Journal of Bone and Mineral Metabolism (2019) 37:512–519
https://doi.org/10.1007/s00774-018-0947-7

ORIGINAL ARTICLE

Effect of a 6-week supervised detraining period on bone metabolism markers and their association with ergometrics and components of the hypothalamic–pituitary–gonadal (HPG) axis in professional male soccer players

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Received: 24 October 2017 / Accepted: 7 August 2018 / Published online: 5 September 2018

Abstract

The aim of this study was to examine the effect of a supervised 6-week detraining period on bone metabolism markers, and their association with ergometrics, and components of the hypothalamic–pituitary–gonadal (HPG) axis in elite male professional soccer players. Sixty-seven soccer players (mean age ± SD 23.4 ± 5.2 years) that were following a supervised training program participated in this study. Players were tested twice: immediately after the conclusion of the competition period, and following the detraining period, for the determination of bone-turnover rates, ergometrics, and components of the HPG-axis. The detraining period resulted in significant reduction in osteocalcin [OC] ($p < 0.001$), C-terminal propeptide of collagen type-I [CICP] ($p = 0.002$), and bone-alkaline-phosphatase [b-ALP] ($p < 0.001$) values, while C-terminal telopeptide [CTX] was increased ($p < 0.001$). No significant relationships were apparent between bone biomarkers and body weight, body-fat %, total testosterone, free testosterone, estradiol, follicle-stimulating hormone, and luteinizing hormone in both experimental sessions ($p > 0.05$). Similarly, despite the deterioration in ergometrics after detraining (all $p < 0.001$), no significant correlations were evident ($p > 0.05$) between bone biomarkers and maximal oxygen consumption, squat jump, countermovement jump, and 20 m sprint performance, and also between % change of bone biomarkers and ergometrics, apart from a weak relationship ($p = 0.041$) between OC and VO_2max of questionable value. Our results suggest that the 6-week soccer off-season detraining period in our study negatively affected bone physiology as reflected by the suppression of bone-formation rate and a parallel induction of bone resorption. The cause of this acute alteration of bone-turnover rates is not related to the examined components of the HPG-axis, although parallels is not associated with the changes in ergometrics.

Keywords Bone metabolism · Detraining · Sex steroids · Ergometrics · Soccer

Introduction

The contribution of physical activity to optimal bone mineral density (BMD) is well established [1]. Exercise training beneficially affects BMD, whereas subsequent training cessation and/or reduction tend to counteract this effect [1–3]. However, BMD, as measured by dual-energy X-ray absorptiometry (DEXA), responds slowly and cannot reflect short-term effects of exercise or the lack of it on bone physiology. On the other hand, markers of bone metabolism respond immediately to changes in physical activity levels and thus they can be used as a dynamic measurement of bone status [2, 3]. Based on these bone markers, it has been repeatedly shown that bone turnover is altered in favor of bone formation following exercise training stress [1, 4].

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00774-018-0947-7) contains supplementary material, which is available to authorized users.

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However, limited and inconsistent evidence exists regarding the effect of short-term detraining on bone metabolism markers and even less in elite competitive athletes [3]. Sartorio et al. [5] failed to observe any significant variation in bone turnover markers between the competition and the rest periods in various professional male and female athletes including soccer players, followed-up for a period of 6 months. On the contrary, training reduction during the off-season soccer period resulted in induction of bone formation attributed to a reduction of the in-season training volume, intensity, and the stress of the competition indicating the reversibility of exercise training on the skeletal system [6, 7]. However, the inferences drawn by these two studies are constrained by a variety of restrictive factors. More specifically, the mode, volume, and intensity of the performed exercise training regimes during the off-season period, and the timeframe between the last training session and blood collection, which are all parameters that are related to the response of bone turnover markers [1, 3, 8], were not reported by the authors. In particular, it has been observed that depending on the type, volume, and intensity of the performed activity, circulating bone markers' levels could be altered even 3–5 days after the last training session [1, 3, 8]. Lastly, in the study by Karlsson et al. [6], blood sampling was not collected at a fixed time of each day, a practice introducing an additional variability since markers of bone turnover exhibit a diurnal periodicity [9].

Other parameters that have been proposed to affect bone metabolism markers are the components of the hypothalamic–pituitary–gonadal axis (HPG). There is a plethora of scientific evidence suggesting that estrogens, and mainly estradiol (E2), may be important in the regulation of bone resorption, whereas both E2 and androgens may play an important role in the maintenance of bone formation markers in the elderly and healthy male individuals [10–12]. However, these findings are not universal. Studies on elderly individuals, adolescent and collegiate athletes, and chronically trained middle-aged men failed to observe any association between bone metabolism markers and total testosterone (TT), free testosterone (FT), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) [10–12]. The latter evidence questions the association between sex steroids and bone turnover markers. This discrepancy is more evident in young and well-trained individuals, suggesting that in this type of population, there seems to be a lack of an association between bone turnover markers and sex steroids.

Based on these data, the aim of the present study was to examine the effect of the specific soccer off-season detraining period on bone turnover markers and specifically on C-terminal telopeptide (CTx), osteocalcin (OC), bone-alkaline-phosphatase (b-ALP), and C-terminal propeptide of collagen type-I (CICP), under close scrutiny of the performed exercise activities regarding both intensity and volume. Furthermore, we examined whether the respective values of the components of the HPG axis—TT, FT, E2, FSH, and LH—correlated with the examined bone turnover markers in the two experimental sessions. Our working hypotheses were that the reduction in training stress could affect bone turnover markers in favor of bone resorption, whereas these markers would not show any association with sex steroids. Furthermore, since some evidences indicate that bone metabolism markers could be related to the level of performance [13, 14], we examined whether this association is evident between bone turnover markers and some physical performance fitness-related variables (maximal oxygen consumption [VO_2max], sprint performance, and jumping ability).

Materials and methods

Participants

Seventy-seven professional male soccer players, members of three Greek Superleague teams, which were under regular and invariable training for more than 5 years, were evaluated for potential inclusion in the study. Exclusion criteria were as follows: (a) any medical or endocrine disorder that could affect their ability to participate in the study and/or affect bone-turnover rates and the HPG axis; (b) suspicion or laboratory evidence of use of exogenous legal or illegal hormonal agents or other illicit substances; (c) players whose contracts were ending before the end of the study; and (d) failure to perform the instructed off-season training regime. As a result of the criteria c and d, a total of ten athletes were excluded from the study. The final number of the participants of the study was 67 professional male soccer players [age 25.85 ± 6.35 years (mean $\pm$ SD) and height 1.81 ± 0.05 m (mean $\pm$ SD)].

Ethics statement

Before testing, verbal explanation was given to each player, concerning the aim of the study and the testing procedures, and written informed consent was obtained. The study was performed in strict accordance with the ethical guidelines of the Helsinki Declaration and was approved by the Ethical Scientific Committee of the University Hospital of Heraklion, Greece.

Training prescription during the off-season period

The duration of the off-season transition period was set to 6 weeks, starting at the end of the competition period. During this recuperation period, participants were instructed to avoid any kind of exercise training for the first 2 weeks.

After this 2-week period, they were instructed to perform low-intensity aerobic running (50–60% of VO_2max) of 20–30 min total duration (30, 20, 2 × 15, 3 × 10, 2 × 10) three times per week, divided by minimum 1 day of rest. This type of activity was selected by the team coaches. All players were tested at two different occasions. The first experimental testing took place 3 days after the end of the competition period in May (pre). The second experimental testing was performed at the beginning of July (post). The first day of each experimental period, after a 12 h overnight fast, anthropometric characteristics were measured (08:30 am) and venous blood samples were obtained from 09:00 to 10:30 am for the determination of the bone turnover markers and the levels of the components of the HPG axis. In the afternoon of the same day (17:00 pm), players were tested for squat jump (SJ), countermovement jump (CMJ), and 20 m sprint performance. The second day of each experimental session, starting at 09:30 am, our participants were tested for the determination of VO_2max. All measurements were performed during the two experimental sessions at the same time of the day and players were tested in the same order to avoid any circadian variation in the measured variables. To ensure that our participants would perform the instructed training regime during the last 4 weeks of the detraining period, all native players were performing the training sessions in specific days under the supervison of their conditioning coach ($n = 57$). All the other players ($n = 10$) were provided with the adequate equipment for the recording and the storage of both the duration and the intensity of the performed training sessions (Polar RS400, Polar Electro, OY, Finland). In addition, players were instructed to perform the last training session 5 days prior to the second experimental testing, i.e., immediately before the beginning of the pre-season preparation period. Detailed nutritional guidelines were given to all players to ensure a high (> 55%) carbohydrate dietary intake during the study, including a list of a variety of foods, based on individual resting-metabolic rates and the calculated daily-energy expenditure as per reported activities [15]. Apart from the provided nutritional guidance, players were instructed to avoid the consumption of any Vitamin D-containing food and calcium supplements and furthermore, the consumption of any performance enhancement supplements throughout the whole detraining period.

Anthropometric measurements and body composition

Height (m) was measured using a stadiometer (Charder HM210D, Charder Electronics CO, LTD, Taiwan) and weight (kg) was obtained using an electronic weight scale (Seca Alpha 770, Seca Vogel, Hamburg, Germany). Body-fat percentage was assessed by skinfold thickness measurement (Lange Skinfold Caliper, Cambridge Scientific Instruments, Cambridge, UK) according to set procedures [15].

Measurement of bone turnover markers

Bone-alkaline-phosphatase (b-ALP; U/L) was measured in serum by an immunoenzymatic assay with the Access Immunoassay System (Beckman Coulter, France) with an intra- and inter-assay CV < 10%. Osteocalcin (OC; ng/mL) was measured in serum by a two-site ELISA recognizing both the intact and the N-terminal mid fragment (N-MID® Osteocalcin Elisa, Nordic Bioscience Diagnostics A/S) with intra- and inter-assay CV < 10%. C-terminal propeptide (CICP; ng/mL) was measured in serum by an enzyme-linked immunoassay (ELISA, METRA® CICP EIA Kit, Quidel) with an intra- and inter-assay CV below 10%. Carboxy-terminal telopeptide of type I collagen (CTX; ng/mL) was measured in serum by the Elecsys β-CrossLaps assay using the ECLIA Elecsys autoanalyzer. The intra- and inter-assay CVs were < 5%. All the samples were analyzed in duplicates.

Measurement of sex steroid levels

Total testosterone (ng/mL), E2 (pg/mL), LH (IU/L), and FSH (mIU/L) concentrations were measured using AIA 21 fully automated immunoassay analyzer (TOSOH-Eurogenetics Tokyo, Japan). Free testosterone (pg/mL) was measured using enzyme-linked immunoabsorbent assays (Alpco Diagnostics, Windham, NH). The sensitivity of the assays for TT, E2, LH, and FSH was 7 ng/mL, 25 pg/mL, 1.0 lU/L, and 0.2 mlU/L, respectively. The intra- and inter-coefficients of variation were 3.1–5.2% and 2.48–5.99% for TT, 2.6–6.1% and 3.8–9.1% for E2, 1.5–2.6% and 4.3–5.6% for FSH, and 1.8–2.5% and 2.1–2.7% for LH. The sensitivity of the assays for FT was 0.17 pg/mL while the intra- and inter-coefficients of variation were 4.7–17% and 5.3–12.4%. All procedures were carried out according to the instructions of the manufacturer. All samples were tested in duplicate.

Ergometry tests

The jumping (SJ, CMJ) and sprinting (20 m) abilities of the soccer players were assessed with a jumping mat (Powertimer, Newtest Ltd., Oulu, Finland) and infrared photoelectric cells (Powertimer, Newtest Ltd., Oulu, Finland), respectively, according to standard procedures [15]. Maximal oxygen consumption (VO_2max) assessment was performed on a motorized treadmill using an automated gas-analysis system (VMAX29, Sensormedics, Yorba Linda, CA), with the use of set procedures of a standard protocol [15].

Statistical analysis

Statistical analysis was performed using software program SPSS 20.0. Results are presented as mean ± SD. The distribution of variables was tested by the Shapiro–Wilk statistical method. Then, Pearson's (for normally distributed variables) and Spearman's (for non-normally distributed variables) correlation coefficients were used to assess the linear relationship between quantitative variables at an alpha level of significance $p < 0.05$. The changes between the experimental periods in the measured parameters within the groups were analyzed by the paired-samples *t* test for normally distributed data, and by Mann–Whitney *U* test for non-normally distributed data with Bonferroni adjustment to control the Type-1 error rate. Results were considered significant at a level of significance $p < 0.0033$ ($a = 0.05/15$). Additionally, effect sizes were calculated and classified to determine the magnitude of changes among experimental conditions as proposed by Cohen [16]: small $d < 0.50$, moderate $d = 0.50$–0.80, and large $d > 0.80$. Statistical power analysis was performed (Stata 13 software, StataCorp LP, USA) to attain 80% power. Analysis was carried out at a confidence level = 95% and confidence interval = 13.6 [16]. Our calculations showed that a sample size equal to 45, much lower than ours, i.e., $n = 67$, was needed to attain 80% power to detect any differences in changes of the measured variables between the two experimental sessions.

Results

Changes in bone metabolism markers, body composition, ergometrics and sex steroids

Our findings revealed that the 6-week detraining period resulted in significant reductions in all measured bone formation markers (OC; $p < 0.001$, b-ALP; $p < 0.001$, and CICP; $p = 0.002$) at the end of the study compared to baseline (Table 1; Fig. 1), while the examined bone resorption markers CTx exhibited a significant increase ($p < 0.001$). Notably, the obtained pre- and post-bone marker values were all within normal reference range (OC 24–70 ng/mL; b-ALP:15-41 U/L; CICP 76–163 ng/mL; CTx; 0.02–0.87 ng/mL), and bone turnover was higher prior to the detraining period compared to the end of the study (Table 1). Notably, according to evidence, active male soccer players have higher bone turnover than age-matched healthy controls [1, 7]. The observed % mean change in each bone marker at the end of the study was 35.9%, 29.3%, and 15.08% reduction for OC, b-ALP, and CICP, respectively, and a 15.2% increase for CTx (Fig. 2).

Analysis of our data failed to reveal any significant alteration in body weight (77.68 ± 7.06 vs. 79.08 ± 7.24; $p > 0.0033$) and body-fat % values (8.81 ± 2.96 vs. 9.69 ± 3.1; $p > 0.0033$) despite a slight increase in both examined body composition parameters at the end of the study compared to baseline.

In regard to sex steroids, no significant changes were observed between their baseline values and those at the end of the study in any of the TT (652.08 ± 141.81 vs. 637.55 ± 133.91 ng/mL, respectively; $p > 0.0033$), FT (10.63 ± 4.63 vs. 11.16 ± 5.58 pg/mL, respectively; $p > 0.0033$), E2 (27.92 ± 13.72 vs. 24.84 ± 12.85 pg/mL, respectively; $p > 0.0033$), LH (4.76 ± 1.95 vs. 4.75 ± 1.60 IU/L, respectively; $p > 0.0033$), and FSH (6.84 ± 5.45 vs. 6.39 ± 4.54 mIU/L, respectively; $p > 0.0033$).

Lastly, analysis of our findings revealed that the detraining period resulted in reduced (Table 2) VO_2max ($p < 0.001$), SJ ($p < 0.001$), and CMJ ($p < 0.001$) values, while 20 m sprint times were significantly increased ($p < 0.001$).

Correlations between bone metabolism markers with body composition, sex steroids and ergometrics

Analysis of our findings failed to reveal any significant correlations between TT, FT, E2, LH, and FSH, and bone turnover markers ($p > 0.05$). Similarly, no significant correlations were evident between body-weight, body-fat % and the ergometrics VO_2max, SJ, CMJ, and 20 m sprint with the examined bone metabolism markers ($p > 0.05$). In addition, no significant correlations were evident between % change

Table 1 Alterations in bone metabolism markers during the study (mean ± SD)

Bone metabolism markers	Pre	Post	*p* value	Effect size (*d*)
Bone formation markers				
OC (ng/mL)	20.02 ± 17.60	12.82 ± 9.75	$p < 0.001$	$d = 0.50$
b-ALP (ng/mL)	36.86 ± 17.93	28.15 ± 15.88	$p < 0.001$	$d = 0.51$
CICP (ng/mL)	136.26 ± 41.48	113.77 ± 15.88	$p = 0.002$	$d = 0.56$
Bone resorption marker				
CTX (ng/mL)	0.74 ± 0.22	0.86 ± 0.19	$p < 0.001$	$d = 0.54$

OC osteocalcin, *b-Alp* bone-alkaline-phosphatase, *CICP* C-terminal propeptide of collagen type-I, *CTX* C-terminal telopeptide

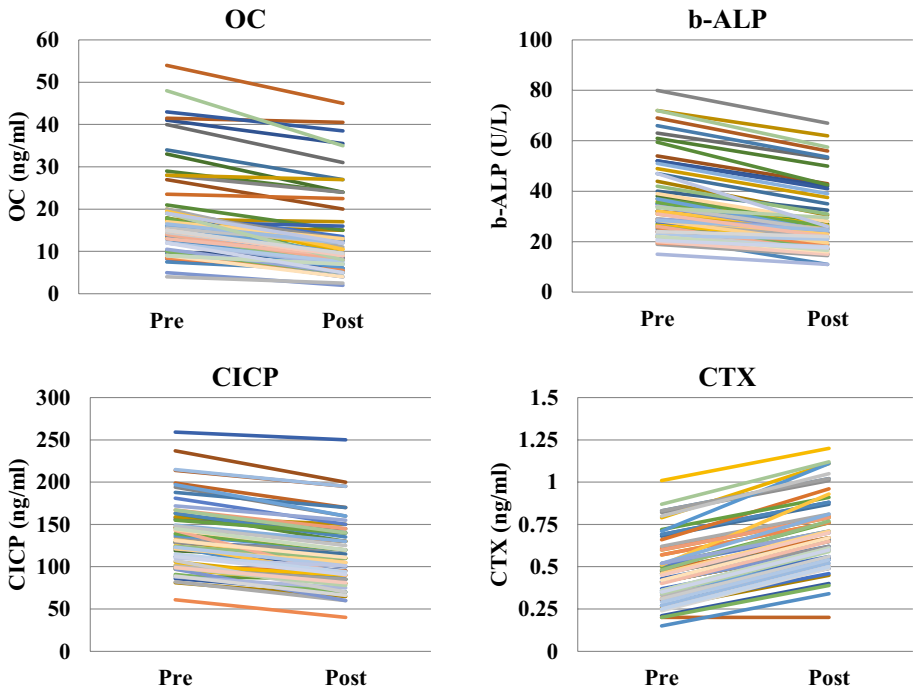

Fig. 1 Pre and post individual values of the examined bone turnover markers. *Pre* first experimental sessions at the end of the in-season period, *Post* second experimental period at the end of the off-season detraining period, *OC* osteocalcin, *CICP* C-terminal propeptide of collagen type-I, *b-ALP* bone-alkaline-phosphatase, *CTx* C-terminal-telopeptide

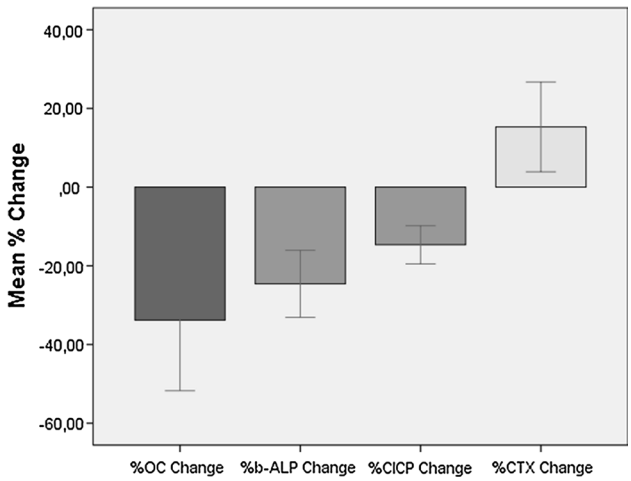

Fig. 2 % Mean change values (±SD) of bone turnover markers during the study. *%OC Change* % mean change in osteocalcin levels, *%b-ALP Change* % mean change in bone-alkaline-phosphatase levels, *% CICP Change* % mean change in C-terminal Propeptide of Collagen Type-I levels, *%CTx Change* % mean change in C-terminal telopeptide levels

of all bone turnover markers and % change of ergometrics (Supplementary Fig. 1) apart from a weak significant association between the % change of OC and the % change of VO_2max ($p=0.041$) of questionable value (Supplementary Fig. 1a).

Discussion

In our study, the massive reduction in training stress during the 6-week detraining period resulted in a significant increase in the bone resorption, as reflected by the marker CTX and a parallel decline of bone formation as reflected by the measure of newly-synthesized collagen CICP, and those of osteoblast-related proteins OC, and b-ALP. No association was observed between both ergometric and sex steroid levels with bone turnover markers, apart from a weak association between the % changes in OC and VO_2max of questionable value. Our findings suggest that the observed alterations in the examined bone turnover markers do not appear to involve

Table 2 Alterations in ergometrics during the study (mean ± SD)

Ergometrics	Pre	Post	*p* value	Effect size (*d*)
VO_2max (mL/kg/min)	59.8 ± 3.40	57.46 ± 3.11	$p<0.001$	$d=0.71$
SJ (cm)	40.2 ± 3.40	37.67 ± 3.18	$p<0.001$	$d=0.76$
CMJ (cm)	41.53 ± 3.68	39.31 ± 3.35	$p<0.001$	$d=0.61$
20 m (s)	3.02 ± 0.65	3.06 ± 0.65	$p<0.001$	$d=0.56$

VO_2max maximal oxygen consumption, *SJ* squat jump, *CMJ* countermovement jump, *20 m* 20 m sprint

the HPG axis and are not related to the examined soccer performance indices and body composition variables.

Our observations are comparable with the findings of the only two, to the best knowledge of the authors, available studies in soccer [6, 7]. Weiler et al. [7] reported that an eight-week off-season soccer period resulted in increased CTx levels whereas, the measured bone formation marker N-terminal propeptide of type I procollagen (PINP) exhibited a decrease in its values. Similarly, Karlsson et al. [6] showed that the four-week off-season transition period resulted in an increase of bone resorption, as reflected by the changes in CTx, and a parallel decrease in bone formation markers, total alkaline phosphatases (t-ALP), and carboxy-terminal propeptide of type I collagen (PICP) but not in OC levels. This lack of response regarding OC was attributed to the different analytical methods used by measuring the specific bone marker, which recognize different OC-derived fragments and have been suggested to limit the use of OC in bone-status assessment due to an induced variability in the obtained results [1]. The aforementioned studies and our own finding clearly suggest that, in soccer, short-term detraining periods do negatively affect bone metabolism, in favor of resorption. Notably, although in accordance with the available literature, the observed biomarkers' levels were within normal range at both experimental sessions, bone turnover was higher prior to the detraining period compared to the end of the study [1, 3, 6, 7]. It should be highlighted that this observed bone biomarkers' response could not be able to lead in the occurrence of stress fractures since, apart from the fact that this effect was within normal physiological range, the mechanical loading was extremely decreased during this period suggesting the absence of excessively repetitive loads on the bone that could result in fatigue-induced bone microdamage and consequently stress fractures.

Although the mechanisms underlying these findings have not yet been clearly established, it could be hypothesized to be related to the transition from a long-term in-season soccer period of continuous training and competitions to a period characterized by an enormous reduction in training load, intensity, frequency, and volume of the performed activities, and in the employment of a single non-impact type (mode) of exercise. It is well demonstrated that training intensity, volume, and frequency are the most important parameters affecting the response of bone biomarkers to exercise [17–19]. Furthermore, the combination of running and resistance-type of activities, as is regularly evident in soccer trainings and competitions [20], is more effective in enhancing bone turnover than when performed in isolation [17, 18]. Therefore, we could hypothesize that the reduction in mechanical loading due to the reduced volume, frequency, intensity, and also mode of performed activities (i.e., only low-intensity running) could have resulted in a reversibility on bone metabolism leading to reduced bone formation and increased bone resorption [1, 17, 18]. This hypothesis is supported by the observed decrease in bone formation and increase in bone resorption in soccer players [6, 7] and elite rowers [21] after the off-season period of reduced activity between two competition seasons, and furthermore, in astronauts after a few weeks of space flight [17]. These findings suggest that bone-turnover reversibility is rapid and profound when mechanical forces acting on the skeleton are markedly diminished.

One of the aims in our study was to control several limitations that were constraining factors in the inferences drawn by the two soccer-detraining studies [6, 7]. More specifically, although it is most likely that training activity exhibited a massive reduction during this period in these two studies, the employed training regime was not recorded. Furthermore, the volume, intensity, and mode of the performed activities were neither controlled nor reported. Since aerobic and/or resistance exercise might affect serum–bone metabolism markers' levels after 3–5 days of recovery [21], the lack of controlling these parameters could have interfered with their findings. In addition, in the study of Karlsson and associates [6], the participants were allowed to consume breakfast, and the blood samples were not collected exactly at the same time of day are factors that have been reported to affect the serum levels of bone biomarkers [4]. In our study, training volume and intensity were controlled. The last training session was performed maximum 5 days prior to each testing, and blood samples were obtained after an overnight fast in the same time of day to avoid any diurnal variability in the obtained results. Based on our observations that were not constrained by the aforementioned limited factors, our findings provide further affirmation to the suggestion that even short-term detraining periods in soccer have a negative effect on bone turnover in favor of resorption.

Although, OC and CTx have been previously studied during short-term soccer-detraining periods, we provide for the first time, to the best of our knowledge, evidence for a reduction in b-ALP and CICP. It is well known that b-ALP is the most widely used bone metabolism marker, since it is involved in all phases of bone mineralization which provides a specific indicator of osteoblast activity, and is one of the most sensitive bone turnover markers to alterations in exercise training volume [1, 22]. On the other hand, changes in bone biomarkers based on collagen fragments, such as CICP, are not always observed [23, 24]. It has been suggested that it takes 6–9 months prior to an observable response in bone formation markers based on collagen fragments [25]. Therefore, it could be argued that for CICP, the timeframe of our study may have been too short. However, our findings are in accordance with a study on adolescent boys (15–17 years) showing CICP to respond already after a 5-week training intervention [26], which is well in line with the timeframe used in the present study. The importance of our findings

regarding CICP is based on the suggestion to be a sensitive marker that is generated from newly synthesized collagen, linked to bone growth and formation, and being indicative of collagen production in vivo [27]. Therefore, the short detraining period, apart from the effects on the two osteoblast-related proteins OC and b-ALP, it affects, in a similar manner, the bone biomarkers based on collagen fragments.

In agreement with the available literature, the off-season detraining period negatively affected exercise performance indices [28]. Interestingly, although this expected performance deterioration was found concomitantly with the negatively altered bone turnover markers, no relationship was evident between bone metabolism and the level of performance as indicated by the ergometrics (VO_2max, 20 m sprint, SJ, CMJ). Our findings do not support the observation that the level of aerobic performance is associated with bone turnover markers [13], and the reports showing a close relationship between optimal bone status and ergometrics in young [28] and elderly [14] populations of both genders, and young female handball players [29]. Notably, although there is evidence indicating that bone turnover markers are related to time to exhaustion in soccer [13], this specific measure serves as an indirect indicator of aerobic capacity, and not VO_2max. In addition, although an association between VO_2max and b-ALP was observed in a study on both genders, this was not evident when only males were included in the regression analysis [28]. Lastly, the evidence that shows that there is a close association between strength, jumping ability, and sprint performance to bone status [28–30] derives from studies that have examined BMD and bone mass, which as indicators of bone status respond slowly and cannot reflect short-term effects of exercise or the lack of it on bone physiology. Therefore, based on our findings, we could speculate that in professional male soccer players, exercise performance indices are not related to bone biomarkers. This is further supported by the observation that even when associations between the % change between bone markers and ergometrics were examined, no relationships were evident, apart from a weak statistically significant relationship of OC with VO_2max of questionable value due to the limited suggested power of this marker in bone status assessment [1]. Overall, these findings are in accordance with the suggestion that physical condition level is only weakly, if at all, correlated with bone formation [26]. These findings suggest that bone turnover is not actually related to performance level and/or measures such as ergometrics, but rather to the characteristics of the performed exercise. Support to this suggestion comes from the findings that in different types of populations of all ages and of various activity profiles, regardless of their level of performance, the employment of weight-bearing activities of increased volume, intensity, and training frequency results in positively altered bone turnover [1, 4, 17].

Although our findings regarding the gonadotropins (LH, FSH) and FT are in agreement with the extremely limited available evidence [11, 31, 32] that failed to reveal any relationship of these hormones with bone metabolism markers [12], the observations regarding the effects of E2 and TT on bone biomarkers add to the existing controversy [10, 12]. Indeed, although studies on elderly individuals and healthy adults [10, 11] indicate that changes in E2 are strongly related to bone resorption, and that both E2 and TT play an important regulatory role in the maintenance of bone formation [11], evidence from well-trained collegiate and middle-aged males failed to support these suggestions [10, 11, 31, 32]. Since both age and physical status of the participants are vital parameters that affect bone metabolism [1, 4], our findings indicate that in well-trained professional soccer players, E2 and TT do not seem to play a regulatory role in bone metabolism, suggesting that the observed negatively affected bone turnover in short detraining periods is not a physiological alteration induced by sex steroids, but an effect of the reduced training stress. This is further supported by the findings that despite the altered bone turnover, in support to available bibliography, no alterations were evident in sex steroids levels during the study [15, 16]. However, we could not exclude the possibility that in long-detraining periods, sex steroids could play a significant role in the modulation of bone metabolism, since the HPG-axis could be less sensitive than bone turnover markers in short-term reductions in training stress.

In conclusion, our findings provide supportive evidence to the suggestion that a short-term off-season soccer-detraining period affects bone turnover rates in favor of resorption in professional soccer players and results in the decline in the measures of both the osteoblast-related proteins (OC, b-ALP), and the examined one of newly synthesized collagen (CICP). These alterations were not related to the observed changes in ergometrics. Similarly, no relationship was observed between bone biomarkers and sex steroids, suggesting that these hormones do not play the main regulatory role in bone health during short-detraining periods. Future research should examine whether this lack of association in professional soccer players between sex steroids, ergometrics, and bone turnover also persists over longer periods of detraining.

Author contributions Study design: TW, FW, GJ, and BO. Study conduct: TW and GJ. Data collection and management: TW, FW, and GJ. Data analysis: FW, KW, TW, and LL. Data interpretation: FW, TW, KW, LL, and GJ. Drafting manuscript: FW and TW. Revising manuscript content: all authors. Approving final version of manuscript: all authors. FW takes responsibility for the integrity of the data analysis.

Compliance with ethical standards

Conflict of interest All authors state that they have no conflict of interest.

References

1. Banfi G, Lombardi G, Colombini A, Lippi G (2010) Bone metabolism markers in sports medicine. Sports Med 40:697–714
2. Maïmoun L, Simar D, Malatesta D, Caillaud C, Peruchon E, Couret I, Rossi M, Mariano-Goulart D (2005) Response of bone metabolism related hormones to a single session of strenuous exercise in active elderly subjects. Br J Sports Med 39:497–502
3. Banfi G, Colombini A, Lombardi G, Lubkowska A (2012) Metabolic markers in sports medicine. Adv Clin Chem 56:1–54
4. Maïmoun L, Sultan C (2011) Effects of physical activity on bone remodeling. Metabolism 60:373–388
5. Sartorio A, Jubeau M, Agosti F, Marazzi N, Rigamonti A, Müller EE, Maffiuletti NA (2006) A follow-up of GH-dependent biomarkers during a 6-month period of the sporting season of male and female athletes. J Endocrinol Invest 29:237–243
6. Karlsson KM, Karlsson C, Ahlborg HG, Valdimarsson O, Ljunghall S, Obrant KJ (2003) Bone turnover responses to changed physical activity. Calcif Tissue Int 72:675–680
7. Weiler R, Keen R, Wolman R (2012) Changes in bone turnover markers during the close season in elite football (soccer) players. J Sci Med Sport 15:255–258
8. Thorsen K, Kristoffersson A, Hultdin J, Lorentzon R (1997) Effects of moderate endurance exercise on calcium, parathyroid hormone, and markers of bone metabolism in young women. Calcif Tissue Int 60:16–20
9. Greenspan SL, Dresner-Pollak R, Parker RA, London D, Ferguson L (1997) Diurnal variation of bone mineral turnover in elderly men and women. Calcif Tissue Int 60:419–423
10. Ackerman KE, Skrinar GS, Medvedova E, Misra M, Miller KK (2012) Estradiol levels predict bone mineral density in male collegiate athletes: a pilot study. Clin Endocrinol 76:339–345
11. Sinnesael M, Boonen S, Claessens F, Gielen E, Vanderschueren D (2011) Testosterone and the male skeleton: a dual mode of action. J Osteoporos 2011:240328
12. Falahati-Nini A, Riggs BL, Atkinson EJ, O'Fallon WM, Eastell R, Khosla S (2000) Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. J Clin Invest 106:1553–1560
13. Karlsson KM, Karlsson C, Ahlborg HG, Valdimarsson O, Ljunghall S (2003) The duration of exercise as a regulator of bone turnover. Calcif Tissue Int 73:350–355
14. Evans RK, Antczak AJ, Lester M, Yanovich R, Israeli E (2008) Moran DS (2008) Effects of a 4-month recruit training program on markers of bone metabolism. Med Sci Sports Exerc 40:S660–S670
15. Koundourakis NE, Androulakis NE, Malliaraki N, Tsatsanis C, Venihaki M, Margioris AN (2014) Discrepancy between exercise performance, body composition, and sex steroid response after a 6-week detraining period in professional soccer players. PLoS ONE 9:e87803
16. Cohen J (1988) Statistical power analysis for the behavioral sciences, 2nd edn. Lawrence Errlbaum Associates, Hillsdale
17. American College of Sports Medicine (2004) Position stand: physical activity and bone health. Med Sci Sports Exerc 36:1985–1996
18. Alghadir AH, Aly FA, Gabr SA (2014) Effect of moderate aerobic training on bone metabolism indices among adult humans. Pak J Med Sci 30:840–884
19. Jürimäe J, Purge P, Jürimäe T, von Duvillard S (2006) Bone metabolism in elite male rowers: adaptation to volume-extended training. Eur J Appl Physiol 97:127–132
20. Stølen T, Chamari K, Castagna C, Wisløff U (2005) Physiology of soccer: an update. Sports Med 35:501–536
21. Malm HT, Ronni-Sivula HM, Viinikka LU, Ylikorkala OR (1993) Marathon running accompanied by transient decreases in urinary calcium and serum osteocalcin levels. Calcif Tissue Int 52:209–211
22. Seibel MJ (2005) Biochemical markers of bone turnover: part I: biochemistry and variability. Clin Biochem Rev 26:97–122
23. Lester ME, Urso ML, Evans RK, Pierce JR, Spiering BA, Maresh CM, Hatfield DL, Kraemer WJ, Nindl BC (2009) Influence of exercise mode and osteogenic index on bone biomarker responses during short-term physical training. Bone 45:768–776
24. Vainionpää A, Korpelainen R, Väänänen HK, Haapalahti J, Jämsä T, Leppäluoto J (2009) Effect of impact exercise on bone metabolism. Osteoporos Int 20:1725–1733
25. Christenson RH (1997) Biochemical markers of bone metabolism: an overview. Clin Biochem 30:573–593
26. Eliakim A, Raisz LG, Brasel JA, Cooper DM (1997) Evidence for increased bone formation following a brief endurance-type training intervention in adolescent males. Bone Miner Res 12:1708–1713
27. Koura HM, Zaki SM, Ismail NA, Salama EE, El Lebedy DH, Effat LK (2014) Relationship between biochemical bone markers and bone mineral density in patients with phenylketonuria under restricted diet. Iran J Pediatr 24:23–28
28. Bevier WC, Wiswell RA, Pyka G, Kozak KC, Newhall KM, Marcus R (1989) Relationship of body composition, muscle strength, and aerobic capacity to bone mineral density in older men and women. J Bone Miner Res 4:421–432
29. Vicente-Rodriguez G, Dorado C, Perez-Gomez J, Gonzalez-Henriquez JJ, Calbet JA (2004) Enhanced bone mass and physical fitness in young female handball players. Bone 35:1208–1215
30. Ginty F, Rennie KL, Mills L, Stear S, Jones S, Prentice A (2005) Positive, site-specific associations between bone mineral status, fitness, and time spent at high-impact activities in 16- to 18-year-old boys. Bone 36:101–110
31. MacKelvie K, Taunton J, McKay H, Khan K (2000) Bone mineral density and serum testosterone in chronically trained, high mileage 40–55 year old male runners. Br J Sports Med 34:273–278
32. Lima F, De Falco V, Baima J, Carazzato JG, Pereira RM (2001) Effect of impact load and active load on bone metabolism and body composition of adolescent athletes. Med Sci Sports Exerc 33:1318–1323