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Hansen, Morten S.; Wölfel, Eva M.; Jeromdesella, Shakespeare; Møller, Jens Jakob K.; Ejersted, Charlotte; Jørgensen, Niklas R.; Eastell, Richard; Hansen, Stinus G.; Frost, Morten

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Once-weekly semaglutide versus placebo in adults with increased fracture risk: a randomised, double-blinded, two-centre, phase 2 trial



Morten S. Hansen,^{a,b,*} Eva M. Wölfel,^b Shakespeare Jeromdesella,^{a,b} Jens-Jakob K. Møller,^c Charlotte Ejersted,^a Niklas R. Jørgensen,^{d,e} Richard Eastell,^f Stinus G. Hansen,^g and Morten Frost^{a,b,h}



^aDepartment of Endocrinology, Odense University Hospital, Denmark

^bClinical Institute, Faculty of Health Sciences, University of Southern Denmark, Denmark

^cOpen Patient Data Explorative Network (OPEN), Odense University Hospital, Denmark

^dDepartment of Clinical Biochemistry, Rigshospitalet, Denmark

^eDepartment of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

^fDivision of Clinical Medicine, University of Sheffield, Sheffield, United Kingdom

^gDepartment of Diabetes and Endocrinology, Esbjerg Hospital, University Hospital of Southern Denmark, Denmark

^hSteno Diabetes Centre Odense, Odense University Hospital, Denmark

Summary

Background Previous studies have indicated that glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RAs) may enhance bone formation and have neutral or beneficial effects on fracture risk. We evaluated the effect of the GLP-1RA semaglutide on the bone formation marker Procollagen type I N-terminal propeptide (PINP) in adults with increased fracture risk.

Methods This randomised, placebo-controlled, double-blinded, phase 2 clinical trial was conducted at two public hospitals in Denmark. We enrolled 64 men and women with increased fracture risk based on a T-score < -1.0 at the total hip or lumbar spine and/or low-energy fracture within three years of recruitment. Participants were randomised (1:1) to receive once-weekly subcutaneous semaglutide 1.0 mg or placebo. The primary outcome was changes in plasma (P)-PINP from baseline to week 52. Primary and safety outcomes were assessed and evaluated for all participants. This trial is complete and registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04702516), NCT04702516.

Findings Between March 24 and December 8, 2021, 55 (86%) postmenopausal women and nine men with a mean age of 63 years (SD 5.5) and BMI of 27.5 kg/m² (SD 4.5) were enrolled. There was no effect on changes in P-PINP from baseline to week 52 between the two groups (estimated treatment difference (ETD) semaglutide versus placebo 3.8 µg/L [95% CI -5.6 to 13.3]; p = 0.418), and no difference in P-PINP levels between groups at week 52 (semaglutide 64.3 µg/L versus placebo 62.3 µg/L [95% CI -10.8 to 15.0]; p = 0.749). The secondary outcomes showed higher plasma levels of bone resorption marker Collagen type I cross-linked C-terminal telopeptide (P-CTX) in the semaglutide group than in the placebo group (ETD 166.4 ng/L [95% CI 25.5–307.3]; p = 0.021). Compared to placebo, lumbar spine and total hip areal bone mineral densities (aBMD) were lower in the semaglutide group after 52 weeks (ETD lumbar spine -0.018 g/cm³ [95% CI -0.031 to -0.005]; p = 0.007; ETD total hip -0.020 g/cm² [95% CI -0.032 to -0.008]; p = 0.001). Treatment differences in femoral neck aBMD were not observed ([95% CI [-0.017 to 0.006]; p = 0.328). Further, body weight was lower in the semaglutide group than in the placebo group after 52 weeks (ETD -6.8 kg [95% CI -8.8 to -4.7]; p < 0.001). Thirty-one [97%] in the semaglutide group and 18 [56%] in the placebo group experienced at least one adverse event, including four serious events (two in each group). No episodes of hypoglycaemia or deaths were reported.

Interpretation In adults with increased fracture risk, semaglutide once weekly did not increase bone formation based on the bone formation marker P-PINP. The observed increase in bone resorption in the semaglutide group may be explained by the accompanying weight loss.

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*Corresponding author. Department of Endocrinology, Odense University Hospital, Denmark.
E-mail address: morten.steen.hansen@rsyd.dk (M.S. Hansen).

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Keywords: Semaglutide; Weight loss; Bone turnover; Cortical bone

Research in context

Evidence before this study

We searched PubMed for articles including randomised controlled trials and meta-analyses on the effect of GLP-1 receptor agonists on bone using the following terms: “semaglutide” OR “lixisenatide” OR “liraglutide” OR “dulaglutide” OR “exenatide” OR “GLP-1RA” AND “bone”. The retrieved articles including their reference lists were reviewed for relevant information. We did not identify studies that were designed to assess the effects of semaglutide on skeletal health. A recent meta-analysis based on nine RCTs showed neutral effects of semaglutide on fracture risk in individuals with type 2 diabetes (relative risk reduction, 0.66; 95% CI 0.13–3.41). Another meta-analysis based on 38 RCTs showed that GLP-1 receptor agonist treatment reduced fracture risk in patients with type 2 diabetes as compared to placebo or other anti-diabetic drugs when treatment duration was longer than 52 weeks (odds ratio, 0.71; 95% CI 0.56–0.91). One RCT reported that liraglutide increased bone formation during weight maintenance in obese women without diabetes, while one RCT reported that liraglutide preserved bone mass during weight loss in patients with type 2 diabetes.

Added value of this study

In adults with increased fracture risk, subcutaneous semaglutide 1.0 mg once-weekly did not increase the bone formation marker P-PINP compared with placebo. Assessment of secondary outcomes showed that bone resorption was increased in the semaglutide group compared

with the placebo group. In addition, bone mass at the lumbar spine and total hip, and tibial cortical thickness were lower, and body weight reduced after 52 weeks in the semaglutide group compared with the placebo group. These changes in bone turnover and bone mass may be explained by lower mechanical loading following weight loss in the semaglutide group or by direct effects of semaglutide on bone.

Implications of all the available evidence

Considering that weight loss is associated with bone loss, there remains a scarcity of studies on the skeletal impact of treatment with GLP-1 receptor agonists, which is commonplace in type 2 diabetes and increasingly used in obesity. Meta-analyses suggest that GLP-1 receptor agonists such as semaglutide are not associated with an increased fracture risk in patients with type 2 diabetes. Albeit based on a limited number of investigations, current evidence suggests that the effect of GLP-1 receptor agonists on bone depends on dosage, duration of use, and the study population e.g., presence of type 2 diabetes or preceding weight loss. While short exposure to GLP-1 receptor agonists may not increase bone resorption, this may be observed with extended use or with more potent GLP-1 receptor agonists, such as semaglutide, possibly as an adaption to lower body weight. Further investigations of the skeletal effects, including fracture risk, of long-term treatment with currently used GLP-1 receptor agonists are warranted.

Introduction

Bone is a dynamic tissue that is degraded and formed in a tightly controlled and coupled process known as bone remodelling.¹ With advancing age, bone resorption continues whereas bone formation declines resulting in lower bone mass which may lead to osteoporosis and a higher risk of fragility fractures.² Contemporary therapeutic strategies for fracture prevention include interventions designed to improve bone mass and reduce fracture risk. These therapeutics target the continuous turnover of the skeleton by impairing bone resorption, increasing bone formation, or achieving both effects simultaneously.³ Yet, the clinical applicability of these treatments can be limited by contraindications, such as impaired kidney function and risk of or established cardiovascular disease, and long-term use may be hampered by diminished effects or rare but severe adverse events.⁴ Given the escalating global incidence of bone fractures,⁵ it is imperative to identify novel therapies for fracture prevention.

Several gut-secreted hormones, including glucagon-like peptide-1 (GLP-1), regulate bone turnover.⁶ GLP-1 mainly promotes insulin secretion in response to nutrient intake and promotes satiety.⁷ Clinically, the ability of GLP-1 receptor agonists (GLP-1RAs) to increase insulin secretion during hyperglycaemia is used to treat type 2 diabetes (T2D). Furthermore, some GLP-1RAs, including liraglutide and semaglutide, are also approved as treatments of obesity as they decrease appetite, leading to lower food intake. It has been suggested that GLP-1RAs may also exert favourable effects on bone. In preclinical studies, liraglutide enhanced human osteoblastogenesis *in vitro*.⁸ Moreover, liraglutide and exendin-4, another GLP-1RA, enhanced bone formation and mitigated bone loss in non-diabetic rodent models of osteoporosis.^{8,9} While these findings support that GLP-1RAs increase bone formation, clinical investigations have only provided circumstantial evidence of beneficial effects on bone mass and fracture risk. In a placebo-controlled trial, liraglutide increased

plasma levels of the bone formation marker Procollagen type I N-terminal propeptide (PINP) when used to maintain body weight after calorie restriction-induced weight loss in obese women.¹⁰ Despite that weight loss is associated with accelerated bone loss,¹¹ and increases the risk of fractures in patients with T2D when obtained by lifestyle interventions,¹² liraglutide reduced body weight without adversely affecting bone mineral density (BMD) in patients with T2D.¹³ These findings indicate that GLP-1RAs may advance bone formation and protect against weight loss-induced bone loss in T2D. Accordingly, register-based data from Denmark showed that GLP-1RAs have neutral effects on fracture risk in patients with T2D.¹⁴ Moreover, a meta-analysis of randomised controlled trials in patients with T2D showed that GLP-1RAs were associated with lower fracture risk than other anti-diabetic drugs or placebo if treatment exceeded 52 weeks.¹⁵ Although the current knowledge of the effects of GLP-1RA treatment on bone in individuals with or without diabetes is limited, preclinical and clinical studies indicate that GLP-1RA may prevent bone loss during weight loss, possibly by promoting bone formation. This study aimed to investigate if semaglutide increases the bone formation marker PINP and exerts beneficial effects on bone mass, microstructure, and strength in men and women with increased fracture risk but without diabetes.

Methods

Study design

This multicentre, randomised, double-blinded, placebo-controlled, phase 2 study was conducted at two hospital departments in The Region of Southern Denmark. Ethical approval was obtained by the regional ethics committee (reference number S-20200048) and the trial was conducted in accordance with the Declaration of Helsinki guidelines and good clinical practice.

Participants

Participants were recruited from outpatient clinics at the study sites and by internet advertisements. Eligible participants were male and female, aged 40–85 years (women had to be menopausal for at least five years prior to inclusion) with increased risk of bone fracture based on a T-score below -1.0 at the total hip or lumbar spine and/or low-energy fracture within three years prior to screening. Key exclusion criteria included type 1 or 2 diabetes, body mass index (BMI) < 20 kg/m², and use of antiresorptive or bone anabolic drugs within 12 months before screening. Full eligibility criteria are provided in [Appendix](#). Participants were instructed to continue their habitual lifestyle concerning exercise and diet but were ensured to have a daily intake of at least 800 mg calcium and 20 µg vitamin D. Adverse events were assessed at each study visit and at the end of the trial. Participants were provided with a blood glucose

meter and instructed to measure blood glucose levels if symptoms that could be caused by hypoglycaemia occurred. Treatment adherence was assessed by manual counting and inspection for drug remains in the used injection pens. All participants provided written informed consent prior to enrolment.

Randomisation and masking

Following completion of all screening procedures, including meeting at least one inclusion criteria and none of the exclusion criteria, participants who consented to randomisation were booked for a baseline visit. At the baseline visit, participants were randomised before receiving any interventions (semaglutide or placebo). Randomisation was done at the primary investigation site (Odense University Hospital) based on a printed randomisation list, which contained consecutive numbers that corresponded to semaglutide (“A”) or placebo (“B”), respectively. The randomisation list was provided by Novo Nordisk, Denmark and was stored in a locked safe at the primary investigation site. Participants were randomly assigned (1:1) to the next available group (semaglutide or placebo) on the randomisation list in block sizes of four using a secure REDCap® database. The REDCap® database provided the next available number (group) on the randomisation list to an investigator masked to group assignment. The investigator then informed an unmasked staff person about the allocated number on the randomisation list. The randomisation list was then accessed by at least two unmasked personnel who picked up the allocated intervention and handed it to the masked investigator. Access to the REDCap® database was restricted to authorised personnel who were masked to group assignment. Data handling was done in the secure REDCap® database by registering study relevant information in predefined records by authorized personnel masked to allocation. Participants, investigators, sponsor, sponsor representative, trial site staff, and statisticians analysing the data were masked to group assignment. Semaglutide and placebo were provided by Novo Nordisk, Denmark in prefilled pens that were visually identical, used the same syringes, and contained the same volume to preserve masking.

Procedures

Participants allocated to the semaglutide group received semaglutide once-weekly subcutaneously, and those allocated to the placebo group received placebo (saline) once-weekly subcutaneously for 52 weeks. Semaglutide doses used for this study corresponded to doses used to treat T2D. Dosages were escalated from an initial dose of 0.25 mg to 0.5 mg after 4 weeks, and from 0.5 mg to 1.0 mg after another four weeks. At any timepoint, dosage could be reduced to the highest tolerable dose in case of intolerable side effects. The final dosage was administered no later than six days before end-of study measurements were done.

To assess participant eligibility, a screening visit was done to obtain health information, such as medical history, height, weight, and HbA_{1c}, and to perform dual-energy X-ray absorptiometry (DXA) scans. For those eligible, a baseline visit was scheduled where participants met in a fasting state between 8 and 10 a.m., had blood samples taken, underwent high-resolution peripheral quantitative computed tomography (HR-pQCT) scans and microindentation by OsteoProbe[®], and were randomised. Another five on-site visits were scheduled after 4, 12, 26, 39, and 52 weeks, respectively. At these visits, blood samples were collected in the morning after an overnight fast, and vital signs including pulse and blood pressure were measured or reported by the participant in case of home measurement. Weight was measured after 12, 26, 39, and 52 weeks, respectively. HbA_{1c} was measured after 26, 39, and 52 weeks. At last visit, follow-up tests including DXA scans, height, weight, HR-pQCT scans, and microindentation were performed for all participants. A total of 21 participants underwent transiliac bone biopsy following tetracycline labelling as described below at last visit. An end-of-trial follow-up phone call for safety assessments was scheduled six weeks after last visit (week 52).

P-PINP was measured using the IDS-iSYS intact PINP assay and P-CTX was measured using the IDS-iSYS CTX (CrossLaps[®]) assay (Immunodiagnostic Systems, plc, Tyne and Wear, UK). Both assays are chemiluminescence immunoassays and were carried out on a dedicated automated analyser, iSYS (Immunodiagnostic Systems) according to the manufacturer's instruction. For each assay the sample aliquots were kept frozen at -80 °C until the day of analysis. None of the samples had previously been thawed, and all analyses were performed immediately after thawing the samples. All samples were analysed using one single batch of each assay. Assay performance was verified using the manufacturers' control specimens. The intermediary precisions expressed as coefficients of variation for P-PINP were 5.4% (18.96 µg/L), 6.5% (48.48 µg/L), and 6.1% (122.10 µg/L). For P-CTX the intermediary precisions were 5.3% (at P-CTX concentration 213 ng/L), 3.4% (869 ng/L), and 3.5% (2113 ng/L).

BMD at the lumbar spine (L1-L4), total hip, and femoral neck, and whole-body scans to assess body composition and fat mass distribution were measured using DXA (Hologic, Inc., Marlborough, MA, USA) by trained laboratory staff using a standard protocol. While three different DXA machines were used in this investigation, each participant was scanned on the same DXA machine at week 52 that was used at baseline. The T-score for each participant was calculated as the difference between the participant's BMD and the mean from a reference population of same sex, divided by the standard deviation of that reference population.¹⁶ The coefficient of variation (CV) for both spine and hip BMD is approximately 1%.

A second-generation HR-pQCT (XtremeCT II, Scanco Medical, Brüttisellen, Switzerland) was used to acquire three-dimensional bone microarchitecture and volumetric BMD (vBMD) at tibia and radius. All participants were scanned on the same HR-pQCT machine. Scans were performed by trained laboratory staff at the primary investigation site. The scanner operated at 68 kVp and 1470 µA. At both tibia and radius, the scan comprised 168 slices, constructing a 3D image of the bone axillary with a length of 10.2 mm. A standard carbon fibre caste was used to immobilize the arm or leg, and a scout view was used to define the measurement region that started at 9.5 mm and 22.5 mm from the endplate of the radius and tibia, respectively. The quality of each scan was immediately assessed by the operator using a 1–5 grade scale, as suggested by the manufacturer. The participant was re-scanned in case of poor scan quality (grade 4–5). Reconstructed images were analysed according to the manufacturer's standard protocol. For assessment of microarchitectural outcomes, images were filtered using a Gaussian filter: sigma 0.8, support 1.0. Fixed bone volume segmentation thresholds were used to extract trabecular and cortical bone (320 and 450 mg hydroxyapatite (HA)/cm³, respectively). The following outcomes were assessed at tibia and radius: total vBMD (mgHA/cm³); trabecular bone volume per tissue volume (BV/TV, %), which was calculated from the trabecular volume density; trabecular thickness (Tb.Th, mm) measured directly as the inter-trabecular distance using a distance transformation method; cortical thickness (Ct.Th, mm) measured directly as the endosteal-periosteal distance using a distance transformation method; and cortical porosity (Ct.Po, mm) measured as void cortical volume divided by total cortical volume. Measurements were assessed automatically by software from Scanco Medical after being checked for potential errors in regions of interest by the technician who obtained the scan. µFE analysis (Scanco Medical FE software version 1.13) was used to estimate failure load (estimated bone strength) (N).

Bone material properties were estimated by impact microindentation using the OsteoProbe[®] (Active Life Technologies, Santa Barbara, CA, USA), which measures bone material strength index (BMSi) on the anterior surface of the tibia plateau as previously described in internationally recognized recommendations for using the OsteoProbe[®].¹⁷

Prior to initiation of the trial, a minimum of 20 participants were planned to undergo transiliac bone biopsy. All 64 participants were informed of the procedure, and 21 participants volunteered to undergo double labelling with tetracycline administered orally using tetracycline hydrochloride 250 mg three times daily for three days twice with a 14-days wash-out period between. Biopsies were collected prior to unblinding using either a 4.0 or 7.0 mm diameter core obtained

across the iliac crest.¹⁸ Within an hour of the procedure, the specimens were separately fixed under vacuum for 4 h and stored in 90% phosphate buffer solution/10% formalin (4%) at 4 °C until the samples were transferred to Holt's solution (sucrose 150 g, acacia gum 5 g, Thymol 75 mg and sterile water 500 ml) and dehydrated overnight. Following dehydration, samples were kept at -20 °C with Tissue-Tek® O.C.T. (Sakura Finetek, USA). Subsequently, 8.0-µm-thick sections were cut using a cryostat (Thermo Scientific Microm HM 560), mounted with VECTASCHIELD® with DAPI (Bio-nordika Denmark) for detailed histomorphometric analyses, which were assessed by authorized personnel blinded to group assignment and patient characteristics.

Outcomes

The primary endpoint was the percentage change in the bone formation marker P-PINP from baseline to week 52. Assessments of changes in P-PINP were also eligible between baseline and weeks 4, 12, 26, and 39.

The bone resorption marker P-CTX was a secondary endpoint that was assessed at similar timepoints as P-PINP. Other secondary endpoints were changes in BMD at the lumbar spine, total hip, and femoral neck, and fat mass distribution assessed as changes in total body mass fat, total body lean mass and the ratio between percentage gynoid fat and percentage android fat. Other secondary endpoints included changes in BMSi; changes in tibial and radial vBMD, cortical and trabecular microarchitecture, including Tb.Th, Tb BV/TV, Ct.Th, Ct.Po, and estimated bone strength; and changes in body weight. Another secondary endpoint was bone formation rate (BFR) assessed by multiplying the mineral apposition rate (MAR) with the mineralized surface per bone surface (MS/BS) using dynamic histomorphometry at week 52.

All adverse events were interpreted and documented by the investigators based on the possibility that a given adverse event was related to semaglutide. If an adverse event was deemed to be related to semaglutide, the adverse event was considered and documented as an adverse reaction.

Statistical analysis

The sample size calculation was based on the assumption that individuals treated for 52 weeks with 1.0 mg semaglutide or highest tolerated dose once weekly compared to placebo, would obtain an increase in P-PINP with at least 16%. This effect size was based on previous reported effects of another GLP-1RA, liraglutide, which significantly increased P-PINP with 16% corresponding to a difference of 7 ng/L (SD 9.3) compared to placebo after 52 weeks treatment in obese women.¹⁰ Assuming 1:1 randomisation and based on two-sample *t* tests with a two-sided α -value of 0.05, a sample size of 58 completers (29 in each group) was estimated to provide at least 80% power to detect the

expected effects. Assuming a 10% drop-out, the study population included 64 participants. Analyses of secondary outcomes were not adjusted for multiple testing.

Efficacy outcomes were assessed using intention-to-treat analysis, and included all participants who were randomised independent of dosage. Safety outcomes were assessed and analysed in all participants who received at least one dose of any study intervention. Efficacy and safety outcomes were assessed in all 64 participants by original assigned groups.

The primary endpoint was analysed using multiple linear mixed models. This was implemented as a mixed model for repeated measures (MMRM) on baseline and all follow-up time points using an unstructured marginal covariance matrix on the patient level. The systematic part of the model consists of treatment and time, and the treatment-time interaction. The model further adjusted for the known prognostic variables age and gender (m/f). The model was fit by restricted maximum likelihood. Confidence intervals and tests employed the Kenward-Roger approximation by its correction to the standard error matrix of the fixed effects and associated degrees of freedom. Model validation was performed by visual inspection of QQ-plots of standardized residuals along with plots of standardized residual versus fitted values to assess variance homoscedasticity. The primary outcome was assessed as the difference between the groups from baseline to the last visit. Efficacy analyses for secondary outcome variables P-CTX and weight were performed in the same manner as for the primary outcome. When there was only one follow-up measurement, the proposed analysis coincides with the ANCOVA model. When at least a baseline and one follow-up measurement were available, parameters were assessed as the difference between changes from baseline in the semaglutide group compared to the placebo group in absolute values (estimated treatment difference (ETD)) or as percentage changes (relative ETD). When only one measurement was available, with the exemption of baseline characteristics, data was analysed using an unpaired *t*-test. We analysed our primary and secondary endpoints based on all randomly assigned participants who completed at least six months of the study and had evaluable pharmacodynamic data.

Statistical analyses were performed using Stata version 18.0 (StataCorp LLC, Texas, USA) or GraphPadPrism version 8.9 (GraphPad Software, LLC, Boston, USA). There was no data monitoring committee. The trial is closed and completed, and is registered with [ClinicalTrials.gov](https://clinicaltrials.gov), number NCT04702516.

Role of the funding source

The sponsor of this study was Odense University Hospital, Denmark. The sponsor representative, Morten Frost, was responsible for trial design, preparing the trial protocol, completion of the trial, the statistical

analysis plan, and analysing the results. The funder of the study and the provider of trial products had no role in study design, data collection, data analysis, data interpretation, or writing of the study report.

Results

Between March 24 and November 24, 2021, a total of 113 individuals underwent screening. Forty-nine were excluded from the study, resulting in the enrolment of 64 participants (Fig. 1). The study was completed on February 2, 2023. Participants were randomly assigned to semaglutide (n = 32) or placebo (n = 32). Predominantly, participants were female (n = 55 [86%]) with a mean age of 63.1 years (46–75, SD 5.5), and a mean BMI of 27.7 kg/m² (21–39, SD 4.5). Forty-seven (73%) participants were included based on a T-score between –1.0 and –2.5, two (3%) were included based on low-energy fracture within three years prior to screening and a T-score > –1.0, and 15 (24%) were included based on a T-score < –2.5. A total of eight participants reported low-energy fracture within three years prior to screening. Randomisation achieved balance in baseline characteristics between groups (Table 1).

There was no difference in changes in the bone formation marker P-PINP from baseline to week 52

between the semaglutide and placebo groups (ETD 3.84 µg/L [95% CI –5.6 to 13.3]; p = 0.418; Fig. 2A, Table 2). This corresponded to a relative ETD of 17.3% ([95% CI –9.0 to 43.6]; p = 0.193; Fig. 2B, Table 2). Between weeks 26 and 39, P-PINP increased in the semaglutide group from 54.3 µg/L [95% CI 46.9–61.6] to 65.4 µg/L ([95% CI 55.4–75.4.3]; p = 0.003; Supplementary Table S1) and remained elevated at week 52 (p = 0.004). In the placebo group, P-PINP increased between week 26 and 52 from 54.8 µg/L [95% CI 44.8–64.8] to 62.3 µg/L ([95% CI 51.6–72.9]; p = 0.031; Supplementary Table S1). No significant differences in P-PINP were observed between the groups at any time points.

The bone resorption marker CTX increased from baseline to week 52 in the semaglutide group compared with the placebo group (ETD 166.4 ng/L [95% CI 25.5–307.3]; p = 0.021; Fig. 2C, Table 2). This corresponded to a relative ETD of 54.8% ([95% CI 17.5–92.1]; p = 0.005; Fig. 2D, Table 2). The increase in P-CTX in the semaglutide group was observed after week 26, with P-CTX increasing from 440.1 ng/L [95% CI 352.8–527.5] at week 26–518.4 ng/L ([95% CI 422.8–613.9]; p = 0.003; Supplementary Table S1) at week 39 and further increasing at week 52–590.6 ng/L ([95% CI 471.5–709.7]; p < 0.001).

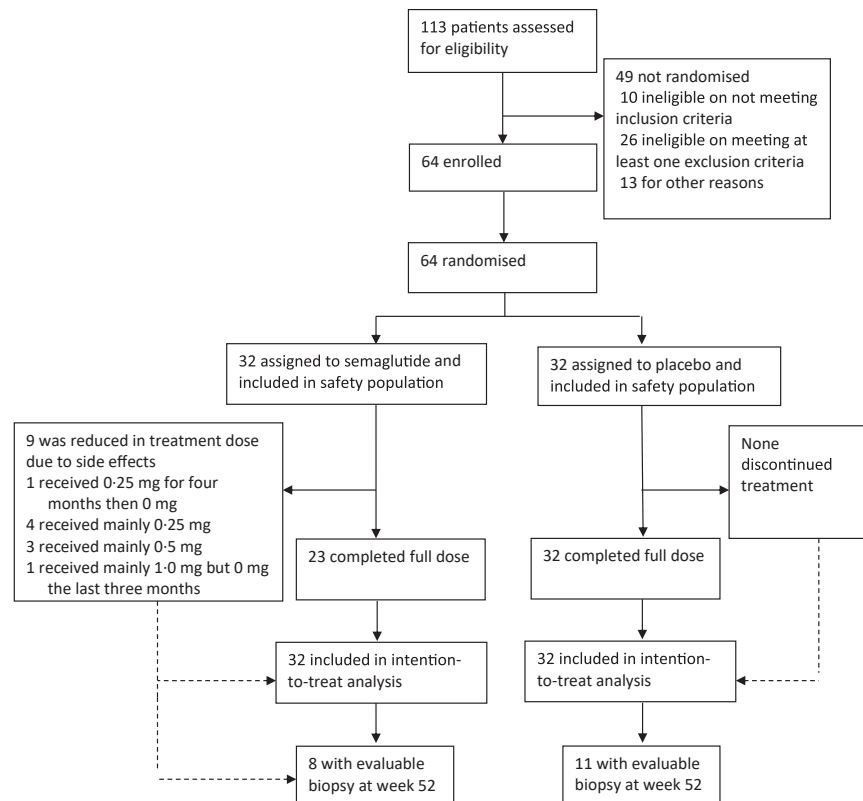


Fig. 1: Trial profile.

	Semaglutide, (n = 32)	Placebo, (n = 32)
Sex		
Female	28 (87%)	27 (84%)
Male	4 (13%)	5 (16%)
Age, years	62.7 (5.6)	63.6 (5.4)
Ethnicity		
White	31 (97%)	32 (100%)
Black	1 (3%)	0
Centre of enrolment		
Odense University Hospital	25 (78%)	24 (75%)
Esbjerg Hospital, University Hospital Southern Denmark	7 (22%)	8 (25%)
Weight, kg	77.4 (14.5)	76.7 (13.2)
Body mass index, kg/m ²	27.9 (4.8)	27.6 (4.3)
Osteopenia	23 (72%)	24 (75%)
Low-energy fracture ^a		
T-score > -1.0	0	2 (3%)
T-score < -1.0	3 (5%)	3 (5%)
Osteoporosis	9 (28%)	6 (19%)
Biochemistry		
HbA _{1c} , %	6.19 (0.4)	6.25 (0.5)
Parathyroid hormone, pmol/L	5.07 (1.9)	4.31 (1.3)
Ionized calcium, mmol/L	1.27 (0.04)	1.26 (0.04)
Creatinine, μmol/L	69.53 (12.0)	69.31 (13.6)
25-OH Vitamin D3, nmol/L	90.13 (26.4)	83.63 (25.4)
DXA		
T-score lumbar spine	-1.85 (0.85)	-1.72 (0.88)
T-score total hip	-1.31 (0.72)	-1.29 (0.57)
T-score femoral neck	-1.79 (0.64)	-1.75 (0.71)

Data are mean (SD). DXA = Dual X-ray energy absorptiometry. ^aAs self-reported within three years prior to screening.

Table 1: Baseline demographics and clinical characteristics in the intention-to-treat population.

Compared with the placebo group, aBMD decreased from baseline to week 52 in the semaglutide group both at the lumbar spine (ETD -0.018 g/cm³ [95% CI -0.031 to -0.005]; $p = 0.007$; Table 2) and total hip (ETD -0.020 g/cm² [95% CI -0.032 to -0.008]; $p = 0.001$; Table 2). There was no difference in changes in femoral neck aBMD between the groups ([95% CI -0.017 to 0.006]; $p = 0.328$; Table 2).

Based on outcomes derived from HR-pQCT scans, there was a decrease in the semaglutide group compared with the placebo group in tibial vBMD (relative ETD $-1.7%$ [95% CI -3.0 to -0.4]; $p = 0.010$; Table 2) and tibial cortical thickness (relative ETD $-1.8%$ [95% CI -3.1 to -0.4]; $p = 0.012$; Table 2). There were no differences between the groups in radial vBMD ([95% CI -1.5 to 0.4]; $p = 0.269$), radial cortical thickness ([95% CI -2.1 to 0.6]; $p = 0.265$), or estimated bone strength at the distal tibia ([95% CI -3.6 to 0.6]; $p = 0.164$) or radius ([95% CI -3.5 to 6.2]; $p = 0.589$; Table 2). Bone material strength index (BMSi) assessed by microindentation was unchanged between groups ([95% CI -9.1 to 6.1]; $p = 0.692$; Table 2).

Bone biopsies from the Iliac crest were collected from 21 participants, of which nine were in the

semaglutide group (43%). The sampling was unsuccessful in two cases (one from each group). This resulted in 19 biopsies eligible for histomorphometric evaluation (Fig. 1). There was insufficient bone material for histomorphometric analysis in one sample from the placebo group. Among the remaining 18 biopsies, eight (44%) were from participants in the semaglutide group. Tetracycline labelling was detectable in all biopsies. Seven exhibited double labelling in both cortical and trabecular bone, and three exhibited single labelling in both cortical and trabecular bone. In the remaining eight biopsies, four exhibited single labelling in cortical bone but double labelling in trabecular bone and vice versa. There were no differences between the groups in trabecular MAR ([95% CI -0.3 to 0.6]; $p = 0.836$), cortical MAR ([95% CI -0.7 to 0.5]; $p = 0.382$), trabecular MS/BS ([95% CI -1.7 to 6.2]; $p = 0.318$), cortical MS/BS ([95% CI -8.4 to 4.6]; $p = 0.791$), trabecular BFR ([95% CI -2.1 to 4.6]; $p = 0.480$), or cortical BFR ([95% CI -13.7 to 3.2]; $p = 0.836$) (Table 2). No visual signs indicated disruption in the quality of newly formed bone with semaglutide.

With semaglutide, body weight decreased from baseline to week 52 compared to placebo (ETD -6.8 kg

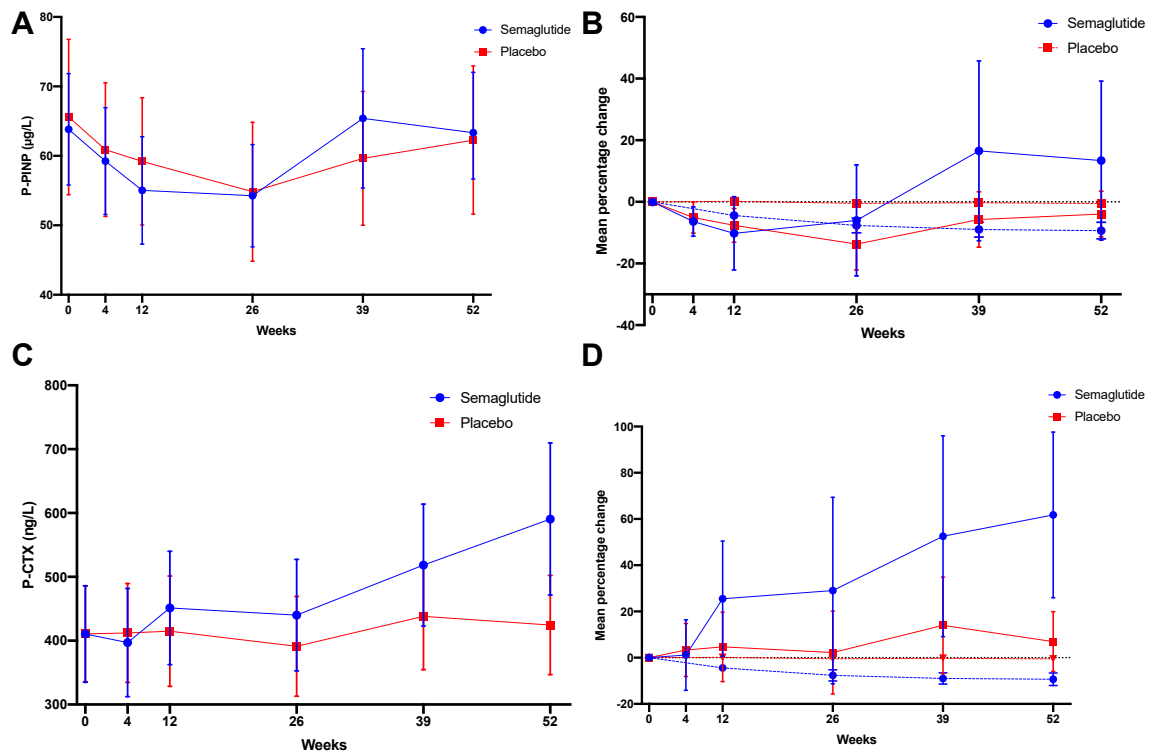


Fig. 2: Changes in P-PINP (A and B), P-CTX (C and D), and body weight (B + D) from baseline to week 52. A: Changes in absolute values for P-PINP semaglutide and P-PINP placebo. B: Mean percentage changes from baseline for P-PINP semaglutide, P-PINP placebo, body weight placebo, body weight semaglutide. C: Changes in absolute values for P-CTX semaglutide and P-CTX placebo. D: Mean percentage changes from baseline P-CTX semaglutide, P-CTX placebo, body weight placebo, body weight semaglutide. Error bars show 95% CI for observed values. Solid lines = Bone turnover markers. Broken lines = Body weight. PINP = Procollagen type I N-terminal propeptide. CTX = Collagen type I cross-linked C-terminal telopeptide.

[95% CI -8.8 to -4.7]; $p < 0.001$; Table 2). This corresponded to a relative ETD of -8.8% ([95% CI -11.7 to -6.0]; $p < 0.001$; Fig. 2B and ure D, Table 2). Specifically, body weight was reduced with semaglutide from baseline to week 39 ($p < 0.001$; Supplementary Table S1) with no significant change between weeks 39 and 52 ([95% CI -0.2 to 0.9]; $p = 0.246$). With semaglutide, changes in body composition were noted, as the lean/fat mass ratio increased from baseline to week 52 (relative ETD 16.1% [95% CI 10.4–21.9]; $p < 0.001$; Table 2) compared to placebo. Additionally, semaglutide induced a shift in the distribution of body fat mass, as the android/gynoid fat percentage ratio decreased from baseline to week 52 (relative ETD -8.7% [95% CI -11.4 to -5.9]; $p < 0.001$; Table 2) compared to placebo.

All participants completed the trial. Adherence was very high in both groups. Dose reduction was done in nine (28%) participants in the semaglutide group due to adverse reactions. No dose reductions were required in the placebo group. There were 31 [97%] of participants in the semaglutide group and 18 [56%] in the placebo group who experienced at least one adverse event. Of these, two in each group were considered serious adverse events

(Table 3). None of the serious adverse events were considered related to semaglutide. Thirty-seven participants [58%] reported treatment-emergent adverse reactions, with 30 in the semaglutide group and seven in the placebo group. These adverse events were primarily gastrointestinal, including nausea, decreased appetite, and constipation (Table 3). There were no deaths and no reported episodes of hypoglycaemia.

Discussion

This study shows that semaglutide 1.0 mg once weekly was not associated with an increase in bone formation in adult women and men without diabetes. Rather, bone resorption increased, and aBMD in the spine and hip as well as vBMD in the tibia decreased in the semaglutide group. Notably, tibial cortical thickness also decreased. By contrast, changes in the distal radius, a non-weight bearing skeletal site, were not observed. The separation of bone formation and resorption may, in part, be attributed to skeletal adaptations resulting from reduced mechanical loading due to semaglutide-induced weight reduction.

	Semaglutide	Placebo	Difference at week 52 [95% CI]	p-value
PINP, µg/L				
Baseline	63.8 (22.2)	65.6 (31.1)		
Week 52	64.3 (21.2)	62.3 (29.5)	2.0 [-10.8 to 15.0]	0.719
ETD	3.8 [-5.6; 13.3]			0.418
Relative ETD, %	17.3 [-9.0; 43.6]			0.193
CTX, ng/L				
Baseline	410.4 (209.6)	410.8 (208.1)		
Week 52	590.6 (330.5)	424.5 (215.5)	166.1 [26.7-305.5]	0.020
ETD	166.4 [25.5; 307.3]			0.021
Relative ETD, %	54.8 [17.5; 92.1]			0.005
Body weight, kg				
Baseline	77.4 (14.4)	76.7 (13.2)		
Week 52	70.2 (14.3)	76.2 (12.6)	-6.0 [-12.8 to 0.8]	0.081
ETD	-6.8 [-8.8; -4.7]			<0.001
Relative ETD, %	-8.8 [-11.7; -6.0]			<0.001
DXA				
Lumbar spine BMD, g/cm²				
Baseline	0.841 (0.09)	0.858 (0.11)		
Week 52	0.828 (0.09)	0.863 (0.12)	-0.035 [-0.09 to 0.02]	0.184
ETD	-0.018 [-0.031; -0.005]			0.007
Relative ETD, %	-2.05 [-3.63; -0.48]			0.012
Total hip BMD, g/cm²				
Baseline	0.785 (0.09)	0.793 (0.08)		
Week 52	0.764 (0.10)	0.793 (0.08)	-0.029 [-0.07 to 0.02]	0.202
ETD	-0.020 [-0.032; -0.008]			0.001
Relative ETD, %	-2.59 [-4.07; -1.11]			0.001
Femoral neck BMD, g/cm²				
Baseline	0.652 (0.07)	0.661 (0.09)		
Week 52	0.642 (0.08)	0.658 (0.08)	-0.016 [-0.06 to 0.02]	0.442
ETD	-0.006 [-0.017; 0.006]			0.328
Relative ETD, %	-1.06 [-2.82; 0.71]			0.236
Lean/fat ratio				
Baseline	1.50 (0.63)	1.51 (0.50)		
Week 52	1.68 (0.65)	1.46 (0.44)	0.218 [-0.06 to 0.50]	0.121
ETD	0.23 [0.15; 0.31]			<0.001
Relative ETD, %	16.1 [10.4; 21.9]			<0.001
Android/gynoid fat % ratio				
Baseline	0.96 (0.12)	0.95 (0.14)		
Week 52	0.90 (0.14)	0.97 (0.15)	-0.07 [-0.15 to -0.01]	0.037
ETD	-0.08 [-0.11; -0.06]			<0.001
Relative ETD, %	-8.7 [-11.4; -5.9]			<0.001
HR-pQCT, tibia				
Total vBMD, mg/cm³				
Baseline	237.4 (40.6)	250.9 (44.5)		
Week 52	231.9 (42.2)	249.0 (45.3)	-17.1 [-39.0 to 4.7]	0.123
ETD	-3.6 [-5.9 to -1.2]			0.003
Relative ETD, %	-1.69 [-2.95; -0.42]			0.010
Trabecular BV/TV, %				
Baseline	0.214 (0.04)	0.222 (0.04)		
Week 52	0.213 (0.04)	0.221 (0.04)	-0.008 [-0.03 to 0.01]	0.437
ETD	0.0 [-0.002 to 0.002]			0.920
Relative ETD, %	0.09 [-0.91; 1.09]			0.858

(Table 2 continues on next page)

	Semaglutide	Placebo	Difference at week 52 [95% CI]	p-value
(Continued from previous page)				
Trabecular thickness, mm				
Baseline	0.246 (0.01)	0.247 (0.02)		
Week 52	0.245 (0.01)	0.248 (0.02)	-0.003 [-0.01 to 0.01]	0.463
ETD	-0.001 [-0.003 to 0.0]			0.146
Relative ETD, %	-0.47 [-1.17; 0.22]			0.178
Cortical thickness, mm				
Baseline	1.21 (0.15)	1.26 (0.22)		
Week 52	1.18 (0.17)	1.25 (0.21)	-0.07 [-0.17 to 0.02]	0.121
ETD	-0.02 [-0.04 to -0.01]			0.009
Relative ETD, %	-1.75 [-3.10; -0.40]			0.012
Cortical porosity, mm				
Baseline	0.034 (0.01)	0.034 (0.01)		
Week 52	0.036 (0.01)	0.034 (0.01)	0.002 [-0.003 to 0.01]	0.471
ETD	0.002 [-0.001 to 0.01]			0.167
Relative ETD, %	4.26 [-4.76; 13.29]			0.349
Estimated bone strength, N				
Baseline	8379 (1607)	8711 (1897)		
Week 52	8133 (1607)	8599 (1993)	-466 [-371.7 to 438.4]	0.307
ETD	-134.6 [-314.7 to 45.4]			0.140
Relative ETD, %	-1.47 [-3.56; 0.62]			0.164
HR-pQCT, radius				
Total vBMD, mg/cm ³				
Baseline	238.3 (52.3)	243.6 (56.2)		
Week 52	235.1 (53.4)	241.0 (54.9)	-5.9 [-33.0 to 21.1]	0.661
ETD	-0.7 [-3.0 to 1.6]			0.522
Relative ETD, %	-0.55 [-1.54; 0.44]			0.269
Trabecular BV/TV, %				
Baseline	0.168 (0.05)	0.176 (0.06)		
Week 52	0.169 (0.05)	0.176 (0.06)	-0.007 [-0.03 to 0.02]	0.588
ETD	0.0 [-0.002 to 0.003]			0.843
Relative ETD, %	0.38 [-1.48; 2.24]			0.682
Trabecular thickness, mm				
Baseline	0.221 (0.01)	0.219 (0.02)		
Week 52	0.221 (0.01)	0.219 (0.02)	0.002 [-0.01 to 0.01]	0.689
ETD	0.0 [-0.002 to 0.001]			0.516
Relative ETD, %	-0.17 [-0.77; 0.43]			0.575
Cortical thickness, mm				
Baseline	0.82 (0.15)	0.83 (0.14)		
Week 52	0.81 (0.15)	0.82 (0.12)	-0.01 [-0.08 to 0.07]	0.826
ETD	-0.004 [-0.02 to 0.01]			0.443
Relative ETD, %	-0.74 [-2.06; 0.58]			0.265
Cortical porosity, mm				
Baseline	0.010 (0.007)	0.009 (0.006)		
Week 52	0.010 (0.007)	0.010 (0.006)	0 [-0.003 to 0.004]	0.703
ETD	0.0 [-0.001 to 0.001]			0.952
Relative ETD, %	-3.95 [-18.79; 10.88]			0.596
Estimated bone strength, N				
Baseline	2650 (875)	2773 (956)		
Week 52	2671 (851)	2765 (969)	-94 [-550.2 to 361.3]	0.680
ETD	28.9 [-82.6 to 140.4]			0.607
Relative ETD, %	1.31 [-3.52; 6.15]			0.589

(Table 2 continues on next page)

	Semaglutide	Placebo	Difference at week 52 [95% CI]	p-value
(Continued from previous page)				
BMSi, N				
Baseline	78.1 (9.8)	74.4 (10.1)		
Week 52	81.1 (8.6)	78.1 (8.6)	3.0 [-1.3 to 7.3]	0.170
ETD	-0.8 [-6.2 to 4.5]			0.761
Relative ETD, %	-1.52 [-9.1; 6.1]			0.692
Dynamic histomorphometry ^a				
Cortical				
MAR, $\mu\text{m}/\text{day}$	0.54 (0.00-0.77)	0.72 (0.00-0.98)		0.382
MS/BS, %	3.56 (1.13-8.44)	2.56 (0.00-12.10)		0.791
BFR, $\mu\text{m}^3/\mu\text{m}^2/\text{day}$	1.91 (0.00-6.61)	1.55 (0.00-18.10)	0.36 (-13.7 to 3.2)	0.836
Trabecular				
MAR, $\mu\text{m}/\text{day}$	0.57 (0.13-0.71)	0.53 (0.00-0.81)		0.836
MS/BS, %	5.13 (1.27-7.80)	1.97 (0.00-6.40)		0.318
BFR, $\mu\text{m}^3/\mu\text{m}^2/\text{day}$	1.94 (0.15-6.24)	2.11 (0.00-3.42)	-0.17 (-2.1 to 4.6)	0.480

Data are mean (SD) or [95% CI] with difference in point estimates at week 52 for semaglutide versus placebo. Dynamic histomorphometry shown as median (interquartile range). ETD and relative ETD shows the treatment effect of semaglutide versus placebo from baseline to week 52. DXA = Dual X-ray energy absorptiometry. BMD = Bone mineral density. ETD = Estimated treatment difference. HR-pQCT = High resolution peripheral quantitative computed tomography. vBMD = Volumetric bone mineral density. BV/TV = Bone volume per tissue volume. BMSi = Bone material strength index. MAR = Mineral apposition rate. MS/BS = Mineralised surface per bone surface. BFR = Bone formation rate. ^aOutcomes assessed at week 52.

Table 2: Pharmacodynamic measures at baseline and week 52 in the intention-to-treat population.

	Semaglutide, (n = 32)	Placebo, (n = 32)
Participants with at least one adverse event	31 (97%) [0.84-1.0]	18 (56%) [0.38-0.74]
Serious adverse events	2 (6%) [0.01-0.21]	2 (6%) [0.01-0.21]
Deaths	0	0
Participants with at least one treatment-emergent adverse reaction	30 (94%) [0.79-0.99]	7 (22%) [0.09-0.40]
Adverse events leading to dose reduction	9 (28%) [0.14-0.47]	0
Adverse events of special interest		
Weight loss	24 (75%) [0.57-0.89]	2 (6%) [0.01-0.21]
Decreased appetite	20 (63%) [0.44-0.79]	0
Nausea	13 (41%) [0.24-0.59]	1 (3%) [0.001-0.16]
Constipation	7 (22%) [0.09-0.40]	1 (3%) [0.001-0.16]
Gastro-oesophageal reflux	4 (13%) [0.04-0.29]	1 (3%) [0.001-0.16]
Abdominal pain	4 (13%) [0.04-0.29]	0
Headache	3 (9%) [0.02-0.25]	0
Dizziness	3 (9%) [0.02-0.25]	0
Fatigue	2 (6%) [0.01-0.21]	0
Vomiting	1 (3%) [0.001-0.16]	0
Diarrhoea	0	0
Hypoglycaemia	0	0
Weakness	0	0
Meteorism	0	0
Flatulence	0	0
Nervousness	0	0
Drowsiness	0	0
Sweating	0	0
Other treatment-emergent adverse reactions		
Gallstones	1 (3%) [0.001-0.16]	1 (3%) [0.001-0.16]
Injection site haematoma	1 (3%) [0.001-0.16]	3 (9%) [0.02-0.25]

Data are number of participants with adverse events (%) [95% CI].

Table 3: Safety profile in the safety population.

We are not aware of outcomes of other studies designed to assess the skeletal effects of GLP-1RAs in individuals without diabetes. A post-hoc investigation showed that liraglutide increased bone formation in obese pre- and postmenopausal women when used to stabilize body weight after calorie-restricted weight loss.¹⁰ By contrast, the present investigation showed no effect of semaglutide on bone formation after a similar study duration. In line with our findings, 26 weeks of treatment with liraglutide had no impact on bone formation in individuals with T2D.¹³ These discrepancies may potentially be explained by differences in the timing of the assessment of bone turnover as liraglutide increased bone formation when used after but not before weight reduction. Longer-term studies are required to determine if the skeletal effects of GLP-1RAs including semaglutide differ between phases with weight loss or weight maintenance.

Previous placebo-controlled studies have reported neutral effects of liraglutide on bone resorption markers in individuals with obesity,¹⁰ pre-diabetes and schizophrenia¹⁹ or T2D.¹³ In contrast, this study showed that semaglutide increased bone resorption substantially. These discrepancies may be attributed to dissimilar weight changes. While we observed an 8.8% weight loss with semaglutide compared to placebo, previous studies with liraglutide reported either no change in body weight¹⁰ or reductions of 3.9%¹³ and 4.5%,¹⁹ respectively. This aligns with the established “mechanostat theory”, which posits that the skeleton adapts to chronically reduced strain levels, such as those induced by weight loss, by increasing bone resorption.²⁰ These adaptive mechanisms are partly driven by increased osteoclast activity following unloading,²¹ as observed after weight loss. Further research is needed to determine if semaglutide directly influences human osteoclast activity. Besides the impact of weight loss, differences in study duration may contribute to the conflicting reports on bone resorption. We observed an increase in bone resorption after 26 weeks, whereas previous studies reported effects on the same bone resorption marker after 13 and 26 weeks, respectively, indicating that effects on bone resorption may have been missed in these.^{13,19} Collectively, these findings emphasize the importance of considering longer study durations of at least 26 weeks when investigating the effects of GLP-1RAs on bone turnover in future studies.

A reduction in bone mass at the lumbar spine and total hip was observed in the semaglutide group after 52 weeks but not in the placebo group. These changes in aBMD were similar to the expected annual reduction in aBMD at the lumbar spine but larger than that of the total hip.²² In contrast to our findings, lumbar spine and total hip aBMD were preserved after 26 weeks of treatment with liraglutide in patients with T2D.¹³ These conflicting reports on the effects of GLP-1RAs on aBMD

may be explained by differences in study populations. In patients with T2D, bone turnover is reported to be lower,²³ which could reduce the effects of GLP-1RAs on bone resorption and bone mass. Previous studies also were of shorter duration and reported smaller weight losses, impeding comparisons between studies. In non-obese individuals without T2D, calorie restriction led to a 10% weight reduction and a 2.2% decrease in both lumbar spine and total hip but not femoral neck aBMD,¹¹ which is in line with the effects seen in the semaglutide group in this study. This suggests that the effects of semaglutide on aBMD are similar to those observed with calorie restriction, further supporting that the main effect on the skeleton is caused by weight reduction. It remains to be investigated if these changes in aBMD translate into changes in fracture risk.

Calorie restricted weight loss is reported to affect both trabecular and cortical bone.²⁴ Decreases in aBMD in the hip and in cortical thickness in tibia indicate that semaglutide mainly affects cortical bone. Cortical bone contributes substantially more to bone stiffness than trabecular bone.²⁵ Although estimated bone strength at the tibia was not changed in the semaglutide group, continuous cortical bone loss is expected to decrease bone strength. Body weight decreased during this study, limiting the opportunity to determine if bone loss continues after stabilisation of the body weight. Bone material properties assessed by microindentation were unchanged with semaglutide, however, the clinical relevance of this measure is uncertain. Weight loss following Roux-en-Y gastric bypass (RYGB) increased bone material properties despite increasing bone turnover and reduced BMD in individuals with and without T2D²⁶ and increased fracture risk substantially.²⁷ Although weight loss may improve the composition of bone tissue, the impact of lower mechanical loading on bone mass may surpass this effect leading to higher fracture risk. Importantly, RYGB is associated with substantial changes that may have adverse skeletal effects, e.g., changes in gut hormone secretion and risks of malabsorption which are not observed with semaglutide. Therefore, further studies on the long-term effects of semaglutide and similar drugs with substantial effects on body weight, bone mass, and fracture risk are warranted.

Overall, the safety profile for semaglutide in this study was consistent with earlier findings in patients with T2D.²⁸ As expected, transient gastrointestinal effects accounted for the most treatment-emergent adverse reactions. While all participants completed the trial, the dose of the investigational drug was reduced in 28% of the participants in the semaglutide group due to adverse reactions. Importantly, this study does not inform of the mechanism behind skeletal changes with semaglutide. Although the observed 17.3% increase in P-PINP from baseline to week 52 in the semaglutide group compared with the placebo group exceeded the

anticipated 16% increase in this marker of bone formation, the confidence interval included zero making an effect of semaglutide on P-PINP unlikely. The effects on bone resorption and aBMD are likely explained by changes in body weight or in combination with direct skeletal effects of semaglutide. The absence of comparable weight loss in the placebo group limits interpretations of a potential direct effect of semaglutide on bone. Notably, exercise may mitigate weight loss-induced bone loss,¹¹ and increased physical activity due to weight loss in this study could have protected against negative effects on the skeletal outcomes. Furthermore, changes in intake of macronutrients may also have contributed to the observed effects. Neither degree of physical activity nor dietary changes were accounted for in this study. Bone loss continued after stabilisation of body weight following calorie restricted-induced weight loss in non-obese men and women.²⁹ With semaglutide, body weight is reported to stabilise after 52–104 weeks of treatment,³⁰ limiting our opportunity to assess skeletal adaptations overshoots after stabilisation of body weight. Additionally, the design of this study deterred us from assessing if bone mass increased after cessation of semaglutide. Finally, the study was conducted at two sites and included a relatively small cohort with limited ethnic diversity which may limit the generalisability of our findings.

In conclusion, this study showed that semaglutide did not increase bone formation based on measures of PINP in adult men and women with increased fracture risk without T2D. Therefore, this study does not support further studies of osteoanabolic effects of semaglutide in individuals without T2D. Secondary outcomes showed increased bone resorption and bone loss, particularly in the cortical bone, which may represent skeletal adaptations to lower mechanical loading following weight reduction, direct effect of semaglutide on bone or a combination.

Contributors

MSH and MF designed the study. MSH and SGH were responsible for recruiting participants. MSH, SJ, CE, SGH, and MF were investigators in the study and were responsible for collecting data. CE and MF performed transiliac bone biopsies. NRJ was responsible for analyses of bone turnover markers and EMW performed the histomorphometric analyses. JJM had access to raw data and was responsible for statistical analyses. MSH, RE, and MF drafted the manuscript. MSH, EMW, RE, SGH, and MF have directly accessed and verified the underlying data reported in the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data sharing statement

Data will be shared with bona fide researchers who submit a research proposal approved by the independent review board and the Danish National Ethics Committee and after receiving a receipt of a signed data sharing agreement. Individual data will be shared in datasets in a de-identified and anonymised format. No expiration date of data request is currently set once data are made available. Results, statistical analysis plan, and study protocol will be uploaded to [ClinicalTrials.org](https://doi.org/10.1016/j.eclim.2024.102624) with publication of this article.

Declaration of interests

EMW, SJ, SGH, JJM, and CE declare no conflicts of interest. MSH and MF have received funding from the Novo Nordisk Foundation. RE receives consultancy funding from Immunodiagnostic Systems, Sandoz, Samsung, CL Bio, Biocon, Takeda, UCB, meeting presentations for Pharmacosmos, Alexion, UCB and Amgen, and grant funding from Alexion. NRJ has received assays and reagents from IDS and Roche for clinical studies. MF has received consultancy funding from Novo Nordisk and is shareholder at Novo Nordisk and Eli Lilly. Novo Nordisk, Denmark, provided the investigational drug and placebo.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.eclim.2024.102624>.

References

- 1 Szulc P, Seeman E. Thinking inside and outside the envelopes of bone: dedicated to PDD. *Osteoporos Int*. 2009;20:1281–1288.
- 2 Ensrud KE. Epidemiology of fracture risk with advancing age. *J Gerontol A Biol Sci Med Sci*. 2013;68:1236–1242.
- 3 Shoback D, Rosen CJ, Black DM, Cheung AM, Murad MH, Eastell R. Pharmacological management of osteoporosis in postmenopausal women: an endocrine society guideline update. *J Clin Endocrinol Metab*. 2020;105:1595–1622.
- 4 Cipriani C, Pepe J, Minisola S, Lewiecki EM. Adverse effects of media reports on the treatment of osteoporosis. *J Endocrinol Invest*. 2018;41:1359–1364.
- 5 Collaborators GF. Global, regional, and national burden of bone fractures in 204 countries and territories, 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019. *Lancet Healthy Longev*. 2021;2:e580–e592.
- 6 Clowes JA, Allen HC, Prentis DM, Eastell R, Blumsohn A. Octreotide abolishes the acute decrease in bone turnover in response to oral glucose. *J Clin Endocrinol Metab*. 2003;88:4867–4873.
- 7 Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab*. 2013;17:819–837.
- 8 Lu N, Sun H, Yu J, et al. Glucagon-like peptide-1 receptor agonist Liraglutide has anabolic bone effects in ovariectomized rats without diabetes. *PLoS One*. 2015;10:e0132744.
- 9 Ma X, Meng J, Jia M, et al. Exendin-4, a GLP-1 receptor agonist, prevents osteopenia by promoting bone formation and suppressing bone resorption in aged ovariectomized rats. *J Bone Miner Res*. 2013;28:1641–1652.
- 10 Iepsen EW, Lundgren JR, Hartmann B, et al. GLP-1 receptor agonist treatment increases bone formation and prevents bone loss in weight-reduced obese women. *J Clin Endocrinol Metab*. 2015;100:2909–2917.
- 11 Villareal DT, Fontana L, Weiss EP, et al. Bone mineral density response to caloric restriction-induced weight loss or exercise-induced weight loss: a randomized controlled trial. *Arch Intern Med*. 2006;166:2502–2510.
- 12 Johnson KC, Anderson A, Beavers KM, et al. The long-term effect of intentional weight loss on changes in bone mineral density in persons with type 2 diabetes: results from the Look AHEAD randomized trial. *Arch Osteoporos*. 2023;18:97.
- 13 Hygum K, Harsløf T, Jørgensen NR, Rungby J, Pedersen SB, Langdahl BL. Bone resorption is unchanged by liraglutide in type 2 diabetes patients: a randomised controlled trial. *Bone*. 2020;132:115197.
- 14 Driessen JH, van Onzenoort HA, Starup-Linde J, et al. Use of glucagon-like-peptide 1 receptor agonists and risk of fracture as compared to use of other anti-hyperglycemic drugs. *Calcif Tissue Int*. 2015;97:506–515.

- 15 Cheng L, Hu Y, Li YY, et al. Glucagon-like peptide-1 receptor agonists and risk of bone fracture in patients with type 2 diabetes: a meta-analysis of randomized controlled trials. *Diabetes Metab Res Rev*. 2019;35:e3168.
- 16 Looker AC, Johnston CC Jr, Wahner HW, et al. Prevalence of low femoral bone density in older U.S. women from NHANES III. *J Bone Miner Res*. 1995;10:796–802.
- 17 Diez-Perez A, Bouxsein ML, Eriksen EF, et al. Technical note: recommendations for a standard procedure to assess cortical bone at the tissue-level in vivo using impact microindentation. *Bone Rep*. 2016;5:181–185.
- 18 Recker RR, Akhter MP, Lappe JM, Watson P. Bone histomorphometry in transiliac biopsies from 48 normal, healthy men. *Bone*. 2018;111:109–115.
- 19 Maagensen H, Larsen JR, Jorgensen NR, Fink-Jensen A, Vilsboll T. Liraglutide does not change bone turnover in clozapine- and olanzapine-treated schizophrenia overweight patients with prediabetes - randomized controlled trial. *Psychiatry Res*. 2021;296:113670.
- 20 Tyrovala JB. The "mechanostat theory" of frost and the OPG/RANKL/RANK system. *J Cell Biochem*. 2015;116:2724–2729.
- 21 Wang L, You X, Zhang L, Zhang C, Zou W. Mechanical regulation of bone remodeling. *Bone Res*. 2022;10:16.
- 22 Finkelstein JS, Brockwell SE, Mehta V, et al. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. *J Clin Endocrinol Metab*. 2008;93:861–868.
- 23 Starup-Linde J, Lykkeboe S, Gregersen S, et al. Differences in biochemical bone markers by diabetes type and the impact of glucose. *Bone*. 2016;83:149–155.
- 24 Liu L, Rosen CJ. New insights into calorie restriction induced bone loss. *Endocrinol Metab (Seoul)*. 2023;38:203–213.
- 25 Schaffler MB, Burr DB. Stiffness of compact bone: effects of porosity and density. *J Biomech*. 1988;21:13–16.
- 26 Blom-Høgestøl IK, Mala T, Kristinsson JA, Brunborg C, Gulseth HL, Eriksen EF. Changes in bone quality after Roux-en-Y gastric bypass: a prospective cohort study in subjects with and without type 2 diabetes. *Bone*. 2020;130:115069.
- 27 Axelsson KF, Werling M, Eliasson B, et al. Fracture risk after gastric bypass surgery: a retrospective cohort study. *J Bone Miner Res*. 2018;33:2122–2131.
- 28 Heise T, Mari A, DeVries JH, et al. Effects of subcutaneous tirzepatide versus placebo or semaglutide on pancreatic islet function and insulin sensitivity in adults with type 2 diabetes: a multicentre, randomised, double-blind, parallel-arm, phase 1 clinical trial. *Lancet Diabetes Endocrinol*. 2022;10:418–429.
- 29 Villareal DT, Fontana L, Das SK, et al. Effect of two-year caloric restriction on bone metabolism and bone mineral density in non-obese younger adults: a randomized clinical trial. *J Bone Miner Res*. 2016;31:40–51.
- 30 Garvey WT, Batterham RL, Bhatta M, et al. Two-year effects of semaglutide in adults with overweight or obesity: the STEP 5 trial. *Nat Med*. 2022;28:2083–2091.