

# Preliminary study of bone biomarkers in elite female ballet dancers

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## ABSTRACT

Elite female ballet dancers are at high-risk of bone stress injuries (BSI) due to high-levels of weight-bearing exercise causing micro-cracks to form in the bone. The consistently high volumes of weight-bearing exercise may lead to a low BMI which reduces oestrogen levels by suppressing the gonadotrophin axis. The combination of high-levels of weight-bearing exercise and low oestrogen may be associated with increased bone remodelling. Investigating the relationship between exercise, BMI, oestrogen, and bone remodelling is crucial for developing prevention and management strategies for BSI.

We conducted a pilot study of 4 weeks in a group of elite female professional ballet dancers and in female controls: the study subjects were monitored for self-reported weekly exercise, BMI, urinary oestrogen metabolites (2OHE1 and 16OHE1) and urinary NTX, a marker of bone resorption and proxy for remodelling. Measurements were collected at baseline, week 2 and 4.

Dancers exhibited significantly higher hours of exercise per week ( $p = 0.004$ ), and a BMI significantly lower ( $p = 0.030$ ) than that of age-matched controls. In contrast, the levels of oestrogen metabolites ( $p = 0.050$ ) and NTX ( $p = 0.460$ ) were comparable in the two groups.

Using NTX as a biomarker of bone remodelling, these results suggest that despite intense weight-bearing exercise, ballet dancers may not undergo higher bone remodelling than controls. The resulting insufficiency of bone remodelling may increase the risk of BSI because micro-cracks might accumulate in the bone faster than damaged tissue could be replaced.

Based on these findings, larger studies will be designed to assess the effect of bone remodelling on BSI risk and recovery in professional ballet dancers, to develop a platform of prevention and treatment strategies including screening programmes to identify dancers at risk.

### Keywords

*ballet, bone, stress injury, remodelling, biomarkers, NTX*

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## Introduction

Elite female ballet dancers are at high-risk of bone stress injuries (BSI) due to high-levels of weight-bearing exercise causing micro-cracks to form and accumulate in bone e.g., metatarsals and tibia. Micro-cracks can cause a stress reaction (e.g., oedema) or propagate and merge into a stress fracture (Burr et al., 1997; Donahue et al., 2000; Taylor & Lee, 2003; Wyon et al., 2014). The bone remodelling process which can repair damage by resorbing damaged bone tissue and forming new bone is stimulated by applied mechanical stress (Maïmoun & Sultan, 2011; Ooi & Sahrir, 2018). Further, high activity levels are also associated with low BMI which in turn reduces oestrogen levels by suppressing the gonadotrophin axis (Valentino et al., 2001). This combination of high-levels of exercise (O’Kane et al., 2006) and low oestrogen (Nazem & Ackerman, 2012) would likely be associated with a high bone remodelling rate. Indeed, long-term levels of both high weight-bearing exercise (O’Kane et al., 2006) and low oestrogen (Nazem & Ackerman, 2012) are associated with high bone remodelling in other elite female athletes e.g., swimmers, rowers, and rhythmic gymnasts (relative to controls). Which raises an interesting question of whether high activity levels

in ballet dancers are associated with high or low bone remodelling rates? To date, bone remodelling rates have not been measured in elite professional dancers despite the potentially key role in BSI.

Bone is a dynamic tissue undergoing constant changes to mass and architecture through bone remodelling which is a balanced process of resorption and formation that removes old and damaged tissue to be replaced with new healthy bone. The metabolites of bone resorption are released into the vascular system and excreted in the urine. For example, cross-linked N-telopeptide (NTX), a biomarker for bone resorption which is applied in clinical settings as a proxy for remodelling (Maïmoun & Sultan, 2011; Ooi & Sahrir, 2018). NTX peptides are formed from bone collagen proteolytic cleavage during resorption by osteoclasts and cathepsin K, which are excreted (Ooi & Sahrir, 2018). Since cellular resorption and formation are coupled the biomarker NTX is used clinically as a biomarker for bone remodelling in patients with Paget’s disease and osteoporosis including people taking antiresorptive medicines like bisphosphonates (Greenblatt et al., 2017). A medicine that is sometimes administered to athletes with BSI. It is potentially feasible to measure bone remodelling rates in ballet dancers with a simple urinalysis.

Therefore, this is a pilot study investigating the relationship between self-reported exercise (hours per week), BMI, urinary oestrogen metabolites (2OHE1 and 16OHE1) and a bone remodelling biomarker (NTX), by comparing professional female ballet dancers with sex-matched controls. The project aims at improving our understanding of the potential role of these factors in predisposing elite professional female ballet dancers to BSI and provide the basis for the design of larger studies potentially impacting clinical care.

The novelty of this project lies in the assessment of bone remodelling biomarkers in elite professional dancers in the context of their exercise, BMI, and hormonal profile. Understanding the impact of these factors on bone remodelling will be crucial for developing prevention and management strategies for BSI. Indeed, BSI prevention would be extremely beneficial for professional female ballet dancers, as overuse injuries account for 64% of the frequent injuries in female ballet dancers (4.4 injuries per 1,000 hours of dance exposure) and can cause an interruption of on average 4 days (Russell, 2013) or a definitively end a career (Nazem & Ackerman, 2012).

## Methods

This pilot study included two cohorts, elite professional female ballet dancers and female controls and was approved for data collection and analysis by the Imperial Research Ethics Committee (Ref: 15IC2468). All subjects provided full consent before data collection. Samples were stored in the Imperial College Tissue Bank.

## Participants

The professional ballet cohort comprised five elite female professional ballet dancers from the Royal Ballet in Covent Garden London (The Royal Opera House), including the ranks of one Soloist, two First Artists and two Artists. The age-matched female control cohort

comprised six subjects from Imperial College London: controls had little or no experience in ballet dancing. The participants included in both cohorts lived and resided in the same geographic location (London).

Following the exclusion criteria of the study, none of the participants had a history of eating disorders, known diseases, family history of bone diseases, fractures or soft tissue injury within the last 3 months leading up to the study, or ever used medication known to affect bone health.

## Data collection

Exercise hours were recorded from training and performance logs. The age, age of menarche and contraceptive were established using lifestyle questionnaires (Bolek-Berquist et al., 2009). Urinary oestrogen metabolites and NTX levels were measured in second morning void urine. The Samples were collected by the participants and subsequently stored at -20°C. Samples were frozen immediately after collection and then transported frozen to the laboratory for storage, where they remained frozen until analysis. Urinary analysis was carried out at the end of the follow up period so all samples could be processed at the same time using the same equipment.

Urinary NTX levels were determined by a competitive inhibition enzyme-linked immunosorbent assay (ELISA, Osteomark® NTx Urine Assay, Alere Scarborough, Inc., 10 Southgate Road, Scarborough, ME 04074 USA, cat. No 9006). Intra- and inter-coefficients of variation were 3.1% and 4.2% respectively. Urinary Oestrogen Metabolites 16OHE1 and 2OHE1 were determined by an ELISA assay (Estramet, IBL international, Flughafenstrasse 52a, D-22335 Hamburg, Germany, Cat. IA52011). The intra- and inter-coefficient sensitivity were 11.2% and 42% for 2OHE1, and 9.2% and 59% for 16OHE1, respectively. The sensitivity range was 0.2-36.1 nM/mM creatinine for 2OHE1, and 0-60 nM/mM creatinine for 16OHE1, respectively. Both assays utilized an automated microplate washer (Biotek Lx50)

and plate reader (Biotek Synergy HT). Appropriate Microman displacement pipettes were used for sample and reagent preparation with Biohit 5 – 100µl electronic multichannel pipettes for reagent application.

### Statistical analysis

Normality was assessed with the Shapiro-Wilks test which revealed non-normal distribution of 2OHE1,16OHE1 and NTX in ballet dancers and controls. Therefore, results are expressed as median ± Interquartile range and the data were analysed with the Mann-Whitney U test. The alpha value  $p < 0.05$  was chosen as the level of significance. Outliers identified using Tukey's method were still included within the analysis. Statistical analyses were carried out using GraphPad-Prism8.

## Results

### Participant's data

Median height, weight and contraception use were comparable in the ballet dancer and control cohorts (Table 1). The two cohorts differed for age and age of menarche ( $p < 0.05$ ). In the ballet group, the age ranged between 28-31 years, with two outliers of 33 and 36 years of age. In contrast, the control group was more homogeneous with all subjects being 27 years old except for one, who was 28. The high uniformity of the control group may explain the statistical difference in age, which, however, is not likely to be clinically relevant. The age of the menarche significantly differed between the two cohorts, as the menarche started on average 2.74 years later in ballet dancers than in the control group.

### Exercise levels and BMI

The professional female ballet dancers all participated in high-intensity exercise for a median of 31 hours per week, while the controls exercised for a median of 5.46 hours per week with low-impact walking. Therefore,

the hours of exercise (Figure 1A) reported by ballet dancers were 4.4-fold higher than those reported by control subjects, resulting in a statistically significant difference ( $p = 0.004$ ) between the two cohorts.

The BMI (Figure 1B) was significantly lower (9.8%;  $p = 0.03$ ) in the professional female ballet dancers than in control subjects. While all the controls (median = 20.67) were within the normal BMI range (18.5 - 25.0), three out of five of the ballet dancers had a BMI below the normal range of 18.5 (median = 18.11).

### Total oestrogen metabolites and 2OHE1/16OHE1 ratio

Oestrogen metabolites were measured at baseline, 2 and 4 weeks, irrespective of the menstrual cycle. Hormonal contraception was used in three out of five ballet dancers and four out of six controls (Table 1). The levels of total oestrogen metabolites (Figure 1C) were similar between ballet dancers and controls, with a median of 21.2 nM/Mm creatinine in female ballet dancers and 9.6 nM/Mm creatinine in controls. There was a trend with 2.2-fold higher total oestrogen metabolites in ballet dancers, when compared to controls (Figure 1C).

Ballet dancers exhibited significantly ( $p = 0.02$ ) higher amounts of 2OHE1 (10.6 nM/Mm creatinine) in comparison to the control group (5.85 nM/Mm creatinine; Figure 1C). The 2OHE1/16OHE1 ratio was similar between ballet dancers (2.35) and controls (1.75; Figure 1D). There was a 1.34-fold higher 2OHE1/16OHE1 ratio in female ballet dancers when compared to controls. The 2OHE1/16OHE1 ratio was within the normal range of 1.5-2.74 in both cohorts.

### Bone resorption (NTX)

Levels of NTX (Figure 1E) were similar between the two cohorts ( $p = 0.46$ ), even though ballet dancers showed a 14.7% higher median NTX (50.40 nM/Mm creatinine) in comparison to the control group (43.95 nM/Mm creatinine). The levels of NTX in the ballet dancers showed high inter-subject variability (IQR 26.0) compared to controls (IQR 16.7). Furthermore,

**Table 1**

Comparison of Subject Characteristics in the female ballet dancers and control cohort. Metadata for female ballet dancers ( $n = 5$ ) and controls ( $n = 6$ ) presented as median $\pm$  interquartile range (IQR) or frequency (%). Height, weight, smoking, and contraception use were comparable in ballet dancers and controls, while age, Body Mass Index (BMI), age of menarche and exercise hours were significantly different in the two cohorts.

Variable	Ballet Dancer ( $n = 5$ )	Control ( $n = 6$ )	<i>p</i> -value Mann-Whitney
Age	31 $\pm$ 4**	27 $\pm$ 0**	0.010
Height	1.66 $\pm$ 0.06	1.67 $\pm$ 0.08	0.710
Weight	49.90 $\pm$ 3.3	57.30 $\pm$ 4.80	0.170
Age of Menarche	17 $\pm$ 3*	13.5 $\pm$ 1.75*	0.020
Participants on contraception	3 (60%)	4 (66%)	0.910
Combined oral pill	1 (20%)	2 (33%)	0.720
Mirena coil	1 (20%)	2 (33%)	0.720
Cerazette	1 (20%)	0 (0%)	0.360

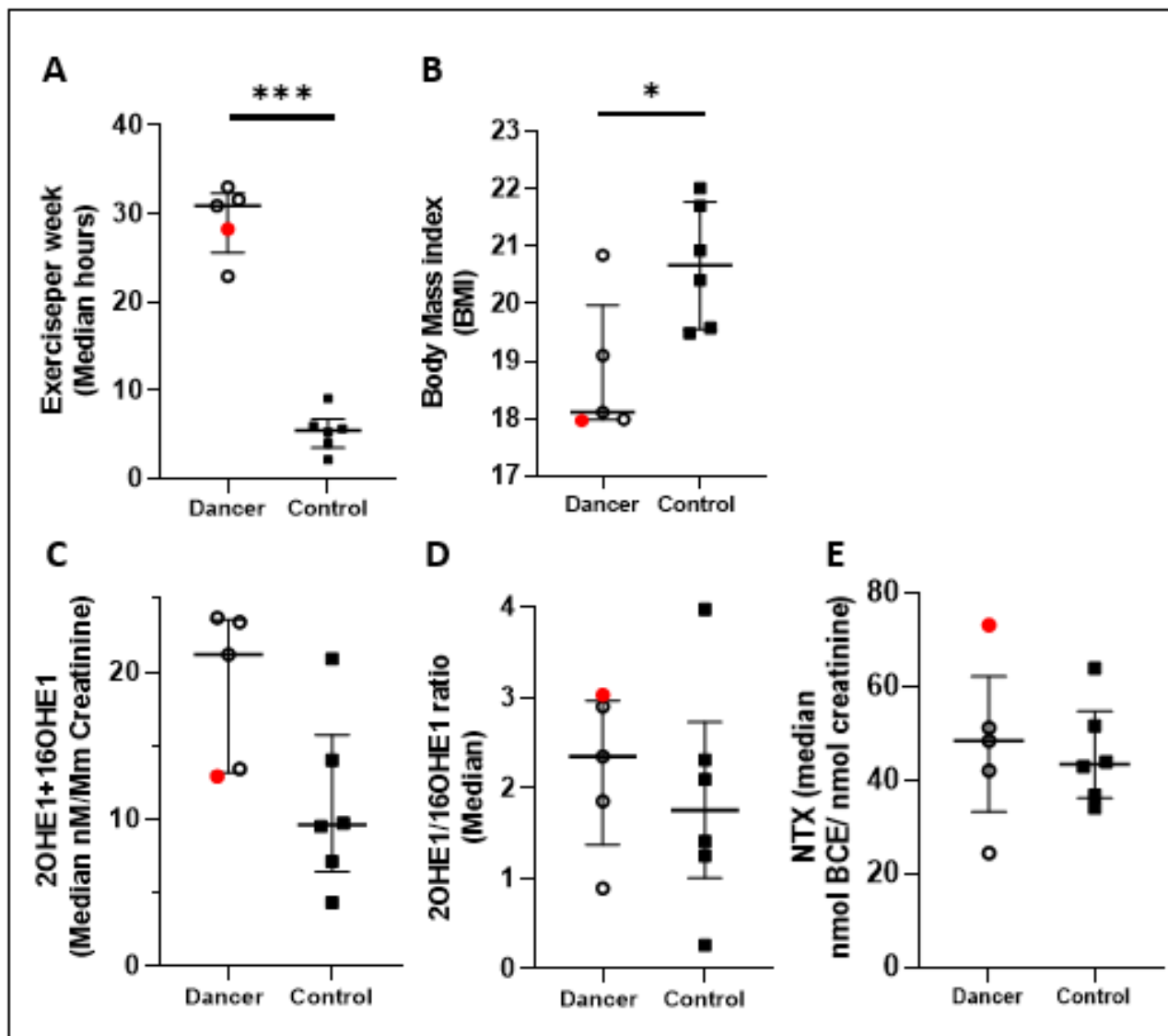
Note: Mann-Whitney U test values significant at \* $p < 0.05$ , and \*\* $p < 0.01$ , respectively.

there was one outlier within the professional dancers (NTX 192 nM/Mm creatinine) whose values were well above the 3<sup>rd</sup> quartile (64.7). This dancer is marked in red in all graphs. due to her very distinct profile, including the lowest BMI and oestrogen metabolite levels, associated with the highest 2OHE1/16OHE1 ratio.

## Discussion

This pilot study explored the feasibility and relevance of an investigation into the relationship between exercise levels, BMI, urinary oestrogen metabolites (2OH, 16OH) and bone remodelling rate (based on urinary resorption marker NTX) in professional female dancers.

A group of professional ballet dancers ( $n = 5$ ) was compared with female controls ( $n = 6$ ). Ballet dancers exercised for a significantly higher number of hours per week ( $p = 0.004$ ) and had a lower BMI ( $p = 0.040$ ) when compared to female controls (Figure 1), which has been previously described (Biernacki et al., 2021). The urinary concentrations of total oestrogen metabolites ( $p = 0.050$ ) and NTX ( $p = 0.460$ ) were similar in female dancers and controls (Figure 1). Therefore, despite the intense weight-bearing exercise which could damage bone, ballet dancers did not exhibit significantly higher bone remodelling rates which would repair the tissue.



**Figure 1** Ballet dancers showed (A) significantly higher levels of weekly exercise and (B) lower BMI, but similar concentrations of (C) urine oestrogen metabolites and (D) NTX vs. controls. Data points represent the median and IQR values of 3 independent measurements taken at the baseline, 2 and 4 weeks in female ballet dancers (◦) (*n* = 5) and age-matched controls (▪) (*n* = 6). Statistical differences were calculated according to the Mann-Whitney U test.

Asterisks denote \**p* < 0.05, \*\**p* < 0.01, and \*\*\**p* < 0.005, respectively. The outlier with high NTX was marked in red in all 4 graphs.

## Bone remodelling in dancers

In this study, the levels of urinary NTX were measured in ballet dancers, providing a non-invasive alternative to previous methods using serum CTX, to assess bone remodelling. CTX is less specific biomarker than NTX for bone resorption because changes in CTX also reflect collagen breakdown in cartilage (O'Kane et al., 2006). Our results are consistent with a previous study, in which the levels of CTX were assessed in young non-professional vocational ballet dancers and found to be slightly higher ( $X1.34$ ), but not significantly different in comparison to controls (Muñoz Calvo et al., 2004). The two studies differ in that the CTX study was carried out in non-professional vocational dancers, which were much younger ( $n = 12$  with a mean age of  $16.4 \pm 2$  years) than the ballet dancers examined in our study and exercised for an average number of hours per week which was lower than professional ballet dancers (20 vs 40 hours/week). Nevertheless, the results of both studies are consistent and suggest that, despite higher activity, dancers may not exhibit higher bone remodelling than control subjects. It should be noted that the study did not control for exercise in the previous 24-48 and it is possible that biomarkers could be increased by strenuous exercise in the run up to sample collection. Furthermore, it is also important to consider that the control group was active with 5.46 hours of exercise per week, while in the UK population only 61.4% reached the national target of 2.5 hours of exercise per week. Future studies should try and match the exercise levels of the control group to that in line with the national guideline.

## Bone remodelling and the female athlete triad

Since the female athlete triad, consisting in amenorrhea, osteoporosis, and low energy availability/disordered eating, is highly prevalent in ballet dancers, it is important to assess whether our study participants suffered from it (Doyle-Lucas et al., 2010; Kaufman et al., 2002; Nazem & Ackerman, 2012; Toro et al., 2009; Valentino et al., 2001; Wyon et al., 2014).

Regarding amenorrhea, the urinary levels of oestrogen metabolites were similar in professional female ballet dancers and controls (Figure 1C and D). However, considering that at least 60% of the study participants were under hormonal contraceptive treatment, it is difficult to assess for amenorrhea or menstrual irregularities. To this end, future studies should include the assessment of current or previous amenorrhea in subjects taking hormonal contraceptives.

Regarding osteoporosis, the comparable levels of NTX observed in professional ballet dancers and controls, except for one ballet dancer, suggest that bone resorption was similar in both cohorts. On the other hand, the difference in the age of menarche and the overall age difference between controls and professional ballet dancers may have affected bone mineral density. To this end, future studies should incorporate bone mineral density measurements, Vit D levels, as well as analysing NTX levels in conjunction with formation marker, such as P1NP (Fisher et al., 2017).

Concerning the low energy availability and disordered eating, subjects with a self-reported active or history of eating disorders were excluded from the study. Future studies should include objective measurements of exercise intensity and diet, including caloric and calcium intake.

Finally, it is important to note that all previous studies were conducted in vocational dancers, which differ from professional dancers in age, eating habits and exercise routine (Wyon et al., 2014). Such a difference in lifestyle and behaviour might be key to explain these discrepancies.

## Oestrogen metabolism pathways in female ballet dancers

In professional female ballet dancers, the high amount of exercise may increase oestrogen metabolism by altering the pathway to favour the A-ring over the D-ring and cause a higher production of the anti-oestrogenic metabolite 2OHE1 over the weakly oestrogenic 16OHE1. The metabolism of circulating estrone is mediated by two mutually exclusive oxidative path-

ways, resulting in the formation of an A-ring or a D-ring: the 2-hydroxy estrone (2-OHE1) is a product of the A-ring pathway while the 16-alpha-hydroxy estrone (16 $\alpha$ -OHE1) results from the D-ring pathway. Both metabolites bind to the oestrogen receptor but the 2-OHE1 metabolite has lower estrogenic activity, due in part to its reduced receptor affinity. The 2:16 $\alpha$ -OHE1 ratio is commonly used as a tool to explore the exposure of a subject to active oestrogen metabolites (Dallal et al., 2013). In this study, female ballet dancers showed significantly higher amounts of 2OHE1 compared to controls. Furthermore, they exhibited a 2.2-fold higher amount of total oestrogen metabolite and a 1.35-fold higher 2OHE1/16OHE1 ratio, which nearly reached statistical significance. This suggests that the dancers metabolise a higher proportion of oestrogen via the A-ring than the D-ring in comparison to controls.

Despite the shift in oestrogen metabolism to favour the less biologically active oestrogens, there was no effect on bone remodelling (Brooks et al., 2004), but the shift could limit the ability of bone remodelling to increase. However, the significance of the increase in 2OHE1 and of the borderline changes in total oestrogen levels are worth further investigation. Collecting data over a longer observation period and establishing a link between the oestrogen levels and the distinct phases of the menstrual cycle may help identify dynamic changes in the oestrogen profile of professional female ballet dancers, which may have gone undetected in this pilot study.

### ***BSI risk and biomarkers of bone remodelling***

The NTX data from our study and published work on CTX both suggest that in dancers BSI (i.e., stress reactions and stress fractures) may result not only because of excessive repetitive loading causing micro-crack formation (i.e., fatigue) but also because of a potentially insufficient bone remodelling. This could increase the risk of micro-cracks accumulating in the bone to cause a stress reaction or propagating and merging into a stress fracture. Hence, the elevated risk of BSI in dancers may be due to a combination of both

fatigue and insufficiency (Romani et al., 2002; Warren et al., 1986). If confirmed in larger studies, this hypothesis could provide a platform to develop novel strategies for preventing, identifying, and managing BSI.

Current diagnostics for BSI rely on the advent of pain. At which point, the BSI pathology may have advanced to a stage where the dancer must cease participation completely. Often BSIs are clinically asymptomatic and therefore difficult to diagnose until a stress fracture occurs. Further, clinicians have difficulty in deciding when it is safe to return to normal training and an early return to high impact activity (e.g., jumping) can cause a BSI recurrence and delay recovery. Therefore, clinical teams need an accurate and objective measure of bone health that can be used to identify BSI early, before pain occurs and before a stress fracture develops. Then monitor continuously to minimize time out due to the injury and optimize return to training and performance whilst minimizing the risk of re-injury.

To this end, it will be important to discover the pathophysiological mechanisms underlying the potentially altered bone in ballet dancers to intervene appropriately and introduce mitigation measures. Adopting a low-cost and sensitive assay such as urinary NTX could allow for early identification and closer monitoring of individual dancers at risk of BSI (Robertson & Wood, 2017).

## **Conclusion**

Our study suggests that professional ballet dancers in this study despite having a 4.4-fold higher level of exercise when compared to controls, did not exhibit higher bone resorption. The clinical relevance of these findings warrants further investigation, as insufficient bone remodelling to repair overuse damage could underlie the high prevalence of bone stress injuries (BSI) in female ballet dancers. Furthermore, elite professional female ballet dancers might not suffer from the female athlete triad as much as their vocational counterparts and do not have alterations in the total oestrogen metabolite levels. Dissecting the molecular basis of BSI susceptibility in professional female ballet



dancers and developing cost-effective, non-invasive, sensitive methods to identify BSI susceptible dancers may allow for the development of pharmacological treatments or the implementation of preventative interventions to restore bone remodelling, such as controlling exercise.

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## Competing interests

The authors have declared that no competing interests exist.

### **Data availability statement**

Please contact the corresponding author for a copy of the raw data.