. Medical history documentation

2. Measurement of anthropometric parameters (Height, Weight, BMI, Waist-to-Hip Ratio, waist to height ratio).
3. Assessment of SARC-F questionnaire, Short-Form 36 (SF-36) Health Survey and Perceived Stress Scale - 14 item (PSS-14) (35) (validated for the Greek population).
4. Bioelectrical impedance analysis (BIA).
5. Resting Metabolic Rate (RMR).
6. Grip strength, Chair stand test and Gait speed.
7. DXA at three skeletal sites (lumbar spine and both hips), calculation of Trabecular Bone Score (TBS) and Whole-Body Scan.
8. Blood sampling.
9. Patients continue to receive liraglutide or tirzepatide.

Monitoring the implementation of the diet and exercise program based on the patient's reports.

4.4 Description of the intervention

(Applicable only in intervention studies).

Clear description of the nature of the intervention and its characteristics [e.g., blinding, randomization, intention-to-treat and/or per protocol analysis, control groups].

4.5 Description of measured variables

List of variables to be recorded according to the research protocol. It is recommended that the data entry form be attached.

4.5 Description of Measured Variables

All variables will be systematically recorded according to the study protocol. These include demographic characteristics, anthropometric data, body composition metrics, laboratory parameters, bone turnover markers, functional assessments, and validated questionnaire responses.

A. Demographic and ClinicalCharacteristics

- **Age** (years)
- **Sex** (male/female)
- **Medical History** (including metabolic, cardiovascular, skeletal, endocrine, and respiratory disorders)
- **Menopausal Status** (for female participants: premenopausal/postmenopausal)
- **Medication Use** (current and past medications including antiresorptives, glucocorticoids, antidiabetics, and others)
- **Smoking Status** (current/former/never)
- **Alcohol Consumption** (average units/week)

B. AnthropometricMeasurements

- **Weight** (kg)
- **Height** (cm)
- **Body Mass Index (BMI)** (kg/m²)
- **WaistCircumference** (cm)
- **HipCircumference** (cm)
- **Waist-to-HipRatio**

C. Body Composition Parameters

Measured by **DXA** and **Bioelectrical Impedance Analysis (BIA)**:

- **Total Fat Mass** (kg and %)
- **Visceral Fat Mass** (if applicable by device)
- **LeanMass** (kg)
- **AppendicularLeanMass** (kg)
- **Bone Mineral Density (BMD)** at:
    - Lumbarspine (L1–L4)
    - Femoralneck
    - Totalhip
- **Bone MineralContent (BMC)**
- **Trabecular Bone Score (TBS)**
- **Whole-BodyScan (WBS)**

D. Laboratory Measurements*(Fasting blood samples)*

- **Glucose** (mg/dL)
- **Insulin** (µU/mL)
- **HbA1c** (%)
- **LipidProfile**:
  - TotalCholesterol (mg/dL)
  - LDL-Cholesterol (mg/dL)
  - HDL-Cholesterol (mg/dL)
  - Triglycerides (mg/dL)
- **Calcium** (corrected for albumin, mg/dL)
- **Phosphate** (mg/dL)
- **25-Hydroxyvitamin D [25(OH)D]** (ng/mL)
- **Parathyroid Hormone (PTH)** (pg/mL)
- **Albumin** (g/dL)
- **Creatinine** (mg/dL)
- **Insulin-like Growth Factor-1 (IGF-1)** (ng/mL)
- **Adiponectin** (µg/mL)
- **Leptin** (ng/mL)

E. Bone Turnover Markers (BTMs)

Bone Formation Markers:

- **Procollagen type 1 N-terminal propeptide (P1NP)**
- **Osteocalcin (OC)**
  Bone Resorption Markers:
- **C-terminal telopeptide of type I collagen (CTX)**
- **Tartrate-resistant acid phosphatase 5b (TRAP5b)**

F. Estimation of Sarcopenia

Following the **EWGSOP2** criteria:

- **GripStrength** (kg)
- **Chair Stand Test** (seconds to complete 5 repetitions)
- **Gait Speed** (m/s over 4 meters)

G. QuestionnairesAdministered

- **SARC-F Questionnaire** (screening tool for sarcopenia)
- **Short-Form 36 (SF-36) Health Survey** (assessing quality of life across eight domains)

- **Perceived Stress Scale (PSS-14)** (evaluation of perceived psychological stress)

4.6 Description of the measurement methods to be used
Reference only to specialized techniques/methods (those beyond the level of the average reader/judge of the research protocol).
Word count range: ***50 – 500***

Whole-body DXA is a validated imaging method that quantifies total and regional fat mass, lean mass, and bone mineral content using low-dose X-rays. It enables precise assessment of body composition and sarcopenia-related metrics such as appendicular lean mass and RSMI.

Trabecular Bone Score (TBS) is a textural index derived from lumbar spine DXA images that reflects bone microarchitecture. Calculated via dedicated software (e.g., TBS iNsight®), TBS complements BMD by providing additional insight into bone quality and fracture risk, particularly in populations where BMD alone may underestimate skeletal fragility.

4.7 Outcomes
Clear listing of variables set as primary and secondary endpoints. Their equivalence with the research hypotheses. If there are more than one, they are to be numbered. Why were they chosen as such?

Primary Endpoints:

- **Changes in Bone Resorption Marker CTX at 3 months:** The primary objective is to evaluate the changes in CTX, a key marker of bone resorption, between the liraglutide and tirzepatide groups at 3 months.

Secondary Endpoints:

- Changes in Body Weight and BMI at 6 months: The secondary objective is to compare body weight and BMI changes between the two groups over 6 months, reflecting the overall effectiveness of the treatments.
- Changes in Bone Mineral Density (BMD) at 6 months: This endpoint aims to assess the impact of the treatments on bone density, crucial for understanding their effects on bone health.
- Body Composition Changes at 6 months: Changes in fat mass and free fat mass will be measured at 6 months to evaluate the treatments' effects on body composition.

<u>Rationale for Choice of Endpoints:</u>

CTX is the primary endpoint because it directly measures bone resorption, a key aspect of bone metabolism. The secondary endpoints—body weight, BMI, BMD, and body composition—will provide additional insights into the overall effects of tirzepatide and liraglutide on both bone health and body composition in patients with obesity.

4.8 Subgroupanalysis
Has it been decided from the beginning of the study that some of the groups (e.g., patient group) will be divided based on certain specific characteristics (e.g., age, gender)?

The division into two groups is based on the collaborative decision between the physician and the patient regarding the optimal therapeutic intervention for weight loss.

4.9 Description of the statistical methods that will be applied to assess the primary and secondary outcomes
Reference to basic and more extensive analysis of specific statistical tests (e.g., bioinformatics, cost-benefit analyses, model building). State their equivalence with the main outcome results. The statistical methodology to be applied should be adequately described in basic research protocols.
Word count range: ***250 – 500***

Statistical Methods

All statistical analyses will be conducted using SPSS (version 27 or later) and/or R software. A two-sided p-value < 0.05 will be considered statistically significant. Prior to analysis, data will be assessed for completeness, and missing data will be addressed using appropriate imputation techniques (e.g., multiple imputation) if missingness exceeds 5%. All continuous variables will be tested for normality using the Shapiro–Wilk test. Data will be presented as mean ± standard deviation (SD) for normally distributed variables and median with interquartile range (IQR) for non-normally distributed data. Categorical variables will be summarized as frequencies and percentages.

For the **primary outcome**, which is the comparative change in serum CTX levels at 3 months between the tirzepatide and liraglutide groups, the analysis will involve an independent-samples t-test if normality is confirmed, or the Mann–Whitney U test for skewed data. Baseline CTX levels will be included as covariates to account for inter-individual variability using an ANCOVA model. Sensitivity analyses will be conducted to adjust for the magnitude of weight loss and baseline BMD.

Secondary outcomes include:
(i) Changes in additional bone turnover markers (P1NP, TRAP5b) at 3 and 6 months, and CTX at 6 months. These will be analyzed using repeated measures ANOVA or linear mixed-effects models to evaluate time × treatment group interactions, adjusting for confounders such as age, sex, baseline values, and percent weight change.
(ii) Comparisons of changes in BTMs between treatment groups at each time point will also be performed using ANCOVA models.
(iii) Changes in BMD at lumbar spine, femoral neck, and total hip, as well as body composition metrics (total fat mass, lean mass, RSMI), will be evaluated using mixed-effects models with random intercepts for individuals. This approach accounts for intra-subject correlations due to repeated measures over time (baseline, 6, and 12 months).
(iv) Between-group comparisons of BMD and body composition changes will be adjusted for the degree of weight loss, age, and menopausal status (where applicable).

To assess the relationship between changes in BTMs and body composition, correlation analyses will be performed using Pearson or Spearman coefficients depending on data distribution. Subgroup analyses may be conducted based on sex, menopausal status, and magnitude of weight loss (e.g., >10% vs. ≤10%).

Additional exploratory analyses may include logistic regression to determine predictors of significant bone loss or sarcopenia onset, incorporating variables such as baseline BMD, adipokine levels (leptin, adiponectin), and inflammatory markers if available.

Statistical assumptions will be checked in all models, and interaction terms will be included where

biologically plausible. If significant heterogeneity of treatment effect is observed, stratified analyses will be conducted. Graphical representation of longitudinal data (e.g., line plots with confidence intervals) will be used to visualize trends and group differences over time.

4.10 Calculation of sample size

Why were the study groups chosen to be of this size? The calculation needs to have been made based on the primary research hypothesis and the corresponding main outcome(s). How is the expected difference between groups documented (references)? In the event of non-existence of data, these should be drawn from related fields. In the few cases where there is a complete lack of data, the conduct of a pilot study needs to precede.
(https://s4be.cochrane.org/blog/2017/07/31/pilot-studies/). Which method and software were used? It is recommended that an image (print screen) be attached with the calculation of the sample size from the program used. Have drop-outs and subgroup analysis been taken into account?

The sample size calculation for this study was based on the least significant change (LSC) of the C-terminal telopeptide of type I collagen (CTX) marker in serum at three months. Using this threshold, we determined that a total of 32 patients per arm would be required to achieve adequate statistical power (80% statistical power (false negative), 5% error (false positive). With anticipated drop-out rate of 10%, about 36 participants/ per group should be randomized, ensuring sufficient power to detect meaningful changes in CTX levels while accounting for potential variability in the biomarker response.

4.11 Site where the study will be conducted

Is the study site appropriate (indicatively: is there equipment, patient recruitment, available expertise)? Will part of the study be required to be conducted in additional settings (clinics, laboratories, private or public settings)? Confirmation of the receipt of the relevant licenses and the signing of the cooperation agreements.

The study will be conducted in three independent endocrinology centers operating within tertiary care hospital settings, which possess the necessary infrastructure and expertise for the implementation of the study. Recruitment of participants is considered feasible due to the regular follow-up of patients with obesity in these centers. All required approvals will be obtained prior to the initiation of the study.

4.12 Additionalresearchers

Will the collaboration of additional researchers be required apart from those mentioned? Their participation is to be justified.

No

4.13 Ethics

For all types of studies other than meta-research an approval by an Independent Research Committee is required.Please, mention the relevant Committee. Are additional permissions required (indicatively: Hospital Scientific Council, Veterinary Service, National Medicines Agency, National Authority for Medically Assisted Reproduction)?
How will the personal data of the participating groups be secured (General Data Protection Regulation (EU) 2016/679 (GDPR))?
Who will have access to the database(s) and for how long? How will confidentiality be ensured?
The "Participant Information and Consent Form" should be submitted as a supplementary document to this "Research Protocol".

The study has been approved by the Independent Research Ethics Committee of one participating hospital, while approval from the corresponding committees of the other two participating centers is currently pending.

Regarding personal data protection, the study is in line with the General Data Protection Regulation (GDPR)

(EU) 2016/679. All personal and medical data of the participants will be anonymized and securely stored in encrypted databases. Only authorized study personnel, including the research team and designated data managers, will have access to the data. Access will be strictly limited to the duration of the study and will be subject to confidentiality agreements.

All participants will receive a "Participant Information and Consent Form" that outlines the details of the study, including their rights and data privacy considerations. This document will be submitted as a supplementary part of the study protocol.

Section 5: Feasibilityofimplementation

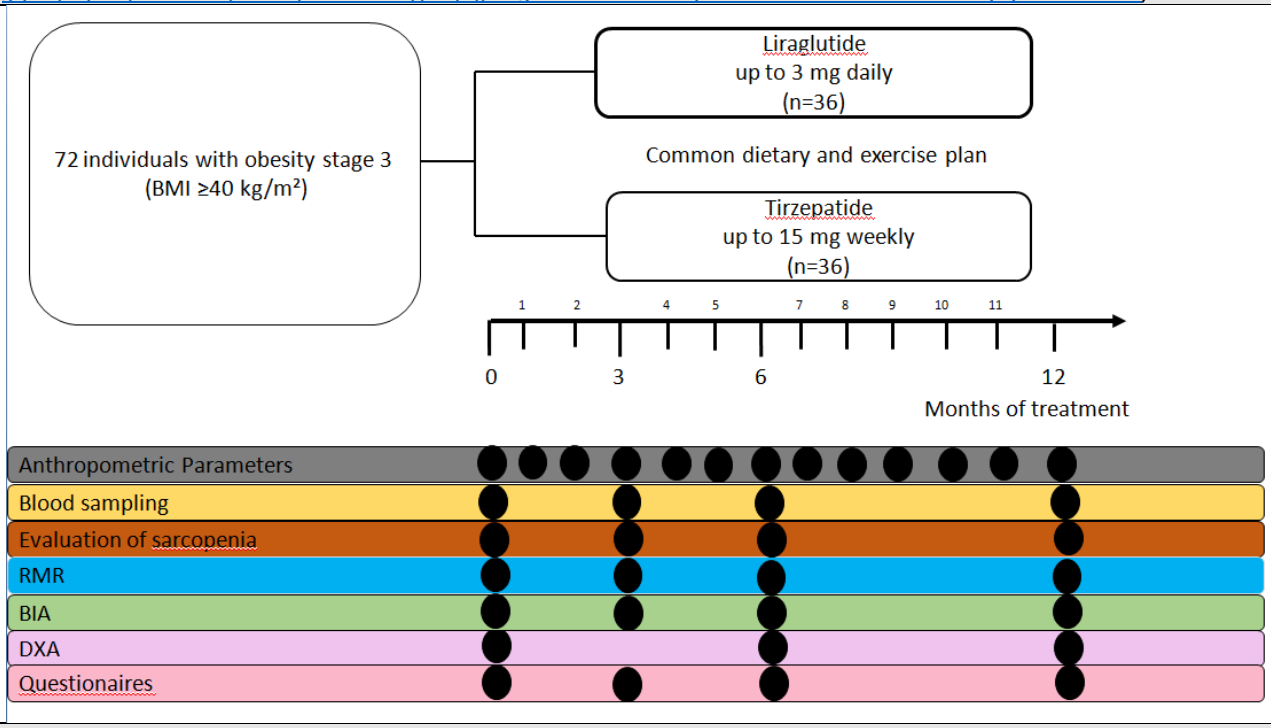
5.1 Estimated completion time based on the number of cases available

Time required from initiation to completion of recruitment of study subjects. Documentation with relevant data.

The recruitment period for individuals with obesity is expected to last up to one year, followed by an additional one-year period of close follow-up.

5.2 Detailed completion schedule

The drawing up of a Gantt chart is advisable(https://www.gantt.comandhttps://support.microsoft.com/el-gr/topic/παρουσίαση-δεδομένων-σε-γράφημα-gantt-στο-excel-f8910ab4-ceda-4521-8207-f0fb34d9e2b6).



5.3 Budget and funding sources

A budget plan is to be submitted stating the costs required (indicatively: equipment needing to be purchased, supplies, software, shipping costs for samples, publication fees). How are additional costs due to be covered?

1. Equipment

Jamar Hydraulic Hand Dynamometer

One-time purchase: €420 per device

Quantity: 3 (one per center)

Total: €1,260

2. Bone Turnover Markers (CTX, P1NP, TRAP5b)

(Per participant × 4 time points)

Cost per set: €50

72 participants × 4 = 288 samples

Total: €14,400

3. Sample Storage and Processing

Cost per sample: €10

72 participants × 4 = 288 samples

Total: €2,880

4. DXA Scan Depreciation Cost

Cost per scan: €10

72 participants × 3 scans = 216 scans

Total: €2,160

Total Cost: €20,700

The requested funding will cover the majority of the direct research costs, while the remaining expenses will be supported by internal resources of the participating centers.

Section 6: Publication strategy and bibliography

6.1 Publications resulting from the research project that will be undertaken

To which journals will the publications be submitted?
What is the publication strategy (Will there be review publications? Will there be primary publications? Will there be technique development publications?).
What are the priorities (where and when will the publications resulting from the research work be submitted)?

The findings from the research project will be submitted to high-impact, peer-reviewed journals that specialize in obesity and bone metabolism. The specific journals to which the publications will be submitted include Bone, The Journal of Clinical Endocrinology & Metabolism (JCEM), Diabetes, Obesity and Metabolism, Frontiers in Endocrinology, and Osteoporosis International.

Publication Strategy:

Primary Publications: The majority of publications will focus on original research, presenting the primary findings regarding the effects of tirzepatide versus liraglutide on bone turnover markers, bone mineral density, and body composition parameters in individuals with obesity. These papers will aim to contribute new knowledge to the field, specifically addressing gaps in the understanding of how weight-loss medications influence bone metabolism.

Review Publications: There may be a review publication that provides a comprehensive summary of the current understanding of GLP-1 receptor agonists and their impact on bone health, including tirzepatide and liraglutide. This publication will aim to synthesize existing literature and provide a broader context for the findings of the current study.

Priorities:

The priority will be to submit primary research articles first, with the goal of contributing to the growing body of evidence regarding the effects of GLP-1 and dual GLP-1/GIP receptor agonists on bone health. The publication of primary research will be prioritized for submission within 6-12 months of completing data collection and analysis.

Review articles will be submitted before the primary research articles.

6.2 References

Formatting as suggested by the New England Journal of Medicine:
(https://paperpile.com/s/the-new-england-journal-of-medicine-citation-style/).
Focus on recent important publications.
References should refer to specific points within the text.
Number of bibliographic references: ***20 - 40***

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Appendix 1: Budget

id	**Expenses** *Supplies, remuneration, travel, publications*	**Amount (in Euros)**
1.	Equipment Jamar Hydraulic Hand Dynamometer One-time purchase: €420 per device Quantity: 3 (one per center)	1.260
2.	Bone Turnover Markers (CTX, P1NP, TRAP5b) (Per participant × 4 time points) Cost per set: €50 72 participants × 4 = 288 samples	14.400
3.	Sample Storage and Processing Cost per sample: €10 72 participants × 4 = 288 samples	2.880
4.	DXA Scan Depreciation Cost Cost per scan: €10 72 participants × 3 scans = 216 scans	2.160
	Total	**20.700**