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Bone marrow metabolism is affected by body weight and response to exercise training varies according to anatomical location

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Abstract

Aim: High body weight is a protective factor against osteoporosis, but obesity also suppresses bone metabolism and whole-body insulin sensitivity. However, the impact of body weight and regular training on bone marrow (BM) glucose metabolism is unclear. We studied the effects of regular exercise training on bone and BM metabolism in monozygotic twin pairs discordant for body weight.

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Methods: We recruited 12 monozygotic twin pairs (mean ± SD age 40.4 ± 4.5 years; body mass index 32.9 ± 7.6, mean difference between co-twins 7.6 kg/m^2; eight female pairs). Ten pairs completed the 6-month long training intervention. We measured lumbar vertebral and femoral BM insulin-stimulated glucose uptake (GU) using ^{18}F-FDG positron emission tomography, lumbar spine bone mineral density and bone turnover markers.

Results: At baseline, heavier co-twins had higher lumbar vertebral BM GU ($p < .001$) and lower bone turnover markers (all $p < .01$) compared with leaner co-twins but there was no significant difference in femoral BM GU, or bone mineral density. Training improved whole-body insulin sensitivity, aerobic capacity (both $p < .05$) and femoral BM GU ($p = .008$). The training response in lumbar vertebral BM GU was different between the groups (time × group, $p = .02$), as GU tended to decrease in heavier co-twins ($p = .06$) while there was no change in leaner co-twins.

Conclusions: In this study, regular exercise training increases femoral BM GU regardless of weight and genetics. Interestingly, lumbar vertebral BM GU is higher in participants with higher body weight, and training counteracts this effect in heavier co-twins even without reduction in weight. These data suggest that BM metabolism is altered by physical activity.

KEYWORDS
bone marrow, bone mineral density, exercise intervention, metabolism, obesity, positron emission tomography

1 | INTRODUCTION

Obesity suppresses bone metabolism and increases the risk of insulin resistance and type 2 diabetes.[1,2] Insulin resistance, in turn, is associated with low bone marrow (BM) metabolism.[3,4] We showed, that 2 weeks of exercise training improves femoral BM glucose and free fatty acid metabolism without changes in BM radiodensity or bone turnover markers.[3] There were no exercise-induced changes in lumbar vertebral BM. We postulate, that 2 weeks of training may not have been long enough to induce changes in these parameters in the previous study. In addition, the 2-week intervention was conducted using a bicycle ergometer, which does not induce high-impact loading that is beneficial for bone health.[5,6] The effects of long-term exercise training on BM metabolism remain elusive.

Genetics have an important role in the variation of exercise capacity and response to the same exercise training protocol.[7] Insulin resistance and the susceptibility to obesity are also influenced by genetics.[8,9] Monozygotic (MZ) twins originate from the same zygote and thereby their DNA sequences are identical. Thus, phenotypic differences within a MZ twin pair can be attributed to acquired lifestyle factors and environmental exposures. Furthermore, MZ twins share similar exposures and environment during early life.

In this study, we examined the effects of 6 months of regular exercise training, including high-impact loading exercise, on BM insulin-stimulated glucose uptake (GU), BM radiodensity, and bone mineral density (BMD) as well as bone turnover markers between MZ co-twins discordant for body weight. We hypothesized that BM GU would be impaired in heavier co-twins and that exercise training would have a beneficial effect on BM metabolism, BMD and bone turnover markers. This study design offers a unique way of studying the impact of acquired weight on bone and BM as the effects of genetics and shared environmental exposures during early life can be excluded in the analyses for causal factors.

2 | MATERIALS AND METHODS

2.1 | Ethics

This study is part of a larger study entitled 'Systemic cross-talk between brain, gut, and peripheral tissues in glucose homeostasis: effects of exercise training (CROSSYS)' (NCT03730610). The study protocol describing study design, participants and recruitment have been previously published.[10] The study was conducted at Turku PET Centre (University of Turku, Turku, Finland), Turku University Hospital (Turku, Finland), University of Turku (Turku, Finland) and Paavo Nurmi Centre (Turku, Finland) between January 2019 and October 2021 according to Good Clinical Practice and in compliance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland (decision 100/1801/2018 §438 and §548). Before any measurements were performed, the purpose and possible risks and benefits of the

study were explained and written informed consent was obtained for all participants.

2.2 | Study design and exercise training intervention

Study design is shown in Figure 1A. The exercise training intervention consisted of 27 ± 2 weeks of progressive exercise training including aerobic endurance, resistance and high-intensity interval training and the details of the exercise intervention have been previously described.[10] After the training intervention, all measurements were repeated. The training adherence based on heart-rate monitor (PolarA370; Polar) data[10] was 88% without a difference between co-twins.

2.3 | Participants

The participants were MZ twin pairs that were recruited from three unique population-based longitudinal twin studies.[10–13] The participants were all born in Finland and were of European descent.

Twelve MZ twin pairs, discordant for body mass index [BMI; eight female, four male pairs; 40.4 ± 4.5 years; mean BMI 32.9 ± 7.6 kg/m^2, mean difference between co-twins 7.6 kg/m^2 (min 2.2 kg/m^2, max 18.4 kg/m^2)], participated in our study. Of the leaner co-twins, five met the criteria for impaired fasting glucose (IFG) and two for impaired glucose tolerance (IGT) as defined by American Diabetes Association guidelines.[14] Of the heavier co-twins, seven met the criteria for IFG and two for IGT. Two co-twins in the heavier group were treated for hypertension. No participants were treated for diabetes, hyperlipidaemia, or used medication that affects bone metabolism. Medication use did not change during the study. All female participants were premenopausal, none used oral contraceptive medications but eight women had hormonal intrauterine devices. Participants were asked not to change their habitual dietary intake or physical activity outside of the intervention during the intervention. There were no differences in the reported amounts of total energy, carbohydrates, protein, and fat between the leaner and heavier co-twins at baseline or after the intervention. In total, three co-twins did not receive the intended intervention, one co-twin because of pregnancy midway through the intervention and one twin pair because of claustrophobic feeling of one co-twin (Figure 1B).

2.4 | Imaging data and euglycaemic-hyperinsulinaemic clamp

BM GU was measured using 2-[^{18}F]fluoro-2-deoxy-D-glucose (^{18}F-FDG) (155 ± 8 MBq) positron emission tomography (PET) imaging during the euglycaemic-hyperinsulinaemic clamp in a fasted state.[10] Before the euglycaemic-hyperinsulinaemic clamp and the PET study protocol, the antecubital veins from both arms were cannulated. One of the two catheters was used for the administration of glucose and insulin during the clamp study and injection of the PET tracer. The other catheter was used to obtain venous blood samples during the study. The tracer injection was given and PET imaging started ~84 min after the start of the clamp and lumbar vertebral and femoral regions were imaged ~56 and ~68 min after the injection, respectively. Computed tomography (CT) images were acquired for anatomical reference. Carimas software (http://turkupetcentre.fi) was used to manually draw three-dimensional regions of interest (ROIs) in the BM cavities of femurs (mid-diaphysis) and lumbar vertebrae (L2-L4) as described earlier.[3] Whole-body insulin-stimulated GU (M-value) was calculated from the glucose infusion rate.[15,16] BM radiodensity was analysed using CT by quantifying the tissue radiodensity in Hounsfield units (HU) from the same ROIs that were used for GU analysis. HU are inversely correlated to the fat content of that specific BM region.[17,18] Quantitative CT (QCT) was used to measure volumetric BMD in lumbar vertebrae L2-L4.[10,19,20] Visceral adipose tissue was measured with magnetic resonance imaging.[10] Carimas software was used to create fat fraction maps in which fat image of the T1 VIBE Dixon scan is divided by the sum of fat and water images. Visceral fat was segmented from fat fraction maps by drawing two-dimensional ROIs in every 5-10 slices starting from the ends of the heads of the femurs and ending to the xiphoid process, and creating a three-dimensional volume of interest from them using the interpolation feature of the Carimas software. Then, all voxels with an intensity value < .5 (i.e. fat fraction over 50%) were excluded and the remaining volume of interest was considered as visceral adipose tissue.

2.5 | Bone turnover markers

Blood samples were collected on the morning of the PET study day between 8 and 10 a.m. after an overnight fast (≥10 h) and EDTA plasma and serum samples were stored at −80°C. Bone formation was assessed by measuring intact N-terminal propeptides of type I collagen (PINP) with IDS-iSYS Intact PINP assay, and bone resorption by measuring C-terminal crosslinked telopeptides of type I collagen (CTX) with IDS-iSYS CTX-I (CrossLaps) assay (both from IDS Ltd, UK).[21] Bone remodelling was assessed by measuring bone-specific osteocalcin with two-site immunoassay as previously described.[22] Assay detects total osteocalcin (TotalOC) and is based on monoclonal antibodies 2H9 and 6F9. We also measured uncarboxylated form of osteocalcin (ucOC), which has been suggested to regulate glucose metabolism.[23] We used an immunoassay based on ucOC-specific recombinant antibody Fab-AP13[24] and expressed the results as the ratio of uncarboxylated to total osteocalcin (ucOC/TotalOC).

2.6 | Other measurements

Body composition was measured using a bioimpedance monitor (InBody 720; Biospace Co.). A 2-h, 75-g oral glucose tolerance test was performed in a fasted state (≥10 h) to assess the participants'

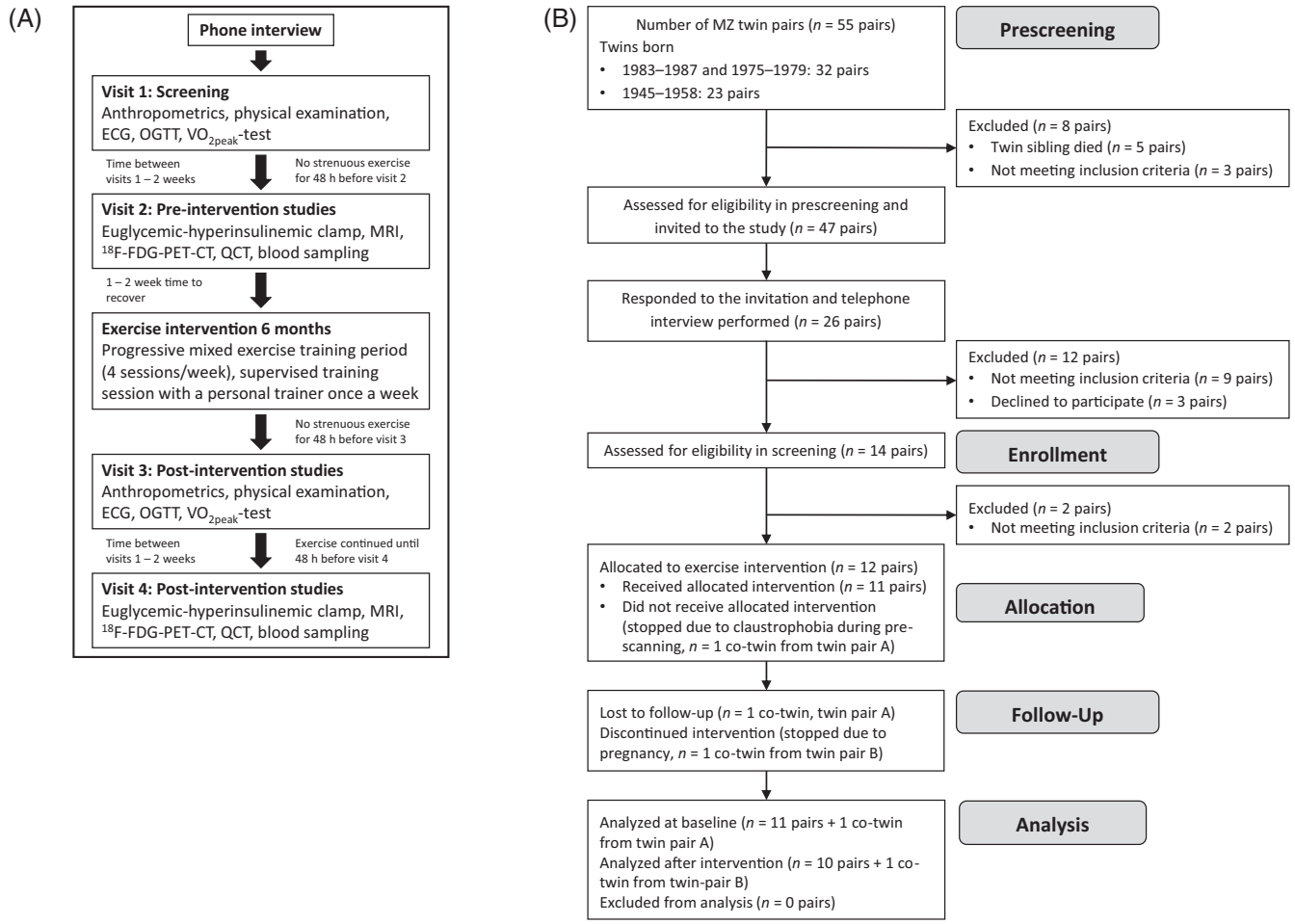

FIGURE 1 (A) Study design. (B) CONSORT flow diagram. (C) Transaxial MRI images showing the difference in body composition between leaner and heavier co-twin of a twin pair. ^{18}F-FDG, 2-[^{18}F]fluoro-2-deoxy-D-glucose; CT, computed tomography; ECG, electrocardiogram; MRI, magnetic resonance imaging; MZ, monozygotic; OGTT, oral glucose tolerance test; PET, positron emission tomography; QCT, quantitative computed tomography; VO_{2peak} test, aerobic capacity.

glycaemic status. Aerobic capacity was determined by an incremental bicycle ergometer test (Ergoline 800s; VIASYS Healthcare) to indicate the baseline aerobic performance as well as the effectiveness of the exercise training intervention.[10]

2.7 | Statistics

The normal distribution of variables was evaluated visually and tested using Shapiro-Wilk test. Logarithmic (log_{10}) [femoral BM GU, lumbar

TABLE 1 Participant characteristics between leaner and heavier co-twins before (pre) and after (post) exercise training intervention

	Leaner co-twins		Heavier co-twins		*p* Value		
Anthropometrics	Pre n = 12	Post n = 10	Pre n = 12	Post n = 11	Baseline	Time	Time × group
Age, years	40.4 (37.5; 43.4)		40.4 (37.5; 43.4)				
Weight, kg	86.4 (72.4; 100.4)	86.9 (72.6; 101.2)	108.7 (94.2; 123.3)	108.0 (93.1; 122.9)	**.001**	.95	.37
BMI, kg/m^2	29.1 (25.2; 33.0)	29.3 (25.3; 33.2)	36.7 (32.7; 40.7)	36.4 (32.4; 40.4)	**<.001**	.92	.41
Whole body fat, %	30.4 (21.3; 39.6)	29.5 (20.3; 38.7)	40.6 (36.5; 44.7)	40.0 (35.9; 44.1)	**<.001**	.37	.72
Visceral adipose tissue, kg	3.38 (2.13; 4.64)	3.22 (2.03; 4.42)	5.83 (4.74; 6.93)	5.46 (4.42; 6.50)	**.002**	.067	.28
Lean mass, kg	33.1 (30.0; 36.3)	33.9 (30.6; 37.2)	35.9 (31.9; 39.8)	36.2 (32.1; 40.3)	**.003**[b]	.14	.10
Systolic BP, mmHg	131.4 (120.4; 143.4)	122.3 (114.1; 131.1)	136.1 (128.4; 144.4)	126.8 (121.2; 132.7)	.38	**.011**[a]	.98[a]
Diastolic BP, mmHg	80.1 (73.3; 86.9)	77.1 (71.2; 83.0)	86.9 (80.2; 93.5)	78.3 (72.5; 84.0)	.074	**.017**	.091
VO_{2peak}, ml/kg/min	32.4 (26.9; 37.8)	35.1 (29.9; 40.2)	25.6 (23.2; 28.0)	28.3 (26.1; 30.6)	**.003**	**.001**	.94
hs-CRP, mg/L	0.81 (0.41; 1,61)	0.56 (0.21; 1.47)	1.42 (0.75; 2.70)	1.14 (0.46; 2.85)	**.005**[a]	.30[a]	.45[a]
Glucose profile							
Fasting glucose, mmol/L	5.5 (5.2; 5.7)	5.5 (5.2; 5.7)	5.7 (5.4; 5.9)	5.8 (5.6; 6.1)	.39	.37	.42
Fasting insulin, mU/L	6.6 (5.1; 8.7)	6.3 (4.3; 9.3)	11.1 (8.7; 14.2)	9.9 (6.9; 14.1)	**.006**[a]	.50[a]	.71[a]
HbA1c, mmol/mol	34.9 (32.9; 36.9)	34.7 (32.2; 37.1)	36.5 (35.0; 38.0)	36.0 (34.2; 37.7)	**.047**[a]	.58	.68
HbA1c, %	5.3 (5.2; 5.5)	5.3 (5.1; 5.5)	5.5 (5.4; 5.6)	5.4 (5.3; 5.6)	**.049**[a]	.59	.67
M-value, µmol/min/kg	37.5 (28.0; 47.0)	46.9 (31.7; 62.1)	23.0 (16.1; 29.9)	31.4 (20.4; 42.3)	**.007**	**.022**	.82

Note: all values are model-based means (95% confidence intervals). *p* Value for baseline indicates the differences between leaner and heavier co-twins. *p* Value for time indicates the change between pre- and post-measurements in the whole study group. *p* Value for the time × group interaction indicates if the mean change in the parameter was different between leaner and heavier co-twins. Statistically significant *p*-values ($p < .05$) are highlighted in **bold**. Eight female and four male twin pairs. For M-value leaner co-twins Pre n = 11, Post n = 10, heavier co-twins Pre n = 11, Post n = 9. For visceral adipose tissue leaner co-twins Pre n = 10, Post n = 10, heavier co-twins Pre n = 8, Post n = 9.

Abbreviations: BP, blood pressure; HbA1c, glycated haemoglobin; hs-CRP, high-sensitivity C-reactive protein; M-value, whole-body insulin sensitivity; VO_{2peak}, aerobic capacity.

[a]Logarithmic.

[b]Square root transformation was performed to fulfil normal distribution assumption.

vertebral BM GU, BMD, lumbar vertebral BM radiodensity, systolic blood pressure, high-sensitivity C-reactive protein (hs-CRP), fasting insulin, glycosylated haemoglobin (HbA1c), CTX, PINP, TotalOC] or square root (lean mass) transformations were performed to fulfil normal distribution.

Statistical analyses were performed using a linear mixed model for repeated time points using compound symmetry covariance structure. The differences between the co-twins were studied with the model, which included one or two within factors; twin effect, i.e. group defined as within-factor (group: leaner and heavier co-twins) and time as within-factor if outcome was measured several times (time; indicating the overall mean change between baseline and measurement after the intervention), and one interaction term (time × group: indicating whether mean change during the study was different between the leaner and heavier co-twins). The statistical unit was defined to be twin.

The analyses were carried out using the intention-to-treat principle and included all the participants. Because of the chosen analysis method, also participants with missing data were included into statistical modelling. Furthermore, model-based means (SAS least square means) and 95% confidence intervals (CI) are reported for all the parameters. Correlations were calculated using Pearson's correlation (Spearman's rank correlation for non-normally distributed data).

The statistical tests were performed as two-sided and the level of statistical significance was set at $p < .05$. The analyses were performed using the SAS System, version 9.4 for Windows (SAS Institute).

3 | RESULTS

Before intervention, heavier co-twins had lower aerobic capacity relative to body weight (VO_{2peak}, $p = .002$) and more visceral adipose tissue ($p = .002$) compared with leaner co-twins (Table 1). At baseline,

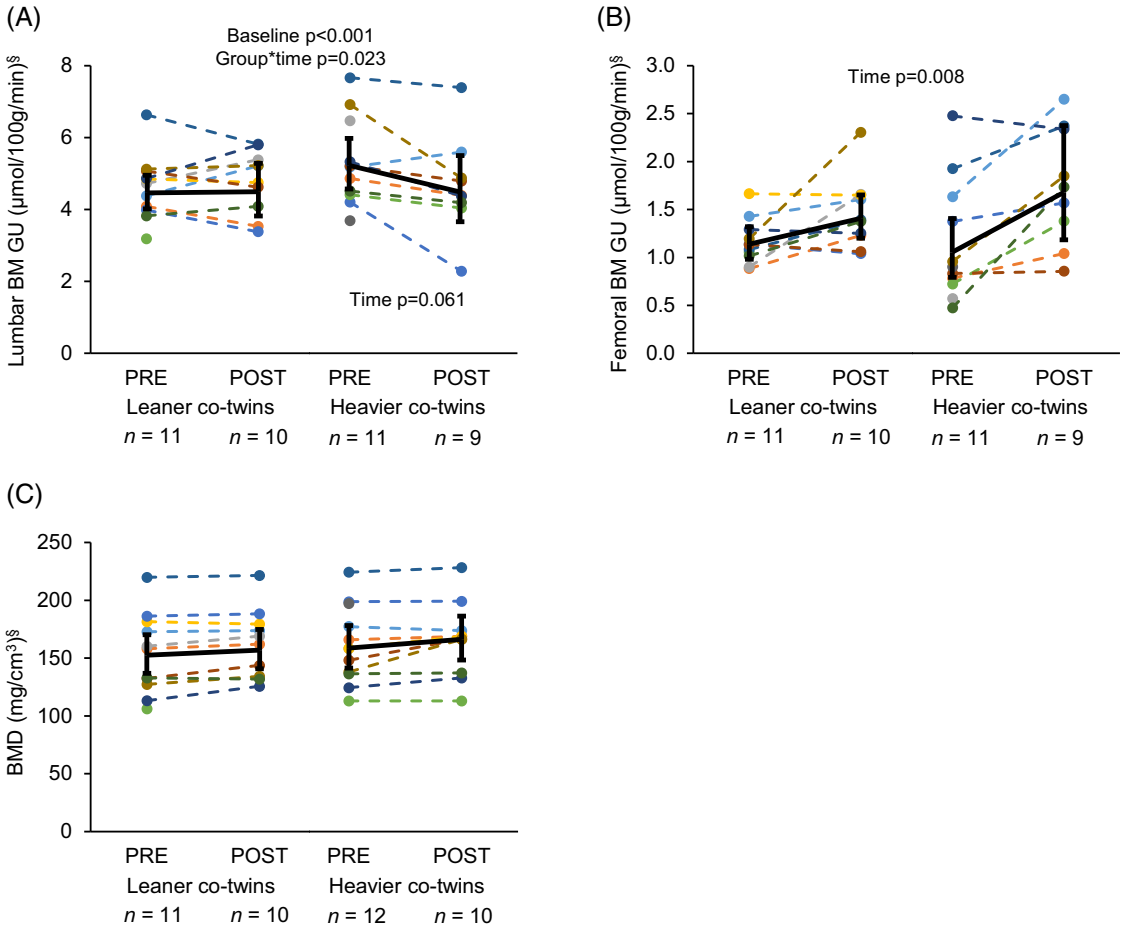

FIGURE 2 (A) Lumbar BM GU is higher in heavier co-twins compared with leaner co-twins at baseline and decreases after training intervention in heavier co-twins. (B) There is no significant difference in femoral BM GU between leaner and heavier co-twins at baseline, and GU increases in both groups after training intervention. (C) There is no difference in BMD between leaner and heavier co-twins before and after training intervention. Data are model-based means with 95% confidence intervals. *p* Value for baseline indicates the differences between leaner and heavier co-twins at baseline. *p* Value for time indicates the change between pre- and post-measurements in the whole study group. *p* Value for group × time interaction indicates if the mean change in the parameter was different between leaner and heavier co-twins. BM, bone marrow; BMD, bone mineral density; GU, insulin-stimulated glucose uptake. §Logarithmic transformation was performed to fulfil normal distribution assumption.

heavier co-twins also had a worse glucose profile manifested as higher fasting insulin and HbA1c, and lower whole-body insulin sensitivity (M-value) than leaner co-twins (all $p < .05$). Training improved aerobic capacity similarly in both groups ($p = .001$, Table 1). However, training had no statistically significant effect on body weight or body composition. Systolic and diastolic blood pressure decreased after training similarly in leaner and heavier co-twins (both $p < .05$). Whole-body insulin sensitivity increased in both groups similarly ($p = .022$).

Heavier co-twins had higher lumbar vertebral BM insulin-stimulated GU ($p < .001$) compared with their leaner co-twins at baseline (Figure 2A). No significant difference was observed between the groups in femoral BM GU (Figure 2B), BMD (Figure 2C), or lumbar vertebral or femoral BM radiodensity. Bone turnover markers TotalOC, PINP and CTX were all significantly lower (all $p < .01$) in heavier co-twins than in leaner co-twins at baseline (Figure 3A-C). There was no significant difference in plasma ucOC or plasma ucOC/TotalOC ratio between the groups (Figure 3D).

In all participants at baseline, lumbar vertebral BM GU correlated negatively with whole-body insulin sensitivity (M-value, $r = -0.52$, $p = .013$) and positively with body composition [BMI ($r = 0.54$, $p = .009$), fat mass ($r = 0.61$, $p = .003$) and whole-body fat percentage ($r = 0.52$, $p = .014$)], HbA1c ($r = 0.53$, $p = .011$) and hs-CRP ($r = 0.77$, $p < .001$). Lumbar vertebral BM radiodensity correlated positively with BMD ($r = 0.61$, $p = .003$), femoral BM radiodensity ($r = 0.59$, $p = .004$), aerobic capacity ($r = 0.68$, $p < .001$), bone turnover markers [serum TotalOC ($r = 0.54$, $p = .012$), PINP ($r = 0.56$, $p = .008$) and CTX ($r = 0.51$, $p = .019$)], and negatively with fat mass ($r = -0.52$, $p = .013$), whole-body fat percentage ($r = -0.50$, $p = .019$), visceral adipose tissue mass ($r = -0.50$, $p = .035$) and hs-CRP ($r = -0.58$, $p = .010$) at baseline. BMD correlated positively with BMI at baseline ($r = 0.45$, $p = .033$).

The 6-month-long training intervention significantly increased femoral BM insulin-stimulated GU in both groups similarly (time $p = .008$) (Figure 2B). Interestingly, training response in lumbar vertebral BM GU was different between the groups (time × group

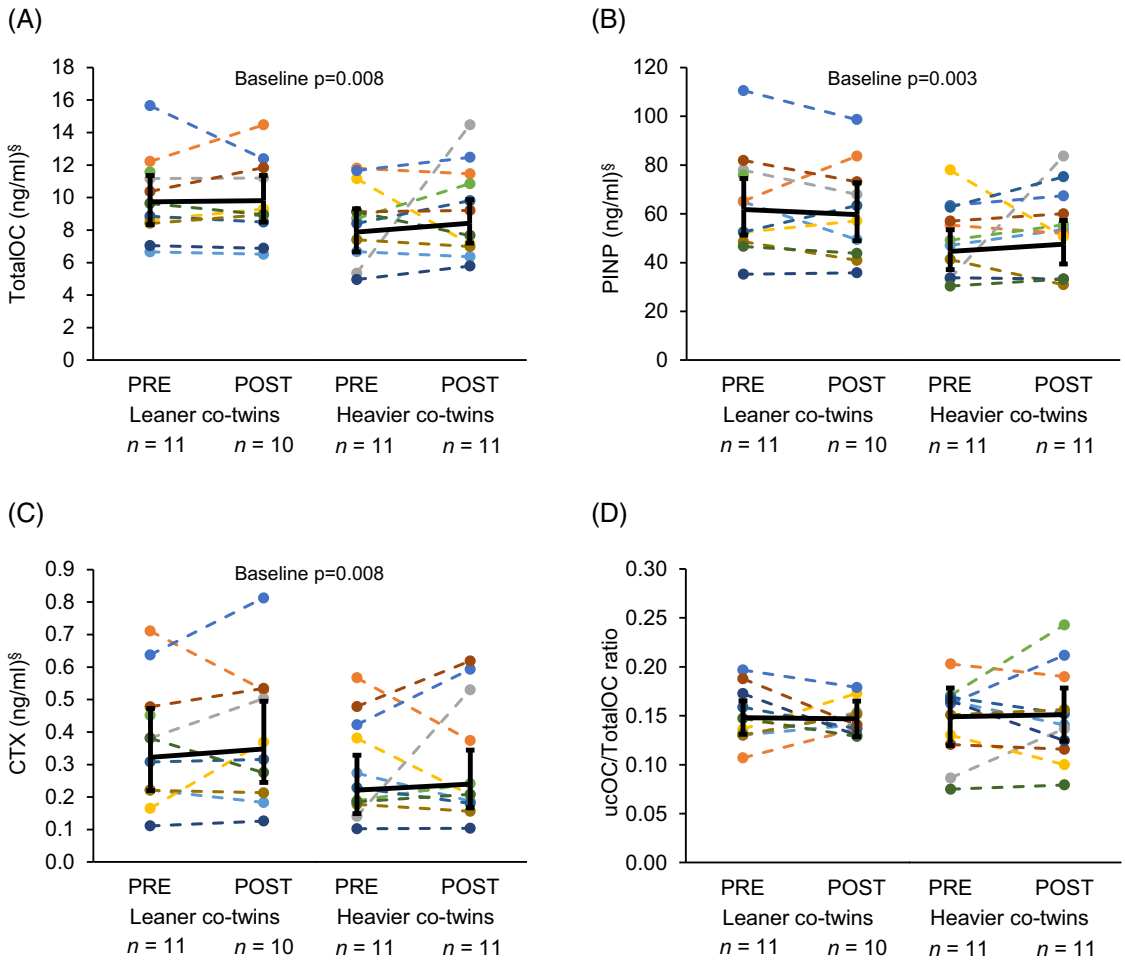

FIGURE 3 Bone turnover markers (TotalOC, PINP and CTX) were lower in heavier co-twins compared with leaner co-twins at baseline. There was no difference between the groups in the plasma ucOC/TotalOC ratio. No exercise effect was seen in bone turnover markers. Data are model-based means with 95% confidence intervals. *p* Value for baseline indicates the differences between leaner and heavier co-twins at baseline. CTX, C-terminal crosslinked telopeptide of type I collagen; PINP, N-terminal propeptide of type 1 collagen; TotalOC, total osteocalcin; ucOC, uncarboxylated osteocalcin. §Logarithmic transformation was performed to fulfil normal distribution assumption.

$p = .023$), as lumbar vertebral BM GU tended to decrease in the heavier co-twins ($p = .061$), while there was no significant change in the leaner co-twins ($p = .90$) (Figure 2A). The training intervention had no effect on vertebral BMD (Figure 2C), or lumbar vertebral or femoral BM radiodensity. Change in lumbar vertebral BM GU correlated positively with the change in hs-CRP after exercise training ($r = 0.61$, $p = .006$).

Serum bone turnover markers TotalOC, PINP, CTX and plasma ucOC correlated positively with each other at baseline ($p < .001$ for all). PINP and CTX correlated negatively with visceral adipose tissue mass ($r = -0.68$, $p = .002$ and $r = -0.52$, $p = .026$, respectively), and similar association was observed for TotalOC ($r = -0.45$, $p = .06$) at baseline. Training had no effect on the levels of the analysed bone turnover markers (Figure 3).

4 | DISCUSSION

Here, we show that training increased insulin-stimulated GU in femoral BM independent of weight and genetics. GU in lumbar vertebral BM was higher in those with higher weight, a phenomenon that was not observed in femoral BM in the present study. Interestingly, this phenomenon was counteracted by regular exercise training. Bone turnover was significantly suppressed in heavier co-twins compared with leaner co-twins, but training had no detectable effect on bone turnover, BMD, or BM radiodensity.

We showed before that femoral BM insulin-stimulated GU is lower in participants with insulin resistance and higher body weight compared with healthy lean participants, similar to other fat depots.[3,25] In this study, femoral BM GU did not differ between leaner and heavier co-twins at baseline (Figure 2B). However, femoral GU at baseline was lower compared with healthy lean participants and in line with the values we published in participants with insulin resistance.[3] Femoral GU was also lower compared with the results in an elderly cohort of frail and control study participants.[26] In this study, participants were allocated to leaner and heavier groups within each twin pair based on BMI. Although the mean difference in BMI between the groups was 7.6 kg/m^2, both groups can be classified as overweight on average (BMI >25) and there were subjects with IFG and/or IGT in both leaner and heavier twin groups. Thus, our data collectively suggest, that femoral BM GU is more strongly associated with glycaemic status than body weight alone.

After training, femoral BM GU increased in leaner and heavier co-twins regardless of genetics and weight at baseline. This is in line with our earlier study in which already 2 weeks of exercise training improved femoral BM GU similarly in both healthy and insulin-resistant participants.[3] As femoral BM has been shown to be an insulin sensitive tissue, this goes in line with the increase in whole-body insulin sensitivity.[25] This suggests that metabolism in femoral BM can be improved by exercise training independent of body weight. BM adipose tissue (BMAT) in humans has been shown to have high basal GU, exceeding that of white adipose tissue in a fasted state as well as during the euglycaemic-hyperinsulinaemic clamp.[3,4] Thus, improved BM metabolism may play a part in whole-body glucose metabolism.

BM has a different composition and different biological functions depending on its location.[4,27] Haematopoietic BM, abundant in axial skeleton, produces blood cells while the BM in long bones in appendicular skeleton, such as femoral BM, may serve as a specialized fat depot and consists in adults mainly of BMAT.[28–30] BMAT can be subdivided into two distinct types: proximal, regulated BMAT (rMAT), which is single adipocytes interspersed with active haematopoiesis, and distal, constitutive BMAT (cMAT), which has low haematopoiesis, larger adipocyte size, develops earlier and remains preserved upon systemic challenges.[31] BMAT volume was shown to be greater in the arms, legs and sternum than in the clavicles, ribs and vertebrae, and that BMAT-rich BM had a radiodensity of <115 HU whereas haematopoietic red BM had a radiodensity of 115-300 HU.[4] In this study, the CT resolution does not allow to differentiate between adipose tissue and other compartments of BM. Furthermore, femoral BM radiodensity is in the range of BMAT-rich BM (leaner 55.8 HU, 95% CI 37.9; 73.6, heavier co-twins 53.1 HU, 95% CI 38.0; 68.1) and lumbar vertebral BM radiodensity that of haematopoietic BM (leaner 165.0 HU, 95% CI 145.7; 186.7, heavier co-twins 152.1 HU, 95% CI 118.2; 195.7).

Unlike femoral BM, lumbar vertebral BM consists of trabecular bone, haematopoietic cells, BMAT and stem cells[32] and has been suggested not to be an insulin sensitive tissue.[25] Haematopoietic BM in axial skeleton is responsible for the formation of blood cells from haematopoietic stem cells.[33] For example, most cells of the immune system are born and mature in the haematopoietic BM and proliferation and differentiation of haematopoietic stem cells into different blood cell types require a robust upregulation of energy metabolism.[33] Intriguingly, in the present study, heavier co-twins had higher lumbar vertebral BM GU than their leaner co-twins at baseline and lumbar vertebral BM GU correlated positively with body weight at baseline. Obesity induces low-grade inflammation[34] and at baseline, heavier co-twins had higher levels of hs-CRP, an inflammation marker, compared with leaner co-twins. Thus, our results suggest that in the heavier co-twins increased low-grade inflammation activates the immune system and haematopoiesis in lumbar vertebral BM resulting in a higher need for energy in BM at baseline. We also found a positive correlation between lumbar vertebral BM GU and hs-CRP at baseline. This suggested link between lumbar vertebral BM GU and low-grade inflammation is supported by the findings of Devesa et al. in a large cohort of more than 700 participants. They showed that lumbar vertebral BM activation (GU measured with ^{18}F-FDG-PET) was associated with increased immune system activation, increased haematopoiesis and with markers of systemic inflammation, such as hs-CRP.[35]

Previously, we found no effect of exercise on lumbar vertebral BM GU when we analysed the effects of short-term exercise training on BM metabolism.[3] We speculate the 2-week intervention to be too short to induce changes in low-grade inflammation. In this study, study groups responded differently to training with respect to lumbar vertebral BM GU (Figure 2A). In heavier co-twins, lumbar vertebral BM GU decreased close to the level of leaner co-twins, while there was no change in leaner co-twins. Change in lumbar vertebral BM GU correlated positively with the change in hs-CRP, suggesting reduced

systemic inflammation and lower GU in lumbar vertebral BM after exercise training. Regular exercise training decreases low-grade inflammation.[36] Our data suggest that the decrease in lumbar vertebral BM GU in heavier co-twins may be explained by reduced need for energy because of the decrease in inflammation-induced haematopoiesis. To our knowledge, this has not been studied before in a preclinical setting and is an interesting topic for future research.

We measured BM radiodensity to assess BM fat content. There was no difference in femoral or lumbar vertebral BM radiodensity between co-twins at baseline, and femoral or lumbar vertebral BM radiodensity did not change in either group regardless of 6 months of regular exercise training. With the used imaging modalities, we cannot differentiate between different tissue types in the BM cavity. However, the lower the HU, the higher the fat content.[17] There was also no significant change in body weight or body composition in either group. In our previous study, femoral BM radiodensity was lower in healthy men compared with men with insulin resistance.[3] Furthermore, 2 weeks of exercise training did not induce changes in any of the groups. This suggests that exercise training without weight loss has no effect on BM adiposity, and that the changes we see in BM metabolism after exercise training may be caused by changes in tissue metabolism rather than changes in fat infiltration.

Obesity is associated with higher BMD.[37] In our study, there was no difference in BMD between co-twins at baseline, and both groups had mean BMD within normal reference interval, that is above the upper limit for osteopenia 120 mg/cm^3 (Figure 2C).[38] BMD correlated positively with BMI at baseline. However, how the effect of exercise on bone is influenced by obesity has not been widely studied.[39] While the benefits of weight-bearing exercise on bone are well established,[5,40] the effects of exercise are mostly explained by baseline BMD level. We found no training effect in BMD in either group. In accordance to our study, Zouhal et al. suggest that in people with overweight or obesity, BMD is not expected to increase, as they already have high BMD values.[39] Six months may also be too short a time to detect significant changes in BMD.[41] Interestingly, when we analysed BMD individually, not taking into account the study participants' twin pair status, there was a statistically significant increase of 4.1% ($p = .005$, 95% CI 1.5; 6.8) in BMD (data not shown). It appears that with our current statistical model and relatively small number of participants we were not able to detect a statistically significant change in BMD in response to exercise training.

We also measured bone turnover markers to assess the effect of exercise training on bone metabolism at the molecular level. Being more dynamic than BMD, bone turnover markers are routinely employed for rapid monitoring of both anti-resorptive and anabolic treatments.[42] At baseline, bone turnover was suppressed in heavier co-twins and in all participants bone turnover markers associated negatively with lumbar vertebral BM radiodensity. These outcomes agree with previous findings.[43] However, exercise training had no effect on bone turnover markers. We previously showed, that bone turnover markers, suppressed by obesity, increased and reached levels beyond normal-weight control subjects 6 months after bariatric surgery, suggesting a high bone turnover rate postoperatively after significant reduction in body weight.[44] It seems that bone turnover markers are more closely associated with changes in weight and body adiposity and not as drastically affected by exercise training per se.

The strengths of this study include the unique, well-controlled study design with MZ twin pairs, as among other factors age, sex and childhood environment can be excluded from the analysis for causal factors. BM insulin-stimulated glucose metabolism was measured with state-of-the-art methods. After the training intervention, there was no change in body weight or fat percentage, which allows us to examine the sole effect of exercise training on the measured parameters. A limitation to this study is that it was performed during the COVID-19 pandemic, and a number of possible study participants declined to participate, which resulted in a relatively small number of twin pairs. Regardless of the significant difference in BMI between the groups (7.6 kg/m^2), both groups were on average overweight and there were co-twins with IFG and/or IGT in both groups. Ideally, the comparison would be performed between lean (BMI <25 kg/m^2) and overweight or obese (BMI >25 kg/m^2) MZ co-twins to elucidate the effects of obesity more profoundly. The length of the intervention was set at 6 months, as this was seen as a sufficient time to study the effects of regular exercise training, but it is possible that there might have been more profound changes in body composition and/or metabolism with a longer intervention period. With used PET/CT modalities, it was not possible to differentiate between the different tissues inside the BM cavity, so further translational research is needed to better understand the role of different tissues in response to increasing body weight and exercise training.

5 | CONCLUSION

When genetic variability is controlled, regular exercise training increases femoral BM glucose metabolism independent of body weight. Interestingly, lumbar vertebral BM glucose metabolism is higher in subjects with higher body weight and regular exercise training counteracts this effect even without reduction in weight. These data suggest, that BM may respond differently to increased body weight and physical activity depending on its biological function and anatomical location.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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DATA AVAILABILITY STATEMENT

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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