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CLINICAL SCIENCES

Effects of a Lifestyle Intervention on Bone Turnover in Persons with Type 2 Diabetes: A *Post Hoc* Analysis of the U-TURN Trial

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ABSTRACT

ABILDGAARD, J., M. Y. JOHANSEN, K. SKOV-JEPPESEN, L. B. ANDERSEN, K. KARSTOFT, K. B. HANSEN, B. HARTMANN, J. J. HOLST, B. K. PEDERSEN, and M. RIED-LARSEN. Effects of a Lifestyle Intervention on Bone Turnover in Persons with Type 2 Diabetes: A *Post Hoc* Analysis of the U-TURN Trial. *Med. Sci. Sports Exerc.*, Vol. 54, No. 1, pp. 38–46, 2022. **Introduction/Purpose:** The increased risk of fractures with type 2 diabetes (T2D) is suggested to be caused by decreased bone turnover. Current international guidelines recommend lifestyle modifications, including exercise, as first-line treatment for T2D. The aim of this study was to investigate the effects of an exercise-based lifestyle intervention on bone turnover and bone mineral density (BMD) in persons with T2D. **Methods:** Persons with T2D were randomized to either a 12-month lifestyle intervention ($n = 64$) or standard care ($n = 34$). The lifestyle intervention included five to six weekly aerobic training sessions, half of them combined with resistance training. Serum markers of bone turnover (osteocalcin, N-terminal propeptide of type-I procollagen, reflecting bone formation, and carboxyterminal collagen I crosslinks, reflecting bone resorption) and BMD (by DXA) were measured before the intervention and at follow-up. **Results:** From baseline to follow-up, s-propeptide of type-I procollagen increased by 34% (95% confidence interval [CI], 17%–50%), serum-carboxyterminal collagen I crosslink by 36% (95% CI, 1%–71%), and s-osteocalcin by 31% (95% CI, 11–51%) more in the lifestyle intervention group compared with standard care. Loss of weight and fat mass were the strongest mediators of the increased bone turnover. Bone mineral density was unaffected by the intervention (ΔBMD, 0.1%; 95% CI, −1.1% to 1.2%). **Conclusions:** A 12-month intensive exercise-based lifestyle intervention led to a substantial but balanced increase in bone turnover in persons with T2D. The increased bone turnover combined with a preserved BMD, despite a considerable weight loss, is likely to reflect improved bone health and warrants further studies addressing the impact of exercise on risk of fractures in persons with T2D. **Key Words:** TYPE 2 DIABETES, BONE TURNOVER, BONE MINERAL DENSITY, EXERCISE, LIFESTYLE INTERVENTION

Fragility fractures are increasingly recognized as an important complication to type 2 diabetes (T2D) despite a normal to increased bone mineral density (BMD) (1–3). The validity of BMD to predict fracture risk in this group of patients has therefore been questioned (1,2,4–7). This has led to a growing interest in identifying other reliable predictors of fracture risk in persons with T2D, and markers of bone quality have received increasing attention. Histological studies have shown that bone turnover is decreased in patients with T2D (8,9), and plasma levels of the bone turnover markers (BTM) osteocalcin (OC) and N-terminal propeptide of type-I procollagen (PINP) (both reflecting bone formation) and carboxyterminal collagen I crosslinks (CTX-I) (reflecting bone resorption) are all lower in persons with T2D compared with healthy controls (10,11). Furthermore, both low PINP and low OC/alkaline phosphatase ratio have been related to

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an increased risk of vertebral fractures in persons with T2D, all in all indicating that low bone turnover could contribute to increased fracture risk with T2D (11–14).

The mechanisms responsible for BTM suppression in T2D are unknown. Several studies have found an association between hyperglycemia and low BTM (15,16). Moreover, *in vitro* studies show direct inhibitory effects of a hyperglycemic environment on osteoblasts (3,11,17,18). Other studies suggest an important role of adipose tissue–bone cross-talk as adipocytes and osteoblasts are derived from a common multipotent mesenchymal stem cell, whereby excess adiposity promotes adipogenesis at the expense of osteogenesis (19,20). Furthermore, low-grade inflammation, a hallmark of T2D pathophysiology, has been hypothesized to contribute to alterations in bone metabolism (21,22). Proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β can synergize with nuclear factor-κB and potentiate bone resorption (23). Conversely, high plasma levels of IL-1 receptor antagonist (IL-1RA), as seen with T2D (24), are believed to suppress bone resorption through inhibition of IL-1β (25,26).

Weight loss leads to increased bone resorption in obese nondiabetic individuals, probably due to "unloading" of the bone, and exercise has therefore been identified as a crucial part of weight loss programs to sustain loading of the bone despite loss of weight (27,28). Current international guidelines recommend lifestyle modifications, including exercise, as first-line treatment for T2D (29). A previous study in elderly persons with prediabetes, randomized to a 16-wk exercise intervention with soccer, showed a substantial increase in both BTM and BMD in response to the exercise intervention (30). However, the effects of prolonged lifestyle interventions with focus on higher volumes of exercise are missing. Furthermore, the effects of lifestyle interventions on bone turnover and BMD in persons with T2D remain to be elucidated.

We have previously identified beneficial effects on glycemic control, body weight, and low-grade inflammation after a 12-month intensive lifestyle intervention in persons with T2D (31,32). Thus, we hypothesized that this exercise-based lifestyle intervention would lead to a substantial increase in bone turnover, statistically mediated by the intervention effects on either glycemic control, weight loss, loss of fat mass, improved maximal oxygen uptake ($\dot{V}O_{2max}$), decreased low-grade inflammation, or perhaps a combination of these factors.

MATERIALS AND METHODS

Study design. The full study protocol has been described previously (33). This study is an explorative analysis of a single center, parallel group, randomized controlled trial where participants were randomized in a 2:1 fashion to either a lifestyle intervention and standard care or standard care alone. The study was designed to investigate changes in glycated hemoglobin (HbA1c) after a lifestyle improvement consisting of a partially supervised 12 months training and diet intervention. Other results from the study have been published previously (31,32,34,35). The study took place at the Copenhagen University Hospital, Rigshospitalet, from April 2015 to August 2016, and participants were recruited from Region Zealand and the Capital Region of Denmark.

The study was approved by the Scientific Ethical Committee of the Capital Region of Denmark (H-1-2014-114) and performed according to the Declaration of Helsinki. Informed consent was obtained in writing and orally from all participants.

Study participants. Inclusion criteria were: 1) T2D diagnosed less than 10 yr ago, 2) body mass index of 25 to 40, 3) taking 2 or fewer glucose-lowering medications. Exclusion criteria were: 1) HbA1c > 9%, 2) insulin-dependence, 3) presence of 1 or more of the following complications: diabetic retinopathy, urine albumin/creatinine ratio ≥300 $mg \cdot g^{-1}$, or plasma creatinine ≥1.47 $mg \cdot dL^{-1}$.

Intervention. At least 6 wk before baseline measurements, all participants had their glucose-lowering, lipid-lowering, and antihypertensive medications titrated by the study endocrinologist to obtain prespecified treatment targets (33). Biguanid (tablet Metformin) was initiated as first-line treatment. If the patient exhibited unsatisfactory glycemic control on metformin alone, a GLP-1 analog (Victoza) was added as second-line treatment. As third-line treatment, one daily injection of insulin glargine biosimilar (Abasaglar initiating dose 0.2 $U \cdot kg^{-1} \cdot d^{-1}$) was added and titrated to an acceptable fasting blood glucose level. A detailed flowchart of treatment algorithms can be found in the study protocol (33).

The treatment target for glycemic control was HbA1c level of 6.5%. If this target was reached at a medical consultation, the glucose-lowering medication dose was halved. In the case of unchanged values or if an additional reduction in HbA1c level was observed at the after medical consultation, the treatment with medication was discontinued. If HbA1c level exceeded 7.5%, the glucose-lowering medication was increased according to the prespecified algorithm (33).

All participants received standard care, including medical counseling, education in T2D, and lifestyle advice by the study nurse every third month throughout the study period. To reach standardization across groups and minimize the risk of bias, prespecified treatment targets and algorithms for regulation of glucose-lowering, lipid-lowering, and antihypertensive medication were controlled by the study endocrinologist, who was blinded to the group allocation (33).

The lifestyle intervention-group additionally underwent an intensive lifestyle intervention consisting of five to six aerobic exercise sessions of 30- to 60-min duration. Furthermore, two to three sessions were combined with 30 min of resistance exercise. Aerobic exercise sessions were performed with an average heart rate of 60% to 80% of maximum the first 4 months and 70% to 90% the last 8 months. Whereas aerobic exercise was structured to hit specific percentages of maximum heart rate in specific time zones, strength exercise was designed to hit specific muscle groups with no detailed training scheme.

The initial 4 months of training were fully supervised after which supervision was gradually reduced. Daily steps and exercise sessions were monitored with a smartwatch (Polar V800; Polar Electro, Kempele, Finland). The lifestyle intervention further consisted of individual and group sessions with a clinical dietician and an individual dietary plan with a macronutrient

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distribution of 45 E % to 60 E % carbohydrate, 15 E % to 20 E % protein, and 20 E % to 35 E % fat (<7 E % saturated fat). During the first 4 months the total energy intake was restricted by approximately 25% based on the Oxford equation and through guidance from a dietitian.

Experimental day. Completed at baseline and 12 months' follow-up. Two days before each experimental day individuals were instructed to refrain from moderate to vigorous intensity exercise and to pause all glucose-, lipid- and blood pressure–lowering medication. Furthermore, no alcohol was permitted during the last 24 h. On the experimental day individuals arrived after a minimum of 8 h overnight fasting. An antecubital vein catheter was inserted, and fasting blood samples were drawn. Ethylene-diamine-tetra-acetic acid plasma tubes were immediately spun at 3500*g* for 15 min at 4°C. Serum was stored at room temperature for a minimum of 30 min before handling.

Bone turnover markers. Serum CTX-I, intact PINP, and OC were measured with a chemiluminescence method using an automated immunoassay system (iSYS, Immunodiagnostic Systems Ltd., Boldon, England). The limit of detection (LOD) for CTX-I was 0.023 $ng \cdot mL^{-1}$ and intra-assay coefficient of variation (CV) was <10%. Propeptide of type-I procollagen LOD was <1.0 $ng \cdot mL^{-1}$ and CV ≤ 5%. Osteocalcin LOD was 0.27 $ng \cdot mL^{-1}$, and CV was <10%.

BMD and body composition. Total body BMD, fat and fat-free masses were assessed based on a whole-body dual-energy X-ray absorptiometry (DXA) scan (Lunar Prodigy Advance; GE Medical Systems Lunar, Milwaukee, WI) before- and after the intervention. All participants were scanned on the same scanner. Coefficient of variation is estimated to <1%. Software (Prodigy, enCORE 2004, version 8.8; GE Lunar Corp, Madison, WI) was used to estimate regional BMD and fat-, and fat-free tissue masses.

Additional measurements. An oral glucose tolerance test was performed using 83 g of glucose monohydrate dissolved in 300 mL of water. Blood samples were drawn at 0, 15, 30, 60, 90, and 120 min. Incremental area under the curve (iAUC) for glucose was calculated for each participant as the AUC above the extrapolated fasting level using the trapezoid rule (36). $\dot{V}O_{2max}$ was assessed using an incremental ergometer bicycle test (Monark 839E; Monark, Varberg, Sweden). The test included a 5-min warm-up followed by an increase of 20 $W \cdot min^{-1}$ until exhaustion (31). $\dot{V}O_2$ was continuously measured by indirect calorimetry (Quark b2; Cosmed, Rome, Italy). Plasma IL-1 receptor antagonist (IL-1RA), IL-6, and TNF-α levels were determined as previously reported and published (31).

Statistical analyses. The original study was designed to investigate changes in HbA1c from baseline to 12 months of follow-up. Thus, the power study was powered to detect a difference of ±0.4% points for HbA1c for the between-group comparison.

This study was performed to explore the effects of an intensive lifestyle intervention on circulating markers of bone turnover, and variable outcomes were assessed through ANCOVA performed with the absolute change of BTM as dependent variables with group (two levels), time (one level), and baseline value of outcome variables as independent variables. Variables were log transformed to reach normal distribution if needed (IL-1RA, TNF-α, IL-6). The log-transformed data were exponentiated, and data are expressed as the ratio of the geometric mean (RGM—reflecting the percent change) change within and between groups. Single-level mediation analyses were performed to assess the role of predefined mediators on the relation between intervention and outcome (37). Models were checked for assumptions of the linear model, including normal distribution of the residuals, homogeneity of variance, linearity, and independence of variables. Single mediation analyses were conducted using the PROCES plug-in (v3.4.1) in SPSS.

Mediators were identified through directed acyclic graphs and were prespecified in a statistical analysis plan before analyses (see Figure, Supplemental Digital Content 1, directed acyclic graph illustrating potential mediators of changes in BTM with lifestyle intervention, http://links.lww.com/MSS/C419). Analyses were controlled for baseline values of outcome variables. Bootstrapping (5000 resamples) was used to obtain bias corrected 95% confidence interval (CI). The proportion of mediation was quantified by dividing the mediation effect with the total effect of intervention on outcome. To determine the multivariate contribution of each covariate to changes in BTM (adjusted outcome variables) we fitted in partial least squares (PLS) regression analyses (38). Partial least squares regression decomposes the explanatory variables into orthogonal linear combinations (PLS components), while simultaneously maximizing the covariance with the outcome variable. Thus, in contrast to ordinary least squares regression, PLS regression can handle completely collinear variables. Monte Carlo resampling with 250 repetitions was used to select the number of PLS components optimizing the predictive performance of the models. The results are displayed in a selectivity ratio (SR) plot indicating positive or negative contributions to changes in BTM. The SR is the proportion of the total explained variance, that each variable explains independent of the other variables. The sign of the SR is determined from the corresponding loading on the predictive target projection component. Confidence intervals were constructed around each SR and used to assess the significance of the SR for each variable. Partial least squares regressions were performed by means of the commercial software Sirius version 11.0 (Pattern Recognition Systems AS, Bergen, Norway). Between group differences in absolute CTX-I suppression in response to an oral glucose tolerance test (OGTT), at follow-up, were analyzed using an ANCOVA, correcting for glucose induced CTX-I suppression at baseline. Between group differences in relative CTX-I suppression in response to an OGTT, were compared using an unpaired *t*-test comparing the relative suppression from fasting levels, only at 12 months of follow-up. Statistical analyses were performed using SPSS version 25 (IBM Corporation), and $P < 0.05$ was considered statistically significant (2-tailed).

RESULTS

Ninety-eight participants were enrolled in the study, 64 were allocated to the lifestyle-intervention group and 34 to

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TABLE 1. Baseline characteristics.

	Standard Care	Lifestyle Intervention	Total
n (M/F)	34 (18/16)	64 (33/31)	98 (51/47)
Age, yr[a]	54 (50–64)	54 (48–59)	54 (49–61)
Body composition			
Bodyweight, kg[a]	97.3 (89.5–104.7)	93.9 (84.0–103.1)	95.1 (85.3–104.2)
Fat mass, kg[a]	37.2 (26.1–45.8)	35.2 (28.1–42.6)	35.2 (27.9–43.7)
Fat free mass, kg[a]	61.7 (52.4–67.2)	59.0 (48.7–68.2)	59.7 (51.0–68.0)
Android fat mass, kg[a]	4.0 (3.0–5.0)	4.0 (3.0–4.8)	4.0 (3.0–5.0)
Gynoid fat mass, kg[a]	5.6 (3.6–7.0)	5.4 (4.1–6.5)	5.5 (4.0–6.6)
Glucose metabolism			
HbA1c, mmol·L^{-1}[a]	50 (43–57)	48 (42–56)	48 (42–56)
iAUC glucose, mmol·L^{-1}·min^{-1}[a]	798.4 (712.5–919.5)	797.9 (683.6–956.6)	798.7 (685.5–951.8)
Systemic inflammation			
CRP > 2 mg·L^{-1}, no. (%)[a]	14 (41)	22 (34)	36 (37)
IL-1RA, pg·mL^{-1}[a,b]	294 (213–587)	299 (202–447)	297.7 (207.0–475.6)
TNF-α, pg·mL^{-1}[a,b]	1.97 (1.56–2.50)	2.02 (1.63–2.42)	2.00 (1.61–2.45)
IL-6, pg·mL^{-1}[a,b]	0.54 (0.39–0.68)	0.55 (0.34–0.75)	0.54 (0.35–0.72)
Fitness			
$\dot{V}O_{2max}$ mL·min^{-1}[a,b]	2511 (2099–3032)	2623 (2147–3145)	2572 (2116–3096)
$\dot{V}O_{2max}$ mL·kg^{-1}·min^{-1}[a,b]	28.1 (22.3–30.9)	26.6 (24.1–33.3)	27.0 (24.0–32.8)
Bone turnover			
CTX-I, ng·mL^{-1}[c]	0.193 (0.119–0.304)	0.213 (0.124–0.320)	0.211 (0.124–0.317)
PINP, ng·mL^{-1}[c]	33.1 (25.1–43.2)	36.4 (27.7–43.9)	35.0 (26.9–43.7)
OC, ng·mL^{-1}[c]	11.8 (10.3–15.8)	14.9 (10.3–19.2)	13.2 (10.3–17.7)
Total BMD, g·cm^{-2}[c]	1.30 (1.25–1.37)	1.27 (1.21–1.34)	1.28 (1.22–1.35)

Data reported as median (IQR).
[a]Baseline characteristics have been published previously (31,32).
[b]Controls, *n* = 32. Lifestyle intervention, *n* = 62. Total, *N* = 94.
[c]Controls, *n* = 27. Lifestyle intervention, *n* = 59. Total, *N* = 86.
F, female; M, male.

standard care. Ninety-three participants completed the follow-up. A detailed flowchart, as well as data on adherence to the intervention, has been published previously (32). Supplemental digital content 2 illustrates a study-specific flow chart (see Figure, Supplemental Digital Content 2, flow of participants through the study, http://links.lww.com/MSS/C420). Participants in the lifestyle intervention group completed 82% of the prescribed exercise sessions during the study period. At 12 months of follow-up, 71% of the lifestyle intervention group and 83% of the standard care group adhered to glucose-lowering medication. Within the lifestyle intervention group, 32 adverse events were reported with the vast majority being mild hypoglycemia and musculoskeletal pain. One participant in the lifestyle intervention group suffered from a hip fracture in relation to an exercise session. Detailed information on medical adherence and adverse events was described previously (32).

Baseline characteristics are shown in Table 1.

As previously published (31,32), from baseline to 12 months of follow-up, $\dot{V}O_{2max}$ increased 6.6 mL·kg^{-1}·min^{-1} (95% CI, 4.4–8.7 mL·kg^{-1}·min^{-1}) more in the lifestyle intervention group compared to the standard care group. The lifestyle intervention group also had a 4.1-kg (95% CI, 1.5–6.8 kg) larger weight loss, including a 5.0-kg (95% CI, 2.1–7.8 kg) larger reduction in fat mass, a decrease in iAUC for glucose (−135.0 mmol·L^{-1}·min^{-1}; 95% CI, −197.5 to −72.5), and in IL-1RA (0.70 RGM; 95% CI, 0.58–0.85), compared with standard care. There were no between-group differences in plasma TNF-α or IL-6 but a significant decrease within the lifestyle intervention group (0.93 RGM;

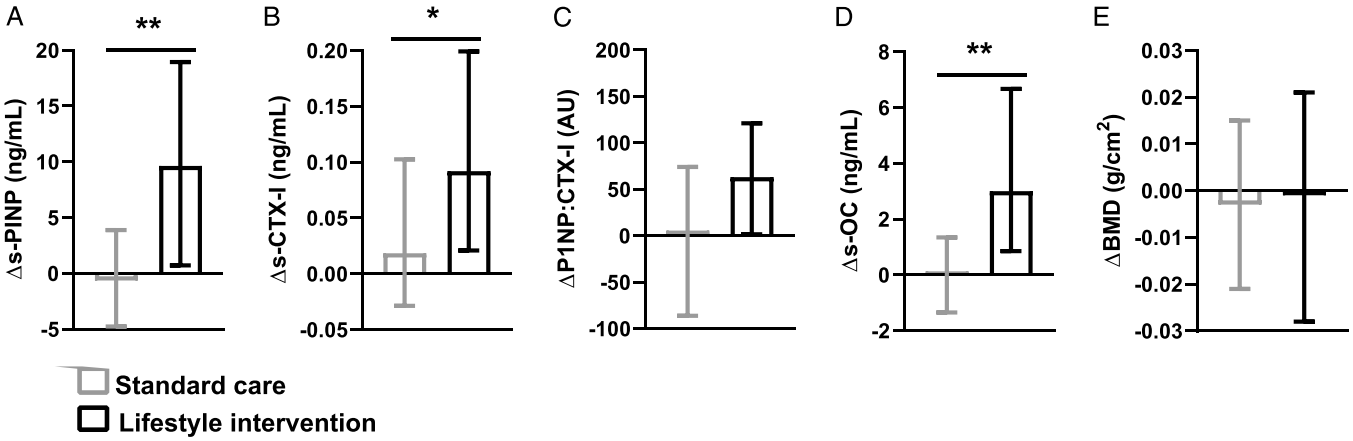

FIGURE 1—Changes in serum levels of BTM and BMD during the intervention in the lifestyle intervention group (*black*) and standard care (*gray*). A, s-N-terminal PINP. B, CTX-I. C, s-PINP/CTX-I ratio. D, s-OC. E, BMD. Data presented as median (IQR). Between-group differences, *$P < 0.05$, **$P < 0.01$.

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TABLE 2. Single-level mediation analyses.

	ΔPINP, ng·mL^{-1}	ΔCTX-I, ng·mL^{-1}	ΔOC, ng·mL^{-1}
	Effect of Mediator, % (95% CI)	Effect of Mediator, % (95% CI)	Effect of Mediator, % (95% CI)
Δ$\dot{V}O_{2\ max}$, mL·kg^{-1}·min^{-1}	35.0* (3.6 to 73.7)	52.7 (−2.2 to 121.4)	33.7* (8.2 to 67.1)
ΔiAUC glucose, mmol·L^{-1}·min^{-1}	−22.2* (−48.9 to −1.0)	−20.2 (−58.7 to 16.1)	8.8 (−15.3 to 31.3)
Δ Body weight, kg	−16.7* (−37.2 to −1.4)	−45.0* (−86.2 to −9.9)	−11.9 (−33.2 to 1.2)
Δ Fat mass, kg	−28.1* (−56.1 to −7.7)	−56.7* (−97.6 to −25.2)	−19.5* (−46.8 to −0.6)
ΔIL-1RA, pg·mL^{-1}	7.4 (−8.7 to 25.7)	−27.2* (−59.5 to −0.4)	7.3 (−5.3 to 24.0)

*$P < 0.05$.

95% CI, 0.89–0.98 and 0.81 RGM; 95% CI, 0.71–0.92, respectively).

Bone turnover and BMD after lifestyle intervention. From baseline to 12 months of follow-up s-PINP increased 34% (95% CI, 17%–50%) more in the lifestyle intervention group compared with the standard care group (Fig. 1A). Serum-CTX-I increased 36% (95% CI, 1%–71%) more in the lifestyle intervention group compared with the standard care group (Fig. 1B). Within the lifestyle intervention group serum-PINP/CTX-I ratio increased by 35% (95% CI, 22%–54%), with no change in the standard care group (4%, 95% CI, −30% to 29%). The change in s-PINP/CTX-I ratio at 12 months of follow-up was larger in the lifestyle intervention group compared with standard care but did not reach statistical significance (53%; 95% CI, −11.6% to 121%) (Fig. 1C).

S-OC increased 31% (95% CI, 11%–51%) more in the lifestyle intervention group compared with the standard care group (Fig. 1D). Bone mineral density did not change during the intervention in either of the groups (between-group difference, 0.1%; 95% CI, −1.1% to 1.2%, Fig. 1E).

Mediators of changes in bone turnover after lifestyle intervention. Single-mediation analyses revealed that increases in s-PINP with lifestyle intervention (ΔPINP) were statistically mediated by both improved $\dot{V}O_{2max}$ (35.0%; 95% CI, 3.6%–73.7%), lower iAUC glucose (22.2%; 95% CI, 1.0%–48.9%), decreased body weight (16.7%; 95% CI, 1.4%–37.2%), and decreased fat mass (28.1%; 95% CI, 7.7%–56.1%). IL-1RA was not a significant mediator of ΔPINP (Table 2).

Increased serum-CTX-I with lifestyle intervention (ΔCTX-I) was statistically mediated by decreased body weight (45.0%; 95% CI, 9.9%–86.2%), fat mass (56.7%; 95% CI, 25.2%–97.6%) and IL-1RA (27.2%; 95% CI, 0.4%–59.5%). Increased $\dot{V}O_{2max}$ was also a mediator of ΔCTX-I, although values did not reach statistical significance (52.7%; 95% CI, −2.2% to 121.4%). Incremental area under the curve glucose was not a statistical mediator of ΔCTX-I.

Increased s-OC induced by lifestyle intervention (ΔOC) was statistically mediated by both improved $\dot{V}O_{2max}$ (33.7%; 95% CI, 8.2%–67.1%) and decreased fat mass (19.5%; 95% CI, 0.6%–46.8%). Neither body weight, iAUC glucose, nor IL-1RA was significantly related to ΔOC.

Multivariate pattern analyses of changes in bone turnover after lifestyle intervention. In multivariate pattern analyses with all mediators fitted as explanatory variables for changes in BTM, we found that increased $\dot{V}O_{2max}$ as well as decreased iAUC glucose, body weight, and fat mass all significantly mediated ΔPINP. Decreased IL-1RA did not mediate ΔPINP. The full model explained 26.5% of the variance (Fig. 2A). Increased $\dot{V}O_{2max}$ as well as decreased body weight, fat mass, and IL-1RA significantly mediated ΔCTX-I. Incremental area under the curve glucose did not mediate ΔCTX-I. The full model explained 17.6% of the variance (Fig. 2B). Increased

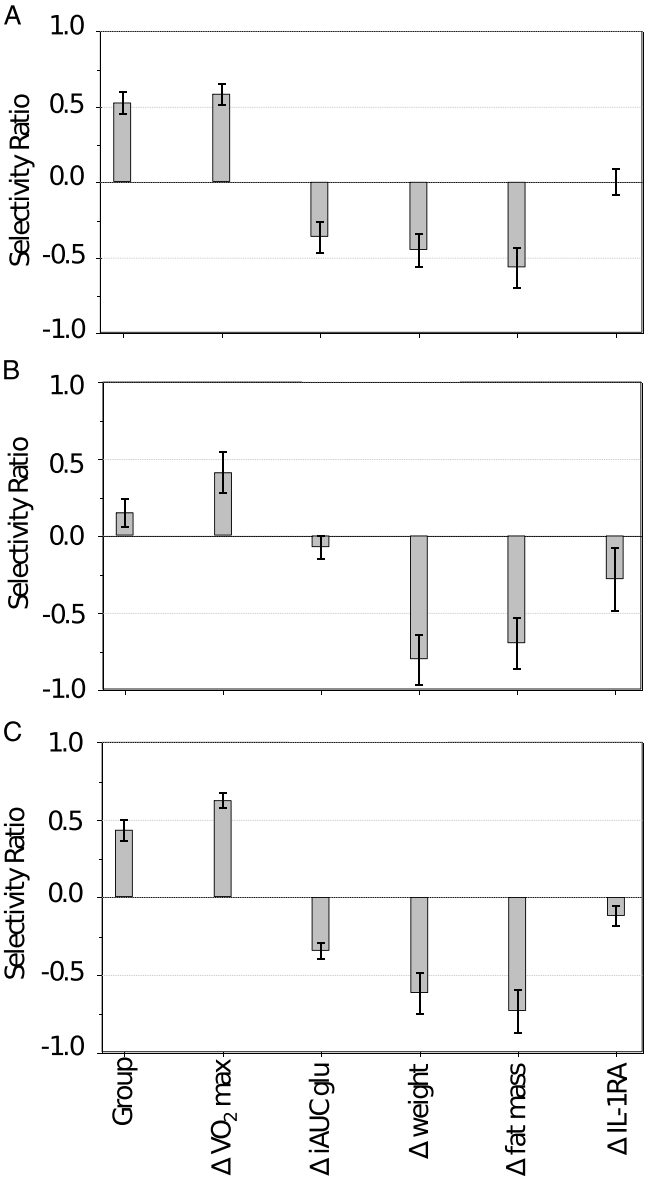

FIGURE 2—Multivariate signature of all explanatory variables for changes in bone turnover with the lifestyle intervention, displayed as a SR plot. A, s-N-terminal PINP. B, CTX-I. C, s-OC. Data presented as SR (95% CI).

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TABLE 3. Association between accumulated time spent on resistance training and changes in BTM during the intervention, in the lifestyle intervention group.

	Accumulated Time Spent on Resistance Training	
	β (95% CI)	*P*
ΔPINP, ng·mL^{-1}	0.06 (–0.08 to 0.20)	0.37
ΔCTX-I, ng·mL^{-1}	0.001 (–0.001 to 0.002)	0.36
ΔOC, ng·mL^{-1}	0.03 (–0.03 to 0.09)	0.34

$\dot{V}O_{2max}$ as well as decreased iAUC glucose, body weight, fat mass, and IL-1RA all significantly mediated ΔOC. The full model explained 16.9% of the variance (Fig. 2C).

Resistance training and changes in bone turnover. During the intervention, the lifestyle intervention group spent a median time of 56.9 min·wk^{-1} (interquartile range [IQR], 40.3–79.4 min·wk^{-1}) on resistance training, corresponding to 21% (IQR, 18%–24%) of total time spent exercising. Accumulated time spent on resistance training was not associated with significant changes in BTM (Table 3).

CTX-I suppression in relation to an OGTT after lifestyle intervention. Neither the absolute nor the relative suppression of CTX-I after oral glucose ingestion at both baseline and follow-up differed between lifestyle intervention and standard care (Fig. 3).

DISCUSSION

The primary findings of this study were that the 12-month exercise-based lifestyle intervention led to a substantial increase in BTM of 31% to 36% compared with standard care, with no changes in BMD. The anabolic BTM PINP and OC increased by 34% and 31%, respectively, whereas the resorptive marker CTX-I increased by 36%, with no significant difference in the PINP/CTX-I ratio, reflecting a balanced increase in bone turnover. We observed an increase of 9.6, 0.09, and 3.0 ng·mL^{-1} in PINP, CTX-I, and OC, respectively, in the intervention group. In line with this, serum levels of bone turnover makers were reported to be 10.5 ng·mL^{-1} (PINP), 0.11 ng·mL^{-1} (CTX-I), and 2.6 ng·mL^{-1} (OC) lower in persons with T2D compared with healthy controls, in a recent meta-analysis including up to 18,000 people (11). Thus, we speculate that the increase in BTM observed in this study likely portrays normalization of BTM rather than pathological increases.

We found that weight loss and loss of fat mass were the strongest statistical mediators of the observed increase in BTM. Previous studies show that weight loss, through caloric restriction alone, leads to increased bone resorption, a loss of BMD and an increased risk of frailty fractures (28,39,40). We found that the 12-month lifestyle intervention led to equal increases in CTX-I and PINP as well as preserved BMD despite a significant weight loss. In accordance with this, it has been shown that combining weight loss with resistance and endurance exercise in persons with obesity and prediabetes attenuated the unfavorable effects of weight loss on bone mass (28,30). Thus, the fact that increases in BTM were balanced and BMD was preserved, despite a reduction in body mass, could suggest that exercise is a beneficial way to prevent the bone loss that usually accompanies weight reductions.

Particularly weight bearing- and resistance-based exercise interventions have been shown to improve bone preservation (41). In this study, changes in BTM were not associated with accumulated time spent on resistance training. However, the exercise intervention in our study was primarily based on endurance exercise in combination with a varying amount of resistance training in two to three of the weekly exercise sessions and resistance training was not based on a predefined progressive resistance training plan. Furthermore, our measure of resistance training was based on accumulated time spent doing resistance training. This might not reflect the actual intensity participants underwent during the session as participants spending more time on resistance training could reflect the ones who trained with lower intensity. Thus, it is possible that this stimulus was insufficient to determine an isolated effect of resistance exercise on BTM in persons with T2D.

We found improved glycemic control to be a significant mediator of markers of bone formation, but not resorption, with the lifestyle intervention, suggesting that the net effect of improved glycemic control on bone turnover was mainly bone formation. In accordance with this, several previous

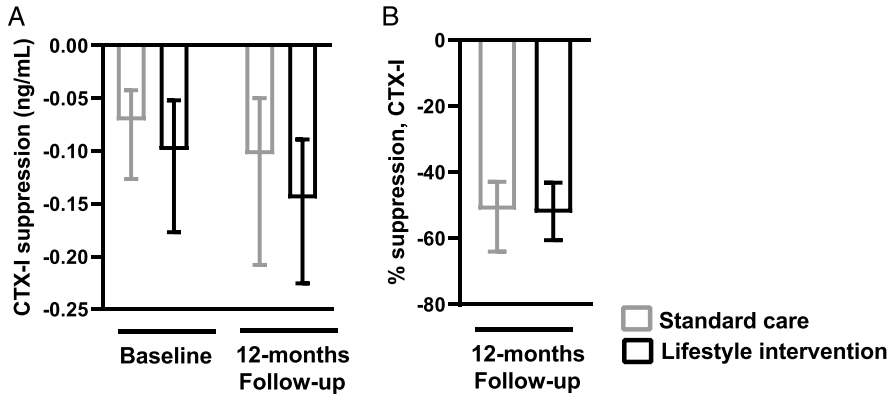

FIGURE 3—s-CTX-I suppression in response to an OGTT. A, Absolute s-CTX-I suppression at 120 min after an OGTT, before and after the intervention in the lifestyle intervention group (*black*) and standard care (*gray*). B, Percentage wise suppression of s-CTX-I at 120 min relative to fasting levels at 12 months of follow-up. Data presented as median (IQR).

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in vivo and *in vitro* studies support a beneficial role for improved glycemic control on the regulation of bone turnover (14,15,42).

Decreased IL-1RA with the lifestyle intervention statistically mediated the increased CTX-I, with limited effect on bone formation markers, indicating that the net effect of decreased IL-1RA plasma levels was bone resorption. Plasma IL-1RA levels reflects the body's response to counterbalance IL-1β levels and IL-1RA works as an IL-1β inhibitor (43,44). Whereas studies in rodents indicate that IL-1β induces bone resorption, IL-1RA or IL-1 blockage inhibits RANKL-induced macrophage to osteoclast differentiation (45,46) all in all pointing towards a resorption inhibiting effect of IL-1RA, which is in accordance with our findings.

Through the included statistical models, we were only able to explain 16%–25% of the variance in BTM in response to the lifestyle intervention. Thus, additional unmeasured factors are believed to contribute to the BTM increase. We used changes in $\dot{V}O_{2max}$ to assess the effects of exercise on bone turnover. However, it is highly likely, that the exercise itself contributes with several additional beneficial effects on bone turnover that were not reflected in $\dot{V}O_{2max}$.

We found CTX-I suppression, 120 min after glucose ingestion, to be comparable between groups. Previous studies, including persons with a similar duration and severity of T2D as this intervention, showed a blunted suppression of BTM in response to an OGTT in persons with T2D compared with healthy controls (47,48). In this study, the effect of the lifestyle intervention on glycemic control was modest due to the pharmacological treat-to-target approach, corresponding to an approximately 17% decrease in iAUC for glucose, after the intervention. This introduced a ceiling effect diminishing the impact of the lifestyle intervention on glycemic control (32). Thus, it is possible that the improvement in glycemic control observed in this study, was inadequate to affect postprandial CTX-I suppression. Furthermore, several participants in the standard care group were treated with GLP-1 analogs and metformin, which could affect their ability to postprandially suppress BTM. However, all medications were paused 48 h before testing. Lastly, we only measured CTX-I at 0 and 120 min after glucose ingestion which might conceal differences in CTX-I suppression between the study groups at other time points.

Previous studies suggest that treatment with GLP-1 analogs leads to BMD maintenance despite weight loss (49,50). Thus, we cannot exclude that GLP-1 analog treatment, in this study, contributed to the preserved BMD in both the lifestyle intervention group and with standard care. However, only 12% of patients in the lifestyle intervention group were treated with GLP-1 analogs at follow-up (vs 42% of the patients receiving standard care) making GLP-1 analogs less likely to substantially impact outcome variables, at least in the lifestyle intervention group. Furthermore, several meta-analyses have failed to prove beneficial effects of GLP-1 analog treatment on risk of fractures (51,52).

Our study has some limitations. The study was designed to test whether lifestyle intervention results in equivalent glycemic control compared with standard care and was therefore not planned nor adequately powered to investigate the assessed outcome variables. Despite the substantial effect of the intervention on bone turnover, the study design did not allow us to detect the clinical impact in terms of risk of fractures. Fracture risk assessment is based on several other components than bone turnover alone and it is highly likely that improvements in physical abilities in the lifestyle intervention group reduces the risk of falls, previously identified as a crucial component of decreasing fracture risk. In this study, we did not track falls or asymptomatic fractures during the study period and are, therefore, only able to speculate on this matter. Furthermore, the study population was relatively young and would, under normal circumstances, not be perceived at increased risk of falls.

We did not collect regional DXA-scans of the lumbar spine and hips, the golden standard to assess bone mass, or any histological or qualitative measures of bone structure, which is a limitation to the study. However, BTM have been shown to independently predict risk of fractures even after controlling for regional BMD (53).

Statistical mediation analyses and PLS regressions were applied to assess possible mediating links between the lifestyle intervention and changes in BTM, however causative conclusions cannot be drawn.

We did not control or measure calcium and vitamin D intake and exposure during the study period. As these have been shown to greatly impact bone metabolism, we cannot rule out that differential exposure during the study period could confound our results (54). Furthermore, effects of long-term exercise on calcium metabolism are poorly understood and could also affect study outcomes (55).

Even though several studies have identified low BTM as a potential contributing factor to the increased fracture risk with T2D the clinical impact of elevating these to levels of nondiabetics remains unknown.

CONCLUSIONS

In conclusion, a 12-month intensive exercise-based lifestyle intervention, in persons with T2D, led to substantial but balanced increases in BTM toward levels seen with normoglycemia. The increase in BTM combined with a preserved BMD, despite a substantial weight loss, is likely to reflect improved bone health and warrants further studies addressing the impact of exercise on risk of fractures in persons with T2D.

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REFERENCES

1. Janghorbani M, Van Dam RM, Willett WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. *Am J Epidemiol*. 2007;166(5):495–505.
2. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. *Osteoporos Int*. 2007;18(4):427–44.
3. Oei L, Zillikens MC, Dehghan A, et al. High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: the Rotterdam Study. *Diabetes Care*. 2013; 36(6):1619–28.
4. de Abreu LLF, Holloway-Kew KL, Sajjad MA, Kotowicz MA, Pasco JA. FRAX (Australia) scores in women with impaired fasting glucose and diabetes. *Bone Rep*. 2019;11:100223.
5. Poiana C, Capatina C. Fracture risk assessment in patients with diabetes mellitus. *J Clin Densitom*. 2017;20(3):432–43.
6. Kanazawa I, Tanaka KI, Takeo A, et al. A scoring assessment tool for the risk of vertebral fractures in patients with type 2 diabetes mellitus. *Bone*. 2019;122:38–44.
7. Napoli N, Chandran M, Pierroz DD, et al. Mechanisms of diabetes mellitus-induced bone fragility. *Nat Rev Endocrinol*. 2017;13(4): 208–19.
8. Krakauer JC, McKenna MJ, Buderer NF, et al. Bone loss and bone turnover in diabetes. *Diabetes*. 1995;44(7):775–82.
9. Manavalan JS, Cremers S, Dempster DW, et al. Circulating osteogenic precursor cells in type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2012;97(9):3240–50.
10. Purnamasari D, Puspitasari MD, Setiyohadi B, Nugroho P, Isbagio H. Low bone turnover in premenopausal women with type 2 diabetes mellitus as an early process of diabetes-associated bone alterations: a cross-sectional study. *BMC Endocr Disord*. 2017;17(1):72.
11. Hygum K, Starup-Linde J, Harslof T, Vestergaard P, Langdahl BL. Mechanisms in endocrinology: diabetes mellitus, a state of low bone turnover - a systematic review and meta-analysis. *Eur J Endocrinol*. 2017;176(3):R137–57.
12. Kanazawa I, Yamaguchi T, Yamamoto M, et al. Serum osteocalcin/bone-specific alkaline phosphatase ratio is a predictor for the presence of vertebral fractures in men with type 2 diabetes. *Calcif Tissue Int*. 2009;85(3):228–34.
13. Jiajue R, Jiang Y, Wang O, et al. Suppressed bone turnover was associated with increased osteoporotic fracture risks in non-obese postmenopausal Chinese women with type 2 diabetes mellitus. *Osteoporos Int*. 2014;25(8):1999–2005.
14. Starup-Linde J, Hygum K, Langdahl BL. Skeletal fragility in type 2 diabetes mellitus. *Endocrinol Metab (Seoul)*. 2018;33(3):339–51.
15. Stage TB, Christensen MH, Jorgensen NR, et al. Effects of metformin, rosiglitazone and insulin on bone metabolism in patients with type 2 diabetes. *Bone*. 2018;112:35–41.
16. Wang L, Li T, Liu J, et al. Association between glycosylated hemoglobin A1c and bone biochemical markers in type 2 diabetic postmenopausal women: a cross-sectional study. *BMC Endocr Disord*. 2019;19(1):31.
17. Tonks KT, White CP, Center JR, Samocha-Bonet D, Greenfield JR. Bone turnover is suppressed in insulin resistance, independent of adiposity. *J Clin Endocrinol Metab*. 2017;102(4): 1112–21.
18. Balint E, Szabo P, Marshall CF, Sprague SM. Glucose-induced inhibition of in vitro bone mineralization. *Bone*. 2001;28(1):21–8.
19. Oshima K, Nampei A, Matsuda M, et al. Adiponectin increases bone mass by suppressing osteoclast and activating osteoblast. *Biochem Biophys Res Commun*. 2005;331(2):520–6.
20. Ducy P, Amling M, Takeda S, et al. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell*. 2000;100(2):197–207.
21. Eleftheriadis T, Kartsios C, Antoniadi G, et al. The impact of chronic inflammation on bone turnover in hemodialysis patients. *Ren Fail*. 2008;30(4):431–7.
22. Koh JM, Khang YH, Jung CH, et al. Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. *Osteoporos Int*. 2005;16(10):1263–71.
23. Kong YY, Feige U, Sarosi I, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature*. 1999;402(6759):304–9.
24. Carstensen M, Herder C, Kivimaki M, et al. Accelerated increase in serum interleukin-1 receptor antagonist starts 6 years before diagnosis of type 2 diabetes: Whitehall II prospective cohort study. *Diabetes*. 2010;59(5):1222–7.
25. Khosla S, Peterson JM, Egan K, Jones JD, Riggs BL. Circulating cytokine levels in osteoporotic and normal women. *J Clin Endocrinol Metab*. 1994;79(3):707–11.
26. Langdahl BL, Lokke E, Carstens M, Stenkjaer LL, Eriksen EF. Osteoporotic fractures are associated with an 86-base pair repeat polymorphism in the interleukin-1-receptor antagonist gene but not with polymorphisms in the interleukin-1beta gene. *J Bone Miner Res*. 2000;15(3):402–14.
27. Villareal DT, Chode S, Parimi N, et al. Weight loss, exercise, or both and physical function in obese older adults. *N Engl J Med*. 2011; 364(13):1218–29.
28. Shah K, Armamento-Villareal R, Parimi N, et al. Exercise training in obese older adults prevents increase in bone turnover and attenuates decrease in hip bone mineral density induced by weight loss despite decline in bone-active hormones. *J Bone Miner Res*. 2011;26(12):2851–9.
29. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of diabetes. *Diabetes Care*. 2015;38(1):140–9.
30. Skoradal MB, Weihe P, Patursson P, et al. Football training improves metabolic and cardiovascular health status in 55- to 70-year-old women and men with prediabetes. *Scand J Med Sci Sports*. 2018; 28(1 Suppl):42–51.
31. Johansen MY, Karstoft K, MacDonald CS, et al. Effects of an intensive lifestyle intervention on the underlying mechanisms of improved glycaemic control in individuals with type 2 diabetes: a secondary analysis of a randomised clinical trial. *Diabetologia*. 2020;63(11): 2410–22.
32. Johansen MY, MacDonald CS, Hansen KB, et al. Effect of an Intensive lifestyle intervention on glycemic control in patients with type 2 diabetes: a randomized clinical trial. *JAMA*. 2017;318(7):637–46.
33. Ried-Larsen M, Christensen R, Hansen KB, et al. Head-to-head comparison of intensive lifestyle intervention (U-TURN) versus conventional multifactorial care in patients with type 2 diabetes: protocol and rationale for an assessor-blinded, parallel group and randomised trial. *BMJ Open*. 2015;5(12):e009764.

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34. MacDonald CS, Johansen MY, Nielsen SM, et al. Dose-response effects of exercise on glucose-lowering medications for type 2 diabetes: a secondary analysis of a randomized clinical trial. *Mayo Clin Proc*. 2020;95(3):488–503.
35. Ried-Larsen M, MacDonald CS, Johansen MY, et al. Why prescribe exercise as therapy in type 2 diabetes? We have a pill for that! *Diabetes Metab Res Rev*. 2018;34(5):e2999.
36. Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. *BMJ*. 1990;300(6719):230–5.
37. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol*. 1986;51(6):1173–82.
38. Wold SRA, Wold H, Dunn WJ. The collinearity problem in linear regression the partial least squares (PLS) approach to generalized inverses. *SIAM J Sci Stat Comput*. 1984;5(3):735–43.
39. Chen X, Zhang J, Zhou Z. Changes in bone mineral density after weight loss due to metabolic surgery or lifestyle intervention in obese patients. *Obes Surg*. 2021;31(3):1147–57.
40. Komorita Y, Iwase M, Fujii H, et al. Impact of body weight loss from maximum weight on fragility bone fractures in Japanese patients with type 2 diabetes: the Fukuoka Diabetes Registry. *Diabetes Care*. 2018;41(5):1061–7.
41. Hinton PS, Nigh P, Thyfault J. Effectiveness of resistance training or jumping-exercise to increase bone mineral density in men with low bone mass: a 12-month randomized, clinical trial. *Bone*. 2015;79:203–12.
42. Shao X, Cao X, Song G, Zhao Y, Shi B. Metformin rescues the MG63 osteoblasts against the effect of high glucose on proliferation. *J Diabetes Res*. 2014;2014:453940.
43. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev*. 2018;281(1):8–27.
44. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol*. 2011;11(2):98–107.
45. Kitazawa R, Kimble RB, Vannice JL, Kung VT, Pacifici R. Interleukin-1 receptor antagonist and tumor necrosis factor binding protein decrease osteoclast formation and bone resorption in ovariectomized mice. *J Clin Invest*. 1994;94(6):2397–406.
46. Lee YM, Fujikado N, Manaka H, Yasuda H, Iwakura Y. IL-1 plays an important role in the bone metabolism under physiological conditions. *Int Immunol*. 2010;22(10):805–16.
47. Maagensen H, Junker AE, Jorgensen NR, et al. Bone turnover markers in patients with nonalcoholic fatty liver disease and/or type 2 diabetes during oral glucose and isoglycemic intravenous glucose. *J Clin Endocrinol Metab*. 2018;103(5):2042–9.
48. Lopes LS, Schwartz RP, Ferraz-de-Souza B, et al. The role of enteric hormone GLP-2 in the response of bone markers to a mixed meal in postmenopausal women with type 2 diabetes mellitus. *Diabetol Metab Syndr*. 2015;7:13.
49. Hygum K, Harslof T, Jorgensen NR, et al. Bone resorption is unchanged by liraglutide in type 2 diabetes patients: a randomised controlled trial. *Bone*. 2020;132:115197.
50. 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(8):2909–17.
51. Driessen JH, de Vries F, van Onzenoort H, et al. The use of incretins and fractures - a meta-analysis on population-based real life data. *Br J Clin Pharmacol*. 2017;83(4):923–6.
52. Zhang YS, Weng WY, Xie BC, et al. Glucagon-like peptide-1 receptor agonists and fracture risk: a network meta-analysis of randomized clinical trials. *Osteoporos Int*. 2018;29(12):2639–44.
53. Tian A, Ma J, Feng K, et al. Reference markers of bone turnover for prediction of fracture: a meta-analysis. *J Orthop Surg Res*. 2019;14(1):68.
54. Jorde R, Stunes AK, Kubiak J, et al. Effects of vitamin D supplementation on bone turnover markers and other bone-related substances in subjects with vitamin D deficiency. *Bone*. 2019;124:7–13.
55. Lombardi G, Ziemann E, Banfi G, Corbetta S. Physical activity-dependent regulation of parathyroid hormone and calcium-phosphorous metabolism. *Int J Mol Sci*. 2020;21(15):5388.

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