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Effects of an Intensive Lifestyle Intervention on Bone Turnover in Persons with Type 2 Diabetes: A secondary analysis of a randomized clinical trial

Abildgaard J[1,2], Johansen MY[1], Skov-Jeppesen K[3,4], Andersen LB[5], Karstoft K[1,6], Hansen KB[7], Hartmann B[3,4], Holst JJ[3,4], Pedersen BK[1], Ried-Larsen M[1]

[1] The Centre of Inflammation and Metabolism and the Centre for Physical Activity Research, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

[2] Department of Growth and Reproduction, Copenhagen University Hospital, Rigshospitalet, Denmark

[3] Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

[4] NNF Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

[5] Department of Sport, Food and Natural Sciences, Faculty of Education, Arts and Sports, Western Norway University of Applied Sciences, Campus Sogndal, Sogndal, Norway

[6] Department of Clinical Pharmacology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark.

[7] Steno Diabetes Center Copenhagen, Gentofte, Denmark.

Corresponding author:

ABSTRACT

Background

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. However, the effects of exercise-based lifestyle interventions on bone turnover and bone mineral density (BMD) in persons with T2D remains to be elucidated.

Methods

Randomized controlled trial including 98 persons with T2D, randomized to either a 12-months intensive lifestyle intervention and standard care (n=64) or standard care alone (n=34). The lifestyle intervention included five to six weekly aerobic training sessions, half of them combined with resistance training, as well as sessions with a clinical dietician. Serum markers of bone turnover (osteocalcin (OC), N-terminal propeptide of type-I procollagen (PINP), reflecting bone formation, and carboxyterminal collagen I crosslinks (CTX-I), reflecting bone resorption) and BMD (by DXA scan) were measured before the intervention and again at follow-up.

Results

From baseline to follow-up, s-PINP increased by 34 % (95 % CI: 17 – 50 %), s-CTX-I by 36 % (95 % CI: 1 – 71 %), and s-OC by 31 % (95 % CI: 11 – 51 %) more in the lifestyle intervention group compared with standard care. BMD was unaffected by the intervention (Δ BMD: 0.1 %, 95 % CI: -1.1 to 1.2 %).

Furthermore, the lifestyle intervention group lost 5.0 kg (95 % CI: 2.1 – 7.8 kg) fat mass and improved their $VO_{2\ max}$ by 6.6 mL/kg/min (95 % CI: 4.4 – 8.7 mL/kg/min).

Conclusions

A 12-months intensive exercise-based lifestyle intervention led to a substantial but balanced increase in bone turnover in persons with T2D. The increase in BTMs 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 in persons with T2D on risk of fractures.

INTRODUCTION

Fragility fractures are increasingly recognized as an important complication to type 2 diabetes (T2D) despite a normal to increased bone mineral density (BMD) in T2D (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 (BTMs) 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 to healthy controls (10, 11). Furthermore, both low PINP and low OC/alkaline phosphatase ratio have been related to 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 BTMs (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 multi-potent 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 non-diabetic 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-week exercise intervention with soccer, showed a substantial increase in both

BTMs 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 following a 12-months 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 $VO_{2\,max}$, decreased low-grade inflammation, or perhaps a combination of these factors.

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) following 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 years 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 ≥300mg/g, or plasma creatinine ≥1.47 mg/dL.

Intervention

At least six weeks prior to baseline measurements, all participants had their glucose-lowering, lipid-lowering, and antihypertensive medications titrated by the study endocrinologist to obtain prespecified treatment targets (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 30-60 minutes duration of which two to three sessions were combined with resistance exercise. The initial four 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 distribution of 45 % - 60 % carbohydrate, 15 % - 20 % protein, and 20 % - 35 % fat (< 7 % saturated fat). During the first four months the total energy intake was restricted by approximately 25% through guidance from a dietitian.

Experimental day

Completed at baseline and 12 months' follow-up. Two days prior to 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. EDTA plasma tubes were immediately spun at 3500g for 15 minutes at 4°C. Serum was stored at room temperature for a minimum of 30 minutes 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/mL and intra assay coefficient of variation (CV) was < 10 %. PINP LOD was < 1.0 ng/mL and CV ≤ 5 %. OC LOD was 0.27 ng/mL and CV was < 10%.

Bone mineral density and body composition

Total body bone mineral content (BMC) and -BMD, and fat- and fat free masses were assessed on the basis of 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. CV is estimated to < 1 %. Software (Prodigy, enCORE 2004, version 8.8; GE Lunar Corp, Madison,WI,) was used to estimate regional BMC, BMD, and fat-, and fat-free tissue masses.

Additional measurements

An oral glucose tolerance test was performed using 83 grams of glucose monohydrate dissolved in 300 ml of water. Blood samples were drawn at 0, 15, 30, 60, 90, and 120 minutes. 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). Maximal oxygen uptake ($VO_{2\,max}$) was assessed using an incremental ergometer bicycle test (Monark 839E, Monark, Varberg, Sweden). The test included a five min warm-up followed by an increase of 20 watts/min until exhaustion (31). VO_2 was continuously measured by indirect calorimetry (Quark b2, Cosmed, Rome, Italy).

Plasma interleukin-1 receptor antagonist (IL-1RA), IL-6, and tumor necrosis factor (TNF)-α levels were determined as previously reported and published (31).

Statistical analyses

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 Analysis of Covariance (ANCOVA) performed with the absolute change of BTMs as dependent variables with group (2 levels), time (1 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 par cent 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 (DAGs) and were prespecified in a statistical analysis plan prior to analyses (*Figure s1*). Analyses were controlled for baseline values of outcome variables. Bootstrapping (5,000 resamples) was used to obtain bias corrected 95% CIs. 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 BTMs (adjusted outcome variables) we fitted in partial least squares (PLS) regression analyses (38). PLS 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 BTMs. The SR is the proportion of the total explained variance, that each variable explains independent of the other variables. The sign of the SRs 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. PLS 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 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 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 standard care. Ninety-three participants completed the follow-up.

A detailed flow-chart as well as data on adherence to the intervention has been published previously (32). *Figure s2* illustrates a study-specific flow chart. Baseline characteristics are shown in *Table 1*.

As previously published (31, 32), from baseline to 12-months follow-up, maximal oxygen uptake ($VO_{2\ max}$) increased 6.6 mL/kg/min (95 % CI: 4.4 to 8.7 mL/kg/min) 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 to 6.8 kg) larger weight loss including a 5.0 kg (95 % CI: 2.1 to 7.8 kg) larger reduction in fat mass, a decrease in iAUC for glucose (-135.0 mmol/l·min, 95 % CI: -197.5 to -72.5), and in interleukin (IL)-1RA (0.70 RGM, 95 % CI: 0.58 to 0.85), compared to 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, 95 % CI: 0.89 to 0.98 and 0.81 RGM, 95 % CI: 0.71 to 0.92, respectively).

Bone turnover and bone mineral density following lifestyle intervention

From baseline to 12-months follow-up s-PINP increased 34 % (95 % CI: 17 to 50 %) more in the lifestyle intervention group compared with the standard care group (*Figure 1A*). s-CTX-I increased 36 % (95 % CI: 1 to 71 %) more in the lifestyle intervention group compared with the standard care group (*Figure 1B*). Within the lifestyle intervention group s-PINP:CTX-I ratio increased by 35 % (95 % CI: 22 to 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 follow-up was larger in the lifestyle intervention group compared to standard care but did not reach statistical significance (53 %, 95 % CI: -11.6 to 121 %) (*Figure 1C*).

S-OC increased 31 % (95 % CI: 11 to 51 %) more in the lifestyle intervention group compared with the standard care group (*Figure 1D*). BMD did not change during the intervention in either of the groups (between group difference: 0.1 %, 95 % CI: -1.1 to 1.2 %, *Figure 1E*).

Mediators of changes in bone turnover following lifestyle intervention

Single-mediation analyses revealed that increases in s-PINP with lifestyle intervention (Δ PINP) were statistically mediated by both improved $VO_{2\ max}$ (35.0 %, 95 % CI: 3.6 to 73.7 %), lower iAUC glucose (22.2 %, 95 % CI: 1.0 to 48.9 %), decreased body weight (16.7 %, 95 % CI: 1.4 to 37.2 %), and decreased fat mass (28.1 %, 95 % CI: 7.7 to 56.1 %). IL-1RA was not a significant mediator of Δ PINP (*Table 2*). Increased s-CTX-I with lifestyle intervention (Δ CTX-I) was statistically mediated by decreased body weight (45.0 %, 95 % CI: 9.9 to 86.2 %), fat mass (56.7 %, 95 % CI: 25.2 to 97.6%) and IL-1RA (27.2 %, 95 % CI: 0.4 to 59.5 %). Neither iAUC glucose nor $VO_{2\ max}$ were significant mediators of Δ CTX-I. Increased s-OC induced by lifestyle intervention (Δ OC) was statistically mediated by both improved $VO_{2\ max}$ (33.7 %, 95 % CI: 8.2 to 67.1 %) and decreased fat mass (19.5 %, 95 % CI: 0.6 to 46.8 %). Neither body weight, iAUC glucose nor IL-1RA were significantly related to Δ OC.

Multivariate pattern analyses of changes in bone turnover following lifestyle intervention

In multivariate pattern analyses with all mediators fitted as explanatory variables for changes in BTMs, we found that increased $VO_{2\ max}$ as well as decreased iAUC glucose, body weight, and fat mass all significantly mediated Δ PINP. Decreased IL-1RA did not mediate Δ PINP. The full model explained 26.5 % of the variance (*Figure 2A*). Increased $VO_{2\ max}$ as well as decreased body weight, fat mass, and IL-1RA significantly mediated the decrease in CTX-I. iAUC glucose did not mediate Δ CTX-I. The full model explained 17.6 % of the variance (*Figure 2B*). Increased $VO_{2\ max}$ as well as decreased iAUC glucose, body weight, fat mass, and IL-1RA all significantly mediated the decrease in OC. The full model explained 16.9 % of the variance (*Figure 2C*).

Resistance training and changes in bone turnover

During the intervention, the lifestyle intervention group spent a median time of 56.9 min (IQR: 40.3 – 79.4 min) per week on resistance training, corresponding to 21 % (IQR: 18 – 24 %) of total time spent exercising. Accumulated amount of resistance training was not associated with significant changes in either s-PINP (p = 0.37), s-CTX-I (p = 0.36), s-OC (p = 0.34) (*Table 3*).

CTX-I suppression in relation to an OGTT following lifestyle intervention

During the study period, the absolute suppression of CTX-I following oral glucose ingestion showed no difference in change between groups (-13 %, 95 % CI: -72 – 46 % in favor of lifestyle intervention group) (*Figure 3A*). Furthermore, the relative suppression of CTX-I in response to glucose ingestion, at follow-up, did not differ between the groups (lifestyle intervention: 51.8 % vs. standard care 53.2 %, mean difference: 1.3 %, 95 % CI: -4.6 – 7.3 %) (*Figure 3B*).

DISCUSSION

The primary findings of this study were that the 12-months exercise-based lifestyle intervention led to a substantial increase in BTMs of 31 – 36 % compared to standard care, with no changes in BMD. The anabolic bone turnover markers 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 ng/ml, 0.09 ng/ml, and 3.0 ng/ml in PINP, CTX-I, and OC respectively, in the intervention group. In line with this, serum levels of bone turnover makers are reported to be 10.5 ng/ml (PINP), 0.11 ng/ml (CTX-I), and 2.6 ng/ml (OC) lower in persons with T2D compared to healthy controls, in a recent meta-analysis including up to18,000 people (11). Thus, we speculate that the increase in BTMs observed in this study likely portrays normalization of BTMs rather than pathological increases.

We found that weight loss and loss of fat mass were the strongest statistical mediators of the observed increase in BTMs. 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-months 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 BTMs 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 BTMs 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-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 and might not reflect the actual intensity participants underwent during the session. Thus, participants spending more time on resistance training could reflect the ones who trained with lower intensity.

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 *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 BTMs in response to the lifestyle intervention. Thus, additional unmeasured factors are believed to contribute to the BTM increase. We used changes in $VO_{2\ max}$ 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 $VO_{2\ max}$.

We found CTX-I suppression, 120 min following glucose ingestion, to be comparable between groups. Previous studies, including participants with a similar duration and severity of T2D as this intervention, showed a blunted suppression of BTMs in response to an OGTT in persons with T2D compared to 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, following 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, a substantial number of participants in the standard care group were treated with GLP-1 receptor agonists and metformin, which could affect their ability to postprandially suppress BTMs. However, all medications were paused 48 hours before testing. Lastly, we only measured CTX-I at 0- and 120-minutes following glucose ingestion which might conceal differences in CTX-I suppression between the study groups at other time points.

Our study has some limitations. The study was designed to test whether lifestyle intervention results in equivalent glycemic control compared to 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.

We did not collect regional DXA-scans of the lumbar spine and hips, the golden standard to assess bone mass, or any histological measures of bone quality which is a limitation to the study.

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

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

CONCLUSIONS

In conclusion, a 12-months intensive exercise-based lifestyle intervention, in persons with T2D, led to substantial but balanced increases in BTMs towards levels seen with normoglycemia. The increase in BTMs 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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	Standard Care	Lifestyle intervention	Total
N (M/F)	34 (18/16)	64 (33/31)	98 (51/47)
Age, years[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[a]	50 (43 – 57)	48 (42 – 56)	48 (42 - 56)
iAUC glucose, mmol/l·min[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, no. (%)[a]	14 (41)	22 (34)	36 (37)
IL-1RA, pg/ml[a, b]	294 (213 – 587)	299 (202 – 447)	297.7 (207.0 – 475.6)
TNF-α, pg/ml[a, b]	1.97 (1.56 – 2.50)	2.02 (1.63 – 2.42)	2.00 (1.61 – 2.45)
IL-6, pg/ml[a, b]	0.54 (0.39 – 0.68)	0.55 (0.34 – 0.75)	0.54 (0.35 – 0.72)
Fitness			
$VO_{2\ max}$ ml/min[a, b]	2,511 (2,099 – 3,032)	2,623 (2,147 – 3,145)	2,572 (2,116 – 3,096)
$VO_{2\ max}$ ml/kg/min[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[c]	0.193 (0.119 – 0.304)	0.213 (0.124 – 0.320)	0.211 (0.124 – 0.317)
PINP, ng/ml[c]	33.1 (25.1 – 43.2)	36.4 (27.7 – 43.9)	35.0 (26.9 – 43.7)
OC, ng/ml[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)
Total BMC, kg[c]	3.36 (2.84 – 3.63)	3.25 (2.70 – 3.68)	3.28 (2.76 – 3.64)

TABLE 1 *Baseline characteristics.*

Data reported as median (interquartile range).

iAUC; incremental area under the curve. IL-1RA; interleukin-1 receptor antagonist. CTX-I; carboxyterminal collagen I crosslinks. PINP; N-terminal propeptide of type-I procollagen. OC; Osteocalcin. BMD; bone mineral density. BMC; bone mineral content.

[a] Baseline characteristics have been published previously (31, 32).

[b] Controls, n=32. Total, n=94

[c] Controls, n=27. Lifestyle Intervention, n=59. Total, n=86.

	Δ PINP, ng/ml	Δ CTX-I, ng/ml	Δ OC, ng/ml
	Effect of mediator, % (95 % CI)	Effect of mediator, % (95 % CI)	Effect of mediator, % (95 % CI)
Δ $VO_{2\ max}$, ml/kg/min	35.0 (3.6 – 73.7)	52.7 (-2.2 – 121.4)	33.7 (8.2 – 67.1)
Δ iAUC glucose, mmol/l·min	-22.2 (-1.0 – -48.9)	-20.2 (-58.7 – 16.1)	8.8 (-15.3 – 31.3)
Δ body weight, kg	-16.7 (-1.4 – -37.2)	-45.0 (-86.2 – -9.9)	-11.9 (-33.2 – 1.2)
Δ Fat mass, kg	-28.1 (-7.7 – -56.1)	-56.7 (-97.6 – -25.2)	-19.5 (-46.8 – -0.6)
Δ IL-1RA, pg/mL	7.4 (-8.7 – 25.7)	-27.2 (-59.5 – -0.4)	7.3 (-5.3 – 24.0)

TABLE 2 *Single-level mediation analyses*.
iAUC; incremental area under the curve. IL-1RA, interleukin-1 receptor antagonist. CTX-I; carboxyterminal collagen I crosslinks. PINP; N-terminal propeptide of type-I procollagen. OC; Osteocalcin.

	Accumulated time spent on resistance training, min.	
	β (95 % CI)	p-value
Δ PINP, ng/ml	0.06 (-0.08 – 0.20)	0.37
Δ CTX-I, ng/ml	0.001 (-0.001 – 0.002)	0.36
Δ OC, ng/ml	0.03 (-0.03 – 0.09)	0.34

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

CTX-I; carboxyterminal collagen I crosslinks. PINP; N-terminal propeptide of type-I procollagen. OC; Osteocalcin.

FIGURE LEGENDS

FIGURE 1 *Changes in serum levels of bone turnover markers and bone mineral density during the intervention in the lifestyle intervention group (black) and standard care (grey).*

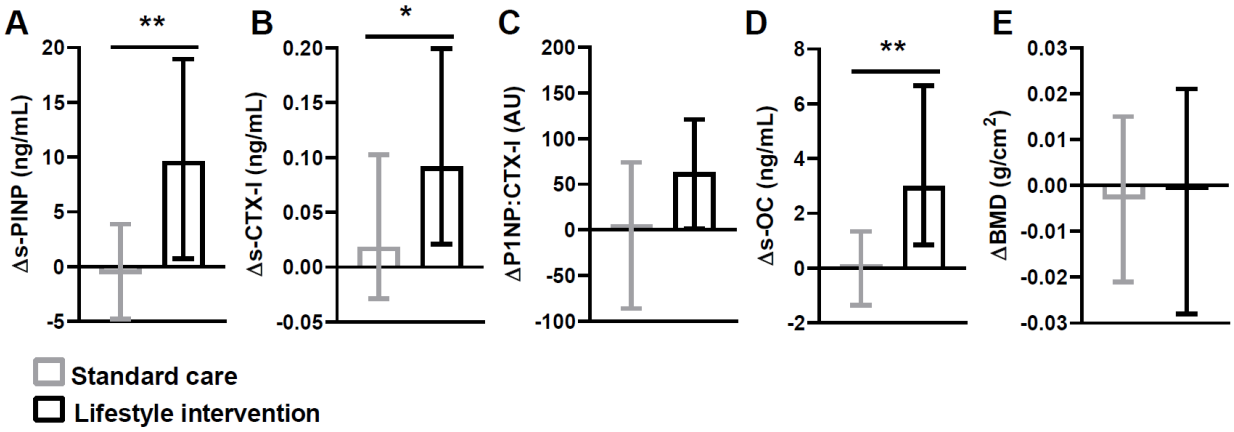
(A) s-N-terminal propeptide of type-I procollagen (PINP). (B) s-Carboxyterminal collagen I crosslinks (CTX-I). (C) s-PINP:CTX-I ratio. (D) s-Osteocalcin (OC). (E) bone mineral density (BMD). Data presented as median (interquartile range). Between group differences, *p < 0.05, ** p < 0.01.

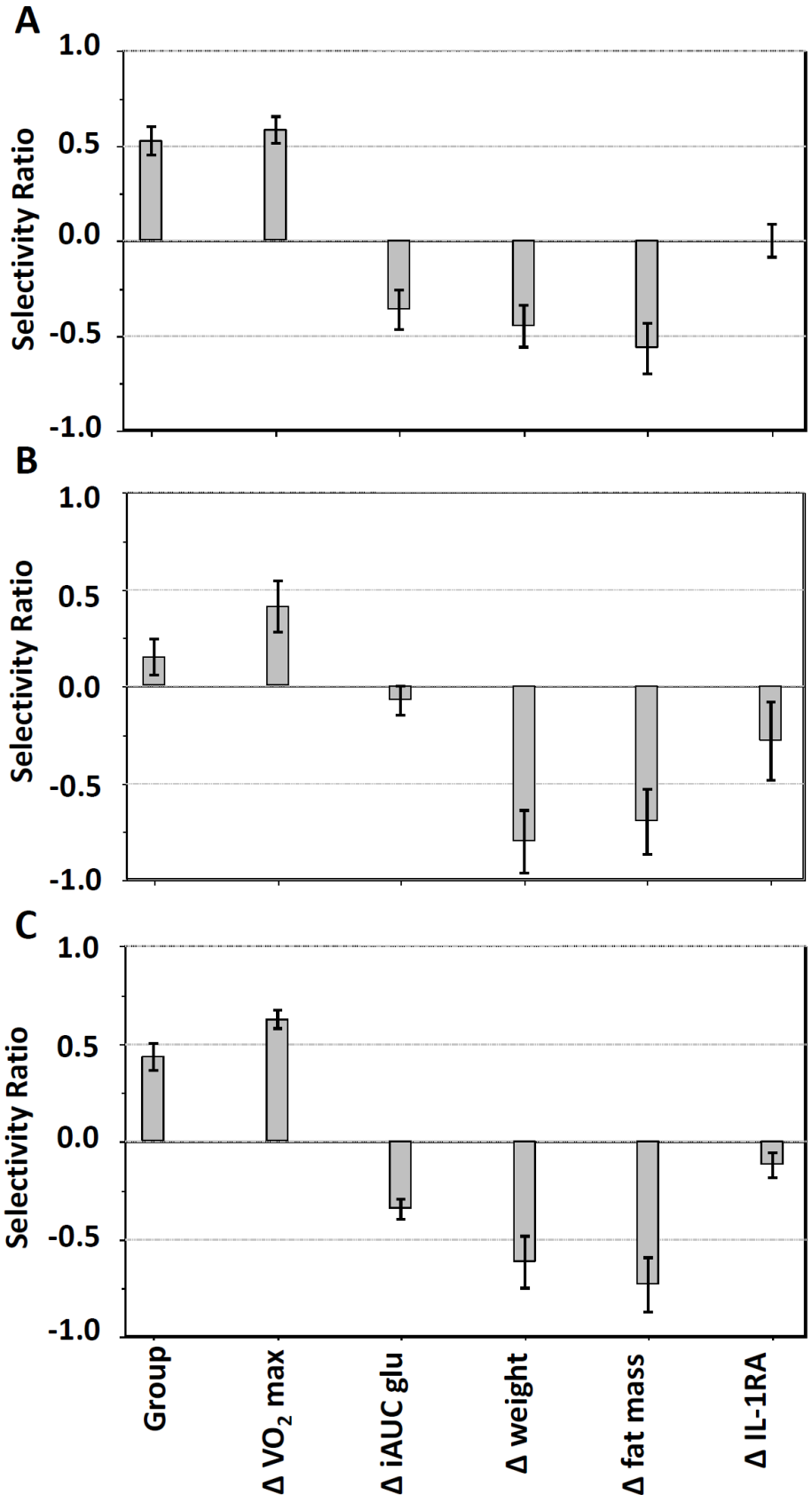
FIGURE 2 *Multivariate signature of all explanatory variables for changes in bone turnover with the lifestyle intervention, displayed as a selectivity ratio (SR) plot.*

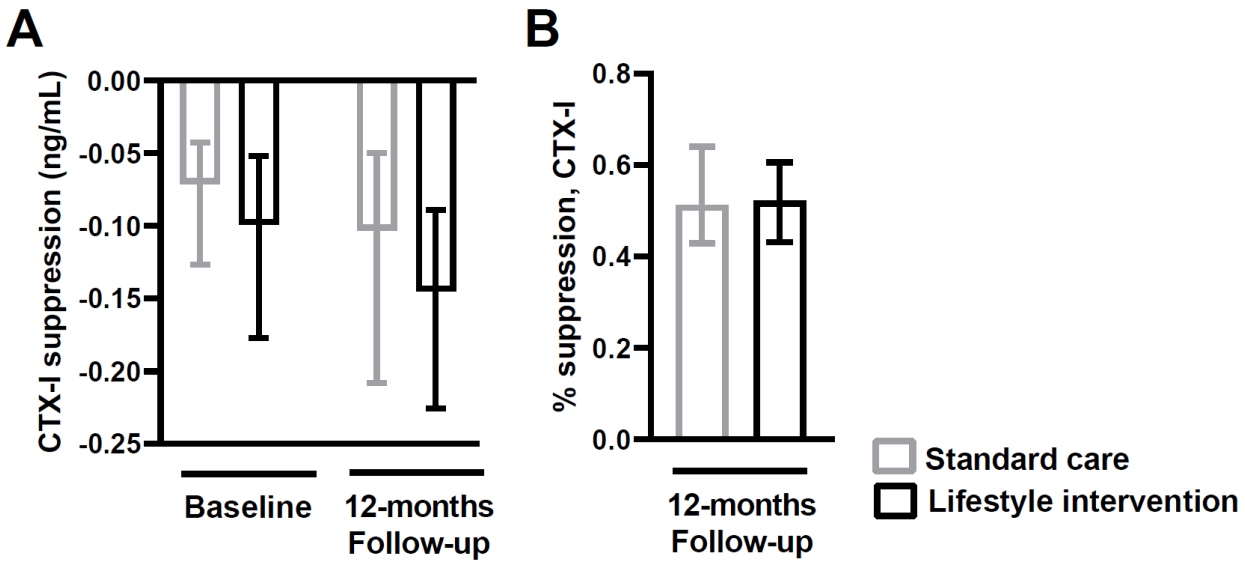
(A) s-N-terminal propeptide of type-I procollagen (PINP). (B) s-Carboxyterminal collagen I crosslinks (CTX-I). (C) s-Osteocalcin (OC). Data presented as SR (95 % CI).

FIGURE 3 *S-Carboxyterminal collagen I crosslinks (CTX-I) suppression in response to an oral glucose tolerance test (OGTT).*

(A) Absolute s-CTX-I suppression at 120 min following an OGTT, before and after the intervention in the lifestyle intervention group (black) and standard care (grey). (B) Percentage wise suppression of s-CTX-I at 120 minutes relative to fasting levels at 12 months follow-up. Data presented as median (interquartile range).




A
B
C
Selectivity Ratio
1.0
0.5
0.0
-0.5
-1.0
Group
Δ VO2 max
Δ iAUC glu
Δ weight
Δ fat mass
Δ IL-1RA


A
CTX-I suppression (ng/mL)
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
Baseline
12-months Follow-up
B
% suppression, CTX-I
0.8
0.6
0.4
0.2
0.0
12-months Follow-up
Standard care
Lifestyle intervention