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Influence of sport type and gender on bone turnover markers in young athletes

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Abstract

Background Exercise is beneficial to bone health. However, little is known about the interaction effect of gender and sport type on bone turnover in young athletes. This study aimed to examine the influence of gender and sports categories (high, medium, and low impact) on bone turnover: reabsorption markers—osteocalcin, calcium, inorganic phosphate (IP), alkaline phosphatase (ALP), and resorption marker—cross-linked N-telopeptides of type 1 collagen (NTx) among a university's undergraduate athletes.

Methods The study was an ex-post facto design involving forty-seven purposively recruited gender- and sport-type-matched undergraduate athletes whose demographic characteristics and BMI were obtained. Participants' 5 mL antecubital blood samples were collected and analysed for serum levels of osteocalcin, calcium, IP, ALP, and NTx using standard laboratory protocols, Bio-Tek spectrometer, and KC4 (3.3) software. Data were analysed using descriptive statistics and two-way ANOVA.

Results The study involved 24 females and 23 males ($n = 47$) aged 22.15 ± 3.35 years with an average BMI of 23.34 ± 4.66 . There was no significant gender effect on the biomarkers. However, there was a significant effect of the sports category on IP ($F = 4.307$, $p = 0.020$), calcium ($F = 6.807$, $p = 0.003$), and ALP serum levels ($F = 11.511$, $p < 0.001$). Specifically, mid-impact sports participants had a higher IP than the low-impact group (mean difference [MD] = 0.81 mg/dL, $p = 0.036$). Low-impact had a higher calcium level than mid-impact (MD = 0.40 mg/dL, $p = 0.022$) and high-impact (MD = 0.49 mg/dL, $p = 0.003$). Conversely, low-impact had lower ALP than mid-impact (MD = -11.13 U/L, $p = 0.013$) and high-impact (MD = -17.44 IU/L, $p < 0.001$).

Conclusion Moderate to high-impact sports positively affected bone turnover in young athletes. However, gender had no significant impact.

Keywords Bone remodelling, Biomarkers, Sports participation, Undergraduates, Sports impact

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Introduction

Physiotherapy involves musculoskeletal health promotion, disease prevention, treatment, and rehabilitation [1, 2]. Physiotherapists need to understand the gendered differences in the musculoskeletal impact of leisure and occupational activities such as sports [3]. Literature has shown that individuals between the ages of 10 and 30 years who engage in high-impact sports have higher bone mineral composition, bone mineral density (BMD), and enhanced bone geometry in specific anatomic regions exposed to the loading patterns of each sport [4].

Sports refer to a subset of exercises undertaken individually or as part of a team whereby participants adhere to a standard set of rules or expectations, and a defined goal exists [5]. Sporting activities can be categorised into high-impact, medium-impact, and low-impact groups based on the extent of associated ground reaction forces [6, 7]. High-impact (basketball and volleyball) and medium-impact sports (football and track events) involve weight-bearing activities [6], while low-impact sports are non-weight-bearing, such as swimming and cycling [6, 8, 9].

Weight-bearing sports involve activities that impose a high mechanical load on the musculoskeletal system, using the antigravity muscles such as jumping, landing, running, shooting, and spiking. Adequate mechanical stress on the bone increases bone mineral content [10]. Non-weight-bearing exercises may have a lesser impact on the musculoskeletal system than weight-bearing exercises [4, 8]. Studies have also shown that in young participants, beneficial skeletal effects on bone metabolism can be attained through plyometric exercises [11] and high-intensity strength and endurance training [11, 12]. Evidence in the literature shows that peak bone mass is attained during the second and third decades of life, and sports participation may lead to adaptive changes that improve bone architecture through increased density and enhanced geometric properties [4]. Athletes and young adults participating in sports may benefit from a life-course improved bone health [13, 14].

Bone turnover markers (BTMs) are a series of protein or protein derivative biomarkers released during bone remodelling by osteoblasts or osteoclasts [15]. Bone turnover is defined as the process of bone resorption and replacement with a new bone with a minor change to the shape of the pre-existing bone [16]. Bone turnover markers are helpful in the prediction of bone loss, prediction of the risk of fracture, and also in monitoring osteoporosis [16]. Calcium and inorganic phosphate (IP) are essential components of the bony inorganic matrix and significant factors in maintaining bone health. Serum total alkaline phosphatase (ALP), osteocalcin, urinary calcium, and hydroxyproline are strong predictors of bone loss. The phosphate deficiency can also lead to loss of bone mass [17].

Several factors, such as smoking, body mass index, hormones [18, 19], and gender [20], can influence bone turnover rates and their biomarkers. Other factors include ageing and pathological disorders [14]. A recent general population systematic review favoured additional exercise to pharmacological treatment in improving BMD and lowering BTMs, but evidence certainty was inconclusive, warranting a recommendation for further studies [21]. Older women have low BMD responses to exercise

[8]; it is important to determine if there are gender differences in BTMs in young athletes. Some existing studies recruited only females and could not analyse gender differences [6, 9, 22, 23]. Therefore, this study aimed to determine the main- and interaction effects of gender and sports categories (in terms of the level of musculoskeletal impact) on bone turnover markers of undergraduate athletes in a Nigerian university. We hypothesised that there would be no significant main- and interaction effects of (a) gender and (b) sports type on participants' serum levels of osteocalcin, calcium, IP, ALP, and cross-linked N-telopeptides of type 1 collagen (NTx).

Methods

Study design

The study was an ex-post facto design involving 47 purposive selected University of Benin undergraduate athletes. The ex-post facto design was suitable for this study since the variables of interest were objectively collected in their existing form without the researchers' intervention or treatment [24]. The rationale for purposive sampling was to ensure we recruited equal men and women across the sports types. However, a man dropped out of the study, making the final sample 47 (24 women and 23 men). The study protocol was approved by the Health Research Ethics Committee of the University of Benin, Benin City, Edo State, Nigeria (Reference number: UBHREC/05/01/2326). A signed individual informed consent was obtained from each participant. The consent form clearly stated the study objectives, participants' right to withdraw from the study, data privacy, and confidentiality. Additionally, participants under 18 years old provided their parent contact for over-the-phone parental consent. The study adhered strictly to the approved protocol and the guidelines of the Helsinki Declaration. The study duration was between 07 June and 30 August 2021.

Participants

Participants were included in the study if they were undergraduate university athletes between 16 and 30 years old and had participated actively (training or competing for at least 2 h daily, 4 days weekly) in one of the selected sporting activities over the last 1 year. They were drawn from low-impact (chess and swimming), mid-impact (soccer and track events), and high-impact games (basketball and volleyball). This categorisation was due to the frequency of jumping and landing in each sport type [4, 6, 22, 23, 25]. Participants were screened out if they were smokers, had a fracture in the previous 12 months, had a systemic musculoskeletal disease such as osteoporosis and arthritis, or taking any medication known to affect bone metabolism. Women with

polycystic ovary syndrome, leiomyomas, or pregnancy were also excluded from the study.

Sample size

The sample size was calculated using G*Power 3.1.9.4 software. The calculation based on two-way ANOVA of two by three independent groups, 95% power, alpha error probability of 0.05, and a moderate effect size of 0.8 gave a sample size of 47 participants.

Protocols and outcome variables

We visited the University Stadium, got permission from the coaches, and approached potential participants after each training day. Eligible and consenting participants provided their names, ages, gender, type of sports, duration of sporting or training, smoking, substance use, and medical histories in a biodata form. Afterwards, each participant was scheduled with a laboratory appointment at the University Medical Centre.

At the laboratory, participants’ weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using a standard BMI apparatus (RGZ-120, made in China; weight/[height]² = BMI) and protocol [26]. A phlebotomist drew participants’ blood samples (5 mL) through an antecubital venepuncture between 8:00 AM and 10:00 AM [27]. It has been recommended that serum samples for biochemical measurements of bone turnover markers are collected under fasting conditions because diet easily affects them [27, 28]. Blood samples were processed and stored in optimum conditions, described in detail in previous studies [29, 30]. Briefly, the blood samples were centrifuged at 3000 rpm for 10 min at 4 °C, and the serum was harvested and stored at – 20 °C. Samples were analysed within 24 h [30]. Serum levels of osteocalcin, calcium, ALP, IP, and cross-linked N-telopeptides of type-1-collagen (NTx) were analysed using commercially available ELISA kits (Osteomark NTx). The results of the bone turnover markers assays were read in triplicate using a Bio-Tek spectrometer and KC4 (3.3) software[®]. The median scores were recorded for statistical analysis.

Statistical analysis

Data were analysed using SPSS version 25 software. Descriptive statistics: frequency, percentage, range, mean, and standard deviation were used to summarise the data. For inferential analysis, the data was checked and confirmed to have no missing variables or significant univariate outliers (standardised Z-score greater than ± 3.29). Next, the data met the assumptions of normal distribution and homogeneity of variance tested using Shapiro-Wilk’s and Levene’s tests, respectively [31]. Therefore, parametric tests were used for inferential analysis. A two-way analysis of variance (ANOVA) test was

used to determine the main- and interaction effects of gender and sports categories on the biomarkers. Where significant *F*-statistic was obtained, we used the Bonferroni post hoc test for pair-wise comparison of the mean values. The alpha level was set at 0.05.

Results

The study involved 47 participants: 24 females (51.1%) and 23 males (48.9%). The participants’ mean±SD age was 22.15 ± 3.35 years, and BMI was 23.34 ± 4.66. Over half of the participants (*n* = 25, 53.2%) were between the ages of 21 and 25 years. Table 1 shows that the participants were equally distributed across sport types and categories: high impact-basketball and volleyball (*n* = 16, 34.0%), mid-impact-soccer and track events (*n* = 16, 34.0%), and low-impact chess and swimming (*n* = 15, 31.9.0%). Participants’ BMI and serum biomarker levels were within the normal laboratory reference ranges, except ALT, which had a slightly lower average than the lower border of the expected range (Table 2). Further analysis in Table 3 shows that irrespective of gender or sports type, many of the respondents’ serum bone turnover markers were within normal ranges.

Table 4 showed no gender differences in all the biomarkers. However, there was a significant main effect of the sports category on calcium (*F* = 6.807, *p* = 0.003), IP (*F* = 4.307, *p* = 0.020), and ALP serum levels (*F* = 11.511, *p* < 0.001). A significant interaction effect of gender*sport-category on ALP was also observed (*F* = 4.546, *p* = 0.016). The Bonferroni post

Table 1 Participants’ sociodemographic characteristics

| Parameters | Frequency | Percentage (%) |
|----------------|-----------|----------------|
| Gender | | |
| Male | 23 | 48.9 |
| Female | 24 | 51.1 |
| Age group | | |
| 16–20 | 15 | 31.9 |
| 21–25 | 25 | 53.2 |
| 26–30 | 7 | 14.9 |
| Sport category | | |
| Low impact | 15 | 32.0 |
| Mid impact | 16 | 34.0 |
| High impact | 16 | 34.0 |
| Sport type | | |
| Chess | 7 | 15.0 |
| Swimming | 8 | 17.0 |
| Football | 8 | 17.0 |
| Track events | 8 | 17.0 |
| Basketball | 8 | 17.0 |
| Volleyball | 8 | 17.0 |

Table 2 Anthropometric data and bone turnover marker values of the participants

| Variable | Minimum | Maximum | Mean \pm SD | Normal range |
|--------------------------|---------|---------|-------------------|--------------|
| Age (years) | 16 | 30 | 22.15 \pm 3.35 | – |
| Body weight (kg) | 46.00 | 135.00 | 71.00 \pm 16.35 | – |
| Height (m) | 1.58 | 1.92 | 1.74 \pm 0.09 | – |
| BMI (kg/m ²) | 16.69 | 41.67 | 23.34 \pm 4.66 | 18.50–24.90 |
| Osteocalcin (mg/dL) | 4.34 | 24.29 | 11.47 \pm 4.85 | 8.00–32.00 |
| NTx (nmol BCE/L) | 1.50 | 55.00 | 15.96 \pm 10.66 | 5.40–24.20 |
| Calcium (mg/dL) | 7.20 | 9.30 | 8.29 \pm 0.45 | 8.60–10.30 |
| IP (mg/dL) | 2.50 | 6.10 | 4.44 \pm 0.94 | 2.80–4.50 |
| ALP (IU/L) | 22.00 | 81.00 | 42.70 \pm 12.82 | 44.00–147.00 |

NTx N-telopeptides of type-1-collagen, ALP Alkaline phosphatase, BMI Body mass index, IP Inorganic phosphate

hoc analyses showed that low-impact sports participants had a significantly higher calcium level than mid-impact (mean difference [MD] = 0.40 mg/dL, $p = 0.022$) and high-impact (MD = 0.49 mg/dL, $p = 0.003$). Conversely, low-impact sport participants had lower ALP than mid-impact (MD = -11.13 U/L, $p = 0.013$) and high-impact (MD = -17.44 IU/L, $p < 0.001$), but no significant difference between high and mid-impact sports (MD = 6.31 IU/L, $p = 0.263$). Finally, mid-impact sports participants had a significantly higher IP than the low-impact group (MD = 0.81 mg/dL, $p = 0.036$), and there was no significant IP difference between the high vs. low or high vs. mid-impact sports.

Discussion

Sporting is necessary for healthy bones throughout the life course. High-impact exercise is believed to induce beneficial osteogenic effects in the skeleton. Little is known about the interaction effect of gender and sport type on bone turnover in young athletes. This study aimed to examine the influence of gender and sports categories (high, medium, and low impact) on bone turnover among a university's undergraduate athletes.

Previous studies on the relationship of bone health with sporting activities have focused majorly on European professional athletes and females [6, 22, 23]. There is a paucity of bone turnover biomarkers research on young African men and women athletes. The present study found no significant main effect of gender on blood levels of osteocalcin and NTx. This observation partially aligned with Evans et al. [32], who revealed that though bone turnover markers increased in both genders, the rate changes were significantly higher in males than in

female military recruits in the first two months of vigorous regimented exercise programmes and levelled up at the fourth month.

Notably, all bone reabsorption and resorption markers were clustered towards the normal ranges except inorganic phosphate, which showed a slightly higher than laboratory reference range for males and low-to-medium-impact sports participants. The present study also showed that bone alkaline phosphatase was higher in high-impact, declined in mid-impact, and lowest in low-impact activities. The finding was consistent with the study conducted among women [22]. The present study also shows that serum inorganic phosphate and alkaline phosphates are higher in males than in females, but the reverse was for calcium. While age affects the serum levels of BTMs [33, 34], the reason for the gender variation in age-matched young athletes is unclear. Some researchers believed it could be due to the osteogenic effect of sex hormones [33] or gendered anthropometric differences [34]. Previous studies on sports-induced changes in inorganic phosphate and alkaline phosphatase were mainly among females [6, 22].

Remarkably, the present study showed that the mean value of calcium was highest in low-impact and lowest in high-impact activities in the studied population and that gender was not a significant factor. The result of the presented study partly agreed with the report of a study that evaluated 1137 adolescents, at 13 and 17 years old, and reported that males only had higher mean values of bone mineral density in those involved in high-impact activities as compared with those engaged in mid and low impact and no statistical difference in the values of females across the categories of sports impacts [25]. We believe that high-impact sports induce more osteoblastic activities, leading to higher bone density and lesser free circulating calcium, hence healthier and stronger bones [4, 23]. Although this observation is true for young male and female participants, a life course research design is necessary to determine at what age and circumstances these benefits start to decline, given the gender differences in the older cohort [33].

The outcomes of the post hoc analyses also showed how serum levels of bone formation markers of calcium, inorganic phosphate, and bone alkaline phosphatase significantly differed across sporting activities among the participants. This finding was in tandem with the reports in the literature, which stated that bone formation markers show changes in athletes depending on the intensity of training [35, 36]. Knowing that medium to high-impact exercise and sports can improve bone homeostasis among young Black athletes increases our curiosity in investigating their correlates with musculoskeletal diseases in older adults, such as osteoporosis

Table 3 Participants’ serum levels of bone turnover markers

| Parameter | Male (n = 23) | Female (n = 24) | High impact (n = 16) | Medium impact (n = 16) | Low impact (n = 15) |
|---|---------------|-----------------|----------------------|------------------------|---------------------|
| Osteocalcin (8.0–32.0 mg/dL)^a | | | | | |
| Mean ± SD | 11.20 ± 4.01 | 11.71 ± 5.63 | 11.23 ± 5.01 | 11.01 ± 5.16 | 12.20 ± 4.60 |
| Range ^b | 4.3–20.0 | 5.1–24.3 | 5.1–24.3 | 5.9–22.2 | 4.3–20.0 |
| Low f(%) | 8(34.8) | 5(20.8) | 5(37.5) | 4(25.0) | 3(20.0) |
| Normal f(%) | 15(65.2) | 19(79.2) | 11(62.5) | 12(75.0) | 12(80.0) |
| High f(%) | – | – | – | – | – |
| Calcium (8.6–10.3 mg/dL)^a | | | | | |
| Mean ± SD | 8.18 ± 0.45 | 8.39 ± 0.44 | 8.10 ± 0.50 | 8.19 ± 0.25 | 8.59 ± 0.44 |
| Range ^b | 7.2–9.3 | 7.5–9.2 | 7.2–9.1 | 7.8–8.6 | 8.0–9.3 |
| Low f(%) | 20(87.0) | 15(62.5) | 11(68.7) | 12(75.0) | 12(80.0) |
| Normal f(%) | 3(13.0) | 9(37.2) | 5(31.3) | 4(25.0) | 3(20.0) |
| High f(%) | – | – | – | – | – |
| IP (2.8–4.5 mg/dL)^a | | | | | |
| Mean ± SD | 4.68 ± 0.94 | 4.20 ± 0.90 | 4.66 ± 0.99 | 4.71 ± 0.53 | 3.90 ± 1.05 |
| Range ^b | 2.6–6.1 | 2.5–5.5 | 2.9–6.1 | 3.9–5.7 | 2.5–5.5 |
| Low f(%) | 1(4.3) | 2(8.3) | 2(12.5) | 1(6.3) | – |
| Normal f(%) | 7(30.4) | 12(50.0) | 5(31.3) | 8(50.0) | 6(40.0) |
| High f(%) | 15(65.3) | 10(41.7) | 9(56.2) | 7(43.7) | 9(60.0) |
| ALP (44.0–147.0 IU/L)^a | | | | | |
| Mean ± SD | 43.87 ± 16.23 | 41.58 ± 8.61 | 50.31 ± 14.08 | 44.00 ± 9.65 | 33.20 ± 7.95 |
| Range ^b | 22.0–81.0 | 27.0–59.0 | 35.0–81.0 | 29.0–61.0 | 22.0–49.0 |
| Low f(%) | 11(47.8) | 17(70.8) | 10(62.5) | 7(43.8) | 11(73.3) |
| Normal f(%) | 12(52.2) | 7(29.2) | 6(37.5) | 9(56.3) | 4(26.7) |
| High f(%) | – | – | – | – | – |
| NTx (5.4–24.2 nmol BCE/L)^a | | | | | |
| Mean ± SD | 19.38 ± 32.58 | 18.69 ± 9.73 | 13.48 ± 9.48 | 19.46 ± 13.61 | 24.48 ± 38.24 |
| Range ^b | 1.60–55.0 | 1.50–30.9 | 3.30–28.5 | 1.50–35.9 | 3.60–55.0 |
| Low f(%) | 5(21.7) | 2(8.3) | 2(12.5) | 2(12.5) | 3(20.0) |
| Normal f(%) | 14(60.9) | 17(70.8) | 9(56.3) | 12(75.0) | 10(66.7) |
| High f(%) | 4(17.4) | 5(20.8) | 5(31.3) | 2(12.5) | 2(13.3) |

IP inorganic phosphate, ALP alkaline phosphatase, NTx N-telopeptides of type 1 collagen, SD standard deviation

^a Laboratory reference range

^b Calculated range

and arthritis. The outcome would inform recommendations for the risk reduction of musculoskeletal disorders by choosing beneficial exercise types from adolescence to adulthood.

Limitation

Participants were sport-type and gender-matched; however, non-probability sampling techniques are prone to sampling bias, which may affect the generalisability of this study. Although participants were all amateur athletes from the university team, differences in the level of personal training, nutritional status, and number of years of sports involvement could also impact the observed results. An age-matched cohort design with one-year

follow-up would have allowed baseline adjusted within and between-groups longitudinal analysis. A larger sample size is required for more statistical power. A longitudinal case-control design will be necessary to track the changes in sporting activities, bone metabolism, and the onset of musculoskeletal disorders through a life course approach.

Conclusion

Irrespective of gender, moderate to high-impact sports positively affected bone turnover in young athletes. Low-impact sports participants had lower bone reabsorption and higher resorption compared to moderate and high-impact sports. Therefore, participation in

Table 4 Two-way ANOVA: main- and interaction effects of gender and sports type on the bone turnover markers

| Parameter | Mean SQUARE | Partial eta squared | F-statistic (df) | p value |
|--|-------------|---------------------|------------------|---------|
| Osteocalcin (mg/dL) | | | | |
| Gender | 2.511 | 0.002 | 0.097(1, 41) | 0.757 |
| Sports-type | 6.321 | 0.012 | 0.244(2, 41) | 0.785 |
| Gender × Sports-type | 3.924 | 0.007 | 0.151(2, 41) | 0.860 |
| Calcium (mg/dL) | | | | |
| Gender | 0.436 | 0.065 | 2.843(1, 41) | 0.099 |
| Sports-type | 1.044 | 0.249 | 6.807(2, 41) | 0.003* |
| Gender × Sports-type | 0.280 | 0.082 | 1.825(2, 41) | 0.174 |
| Inorganic phosphate (mg/dL) | | | | |
| Gender | 2.373 | 0.073 | 3.228(1, 41) | 0.080 |
| Sports-type | 3.167 | 0.174 | 4.307(2, 41) | 0.020* |
| Gender × Sports-type | 0.830 | 0.052 | 1.129(2, 41) | 0.333 |
| Alkaline phosphatase (IU/L) | | | | |
| Gender | 30.951 | 0.007 | 0.297(1, 41) | 0.588 |
| Sports-type | 1197.702 | 0.360 | 11.511(2, 41) | <0.001* |
| Gender × Sports-type | 473.043 | 0.182 | 4.546(2, 41) | 0.016* |
| N-telopeptides of type-1-collagen (nmol BCE/L) | | | | |
| Gender | 20.541 | 0.001 | 0.037 | 0.849 |
| Sports-type | 522.484 | 0.044 | 0.940 | 0.399 |
| Gender × Sports-type | 898.391 | 0.073 | 1.616 | 0.211 |

* F-statistic was significant at $p < 0.05$. df = degree of freedom

moderate to high-impact sporting activities is recommended for young adults to attain peak bone formation and maintain a life-course benefit of healthy bones. Low-impact sports professionals should undertake additional moderate to high-impact physical activities periodically or during the off-season.

Abbreviations

| | |
|-------|--|
| ALP | Alkaline phosphatase |
| ANOVA | Analysis of variance |
| BMD | Bone mineral density |
| BMI | Body mass index |
| BTM | Bone turnover marker |
| IP | Inorganic phosphate |
| MD | Mean difference |
| NTx | Cross-linked N-telopeptides of type 1 collagen |
| SD | Standard deviation |

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Authors' contributions

All authors contributed equally to the conception, design, data acquisition, statistical analysis, article drafting, and critical revision. All authors approved the final manuscript for publication. All authors have agreed to be personally accountable for the author's contributions and ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated, resolved, and the resolution documented in the literature.

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Availability of data and materials

The datasets analysed during the current study are available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate

The authors obtained ethical approval from the Health Research Ethics Committee of the University of Benin, Benin City, Edo State, Nigeria (Reference number: UBHREC/05/01/2326). The objectives of the study were clearly explained to each participant, who then signed an informed consent form.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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