

# A HIGH LEVEL OF VOLLEYBALL PRACTICE ENHANCES BONE FORMATION MARKERS AND HORMONES IN PREPUBESCENT BOYS

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**ABSTRACT:** Objectives: To examine the effects of volleyball on hormones and biochemical markers before puberty. Methods: 130 prepubescent white boys were investigated in this study. 80 prepubescent volleyball players were divided into two groups according to the duration of training: 40 (age:  $11.5 \pm 0.6$  years), representing the high-level training group (HLG), completed 6 to 8 hours of training/week; 40 (age:  $11.2 \pm 0.7$  years), representing the low-level training group (LLG), completed 3 to 5 hours of training/week. The other 50 non-athletic boys (age:  $11.3 \pm 0.2$  years) were used as control subjects (C). Results: Serum concentration of growth hormone (GH), insulin-like growth factor 1 (IGF-1) and carrier protein 3 (IGFBP-3), cortisol, bone formation markers (osteocalcin [OC] and bone alkaline phosphatase [BAP]), and a bone resorption marker (cross-linked C-terminal telopeptide of type I collagen [CTX]) were measured. No difference in CTX was observed among the three groups. However, the HLG presented higher levels of bone formation markers (OC, BAP) compared to controls. Hormonal concentrations of GH, IGF-1, IGFBP-3, and cortisol were higher in HLG than in controls. Conclusion: Volleyball did not lead to enhanced bone turnover markers and anabolic hormones of bone after a low-training level when compared to controls. Indeed, a high-training level induces enhanced bone formation markers and basal concentration of anabolic (GH, IGF-1, and IGFBP-3) and catabolic (cortisol) hormones of bone metabolism. Therefore, basal hormone concentrations and bone formation markers were directly related to the intensity and the duration of the training level.

**KEY WORDS:** bone turnover markers, growth factors, IGF-1, IGFBP-3, steroid hormones, glucocorticoids, volleyball, prepubescent boys

## INTRODUCTION

Bone turnover can be viewed as the net product of two counteracting metabolic processes, bone formation and bone resorption. The balance between osteoblastic activity of bone formation and osteoclastic activity of bone resorption depends on age, nutrition, and physical activity. Physical exercise is a known source of bone turnover and is recommended for preventing bone metabolism problems.

It is recognized that weight-bearing exercises ensure increased bone turnover in adulthood [40]. This gain is clearly exhibited through the increase in bone formation markers [22] and the basal level of anabolic and catabolic hormones affecting bone [44]. Bone formation markers are more sensitive to physical activity than bone resorption [6]. We chose to study two markers of bone formation, osteocalcin (OC) and bone alkaline phosphatase (BAP), and a single bone resorption marker, cross-linked C-terminal telopeptide of type I collagen (CTX). BAP increased after 1 month of an aerobic exercise programme and normalized after 2 months, but OC increased after 2 months of an anaerobic exercise programme [6]. Among healthy premenopausal women, minor changes in physical activity are associated with a clear effect on bone formation markers [3].

However, most studies have shown that physical activity during the prepubertal stage is insufficient for modifying serum concentrations of bone metabolism markers [28,47]. In fact, growth hormone (GH), insulin-like growth factor-1 (IGF-1) and carrier protein 3 (IGFBP-3) are considered as the most important anabolic hormones in the body [27], and positively affect bone turnover by stimulating osteoblast proliferation and differentiation [41].

The GH/IGF-1 axis is the key regulator of somatic growth in humans. GH action on bone is mainly mediated through IGF-1. Elevated rates of GH, IGF-1 and IGFBP-3 have a beneficial effect on bone acquisition in young and adult athletes [5,39]. Links between physical activity and IGF-1 and IGFBP-3 secretion were observed in young adult women; gymnasts who practise a high-impact sport have higher IGF-I values and IGF-I/IGFBP-3 than runners and controls [44]. The plasma IGF-1 and IGFBP-3 increased after endurance exercise [33] and intensified training [34]. At early puberty, soccer practice induces an increase in the rate of secretion of GH, IGF-1 and IGFBP-3 [38]. Contrary to the role played by GH, IGF-1 and IGFBP-3, cortisol with catabolic characteristics also deserves attention. It has important

metabolic functions such as influencing the metabolism of glucose, proteins and lipids. [20]. Cortisol, the main glucocorticoid form in humans, is a catabolic hormone secreted from the adrenal cortex in response to physical and psychological stress. It plays important roles during and after acute exercise [13], including taking part in gluconeogenesis and accelerating the mobilization and use of fats to produce energy. While an elevated rate of the cortisol level may lead to the exhaustion of the athlete [46].

In fact, there is a divergence in results obtained in studies involving cortisol. Some investigations that used strength training among older adults showed a reduction in its levels [24], but others found no changes [23,43]. However, in young athletes, some investigations have demonstrated that intensive physical training stimulates cortisol secretion, and increases its basal rate [19], but others showed no variation [18].

In prepubescent girls, too intense exercise can cause hormonal disturbances clearly seen in a decrease in the IGF-1 and IGFBP-3 levels, responsible for delayed puberty [19], followed by a postponed acquisition of bone mass, despite the effect of osteogenic exercise [10]. To our knowledge, this hormonal disturbance has never been found in boys. Causing no hormonal troubles, an optimal intensity exercise allows the most appropriate response to stress. Volleyball is a highly osteogenic sport in adolescents and adults, particularly in weight-bearing bones [2,11,42]. It combines anaerobic phases of intense activity (sprints) and multiple impacts (jumping), and involves frequent bouts of intense activities such as jumping, diving, and lateral movement, and these activities are coupled with short rest periods.

Many investigations have aimed to study the effect of different levels of physical training on bone modelling and hormone parameters (GH, IGF-1, IGFBP-3, and cortisol) among pubescent and adult athletes. This is the first study to investigate the influence of different levels of volleyball practice on hormonal balance and its effects on bone turnover markers in the growing skeleton among prepubescent volleyball players. We therefore hypothesized that hormones in relation to volleyball activity generate a positive osteogenic effect in bone turnover markers during pre-puberty.

## MATERIALS AND METHODS

**Subjects.** One hundred and thirty Tunisian boys were recruited from several schools and Tunisian volleyball clubs, all resident in the city of Sousse. All subjects, whose ages ranged from 10 to 12, were divided into three groups. 80 were volleyball players in 2 local clubs for at least 18 months in addition to physical education at school; 40, whose ages ranged from 10.9 to 12.1, and who completed 6 to 8 hours of training plus one competitive game per week, constituted the high-level training group (HLG); and 40, whose ages ranged from 10.5 to 11.9, and who completed 3 to 5 hours of training plus one competitive game per week, constituted the low-level training group (LLG). The other 50 subjects, whose ages ranged from 11.1 to 11.5, were assigned to the control group (C). They participated only in the compulsory physical education curriculum at school (two weekly sessions of 50 min each).

In general, training volleyball sessions lasted 1 h 30 min, including about 15-20 min of warm up, low-intensity games and stretching exercises, 15-25 min of technical volleyball exercises (passing actions, smashing, blocking, and running with fast accelerations), 20-30 min of match practice, and 10 min of active recovery.

Each boy having a chronic disease that might affect either the physical exercise or bone metabolism was automatically excluded from this study. The study was approved by the Independent Ethics Committee of Farhat Hached Tunisian Hospital, and written informed consent was obtained from both parents of each participant.

### *Anthropometric measurements*

Height and weight were measured in light indoor clothing without shoes. Height was measured to the nearest 0.01 m using a wall stadiometer weight was measured to the nearest 0.1 kg using a balance.

### *Calcium intake*

Calcium intakes of each subject were measured by the method of recording food for three consecutive days. At each meal the patient mentioned what he had eaten, then with the program Bilnut, SCDA Nutrisoft (Cerelles, France) we calculated the amount of calcium consumed per day, expressed in  $\text{mg} \cdot \text{day}^{-1}$ .

### *Parameters of physical activity*

#### *$\dot{V}O_2 \text{ max}$*

The fitness level of children was evaluated by indirect calculation of the maximal oxygen uptake ( $\dot{V}O_2 \text{ max}$ ) through the 20-m shuttle run test as devised by Luc Léger. Subjects were required to run back and forth on a 20-m course and be on the 20-m line at the same time that a beep is emitted from a tape. The frequency of the sound signals increases in such a way that running speed starts at  $8.5 \text{ km} \cdot \text{h}^{-1}$  and is increased by  $0.5 \text{ km} \cdot \text{h}^{-1}$  each minute. The length of time the subjects were able to run was recorded to calculate the  $\dot{V}O_2 \text{ max}$  [31].

#### *Basal physical activity level*

Bratteby's questionnaire estimated the level of daily physical activities during a typical day, without volleyball training. The level of physical activity (PAL) was calculated by the following formula:  $\text{PAL} = \text{TEE} / \text{BMR}$  (PAL= physical activity level; TEE= total energy expended; BMR= basal metabolic rate) [8].

#### *Peak power of lower limbs*

We measured the peak power of lower limbs (squat jump [SJ], counter movement jump [CMJ] and horizontal jump [HJ] by using the sergeant test [1].

#### *Pubertal status*

Tanner pubertal status was determined by serum rates of follicle stimulating hormone (FSH), luteinising hormone (LH), and testos-

terone [25] and confirmed by a clinical method of recognized validity and reliability [15].

Only children with testosterone < 0.6 (mUI · ml<sup>-1</sup>), FSH < 4.6 (mUI · ml<sup>-1</sup>) and LH < 4.8 (mUI · ml<sup>-1</sup>), corresponding to Tanner's stage I, were considered as prepubescent.

**Biochemistry**

For each participant, blood samples of 18 cc were conducted between 8 am and 9:30 am and withdrawn following overnight fasting. Immediately, the serum was centrifuged (2100 g for 10 min), then it was isolated and frozen at -80°C.

We measured serum concentration of the hormone gonadotropins (FSH, LH and testosterone), growth hormone (GH), insulin-like growth factor 1 (IGF-1) and carrier protein 3 (IGFBP-3), cortisol, markers of bone formation (osteocalcin [OC], and bone alkaline phosphatase [BAP], and a marker of bone resorption (CTX).

Serum FSH, LH, GH, IGF-1 and IGFBP-3 levels were measured in one laboratory by immunoradiometric assay (IRMA) using a commercial kit (IRMA STH IMMUNOTECH FRANCE). The intra-assay coefficients of variation (CVs) were <5% and the inter-assay CVs were <10%. The levels of testosterone and cortisol were measured by radioimmunoassay (RIA) method (RADIOIMMUNOASSAY kit, IMMUNOTECH France). The intra-assay coefficients of variation (CVs) were <4% and the inter-assay CVs were <10%. The measurement of serum cortisol levels was performed by RIA using commercially available kits; the intra-assay coefficient of variation was <9.2% and the inter-assay coefficient of variation was 3.4%.

For the markers of bone formation, serum OC was measured by N-MID Osteocalcin ELISA; inter and intra-assay CVs were 12.6% and 3.9%, respectively. Serum BAP was determined by the immunoenzymetric test Ostase BAP ELISA; inter and intra-assay CVs were 13.1% and 7.6%, respectively. Finally, CTX (a marker of bone resorption) was measured by the Serum CrossLaps One Step ELISA (Nordic Bioscience, Herlev, Denmark); inter and intra-assay CVs are <8%.

**Statistical analysis**

Anthropometry, age, dietary calcium intake, hormonal and physical activity data were analysed by one-way analysis of variance (ANOVA) followed by Fisher's LSD post hoc test between HLG, LLG and C. The analyses of covariance (ANCOVA) entering weight, height and gonadotropin hormones (FSH) as covariates were performed to evaluate differences in hormonal and biochemical markers among HLG, LLG and C. Data were expressed as means ± SD and differences were considered significant at the 0.05 level. The Statistica software (StatSoft, version 6.0, 2001, France) was used for all analyses.

**RESULTS**

**Anthropometric variables.** The average age, height, weight, lean mass, calcium intake and pubertal status data are summarized in Table 1. At the time of measurement all children remained prepubescent. No age or dietary calcium intake differences were observed among the three groups, but the HLG group was heavier and taller than the LLG and control groups (p <0.001).

**TABLE 1. CHARACTERISTICS OF STUDY SUBJECTS: ANTHROPOMETRY, AGE, CALCIUM INTAKE, AND PUBERTAL STATUS**

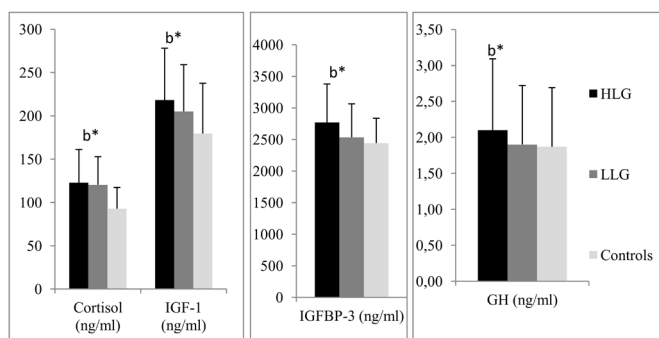
	HLG	LLG	Control(C)	Comparison between groups		
	N = 40	N = 40	N = 50	a	b	c
Age (year)	11.5 ± 0.6	11.2 ± 0.7	11.3 ± 0.2	ns	ns	ns
Height (m)	1.51 ± 0.06	1.45 ± 0.04	1.43 ± 0.05	a***	b***	ns
Weight (kg)	40.1 ± 6.3	35.5 ± 4.6	33.2 ± 2.8	ns	b***	c***
BMI	17.75 ± 2.22	16.96 ± 1.99	16.13 ± 1.26	ns	b***	ns
Calcium intake/d (mg)	712.52 ± 82.75	710.10 ± 117.87	716.88 ± 85.05	ns	ns	ns
FSH (mUI · ml <sup>-1</sup> )	3.84 ± 0.93	4.07 ± 0.82	3.27 ± 1.11	ns	b**	c***
LH (mUI · ml <sup>-1</sup> )	2.13 ± 0.40	2.07 ± 0.58	2.01 ± 0.48	ns	ns	ns
Testosterone (mUI · ml <sup>-1</sup> )	0.26 ± 0.16	0.25 ± 0.18	0.20 ± 0.15	ns	ns	ns

Note: Data are presented as mean ± standard deviation, a: comparison between HLG and LLG, b: comparison between HLG and C, c: comparison between LLG and C, \*: p <0.05, \*\*: p <0.01, \*\*\*: p <0.001, ns: not significant

**TABLE 2. PHYSICAL CHARACTERISTICS OF THE STUDY SUBJECTS**

	HLG	LLG	Control(C)	Comparison between groups		
	N = 40	N = 40	N = 50	a	b	c
PAL	35.94 ± 2.76	36.41 ± 2.93	37.02 ± 2.67	ns	ns	ns
VO <sub>2</sub> max (ml · Kg <sup>-1</sup> · min <sup>-1</sup> )	50.81 ± 3.55	49.68 ± 2.98	47.65 ± 2.8	ns	b***	c*
HJ (cm)	166.64 ± 16.05	146.14 ± 6.93	137.54 ± 10.83	a***	b***	ns
SJ (cm)	26.74 ± 5.85	23.41 ± 5.36	20.19 ± 5.44	a**	b***	c*
CMJ (cm)	30.34 ± 5.78	26.05 ± 5.13	22.42 ± 4.72	a***	b***	c***

Note: Data are presented as mean ± standard deviation, a: comparison between HLG and LLG, b: comparison between HLG and C, c: comparison between LLG and C, \*: p <0.05, \*\*: p <0.01, \*\*\*: p <0.001, ns: not significant



**FIG. 1.** COMPARISON OF HORMONES BETWEEN THE THREE GROUPS

Note: b: comparison between HLG and C; \*:  $p < 0.05$ , \*\*:  $p < 0.01$

No significant differences in LH and testosterone levels were found among the three groups, but a significantly higher FSH was observed in the training groups compared to the control group. Individual biochemical markers and hormone results were therefore adjusted for FSH, height and weight.

#### Physical fitness

Physical fitness data are summarized in Table 2. No significant difference in the basal physical activity was found among the three groups. However, volleyball player groups had higher  $\dot{V}O_2\max$ , SJ, and CMJ than controls. Nevertheless, there were no differences in HJ between LLG and controls. However, for volleyball groups, the HLG had a higher HJ, SJ, and CMJ than LLG. But there were no differences in  $\dot{V}O_2\max$  between HLG and LLG.

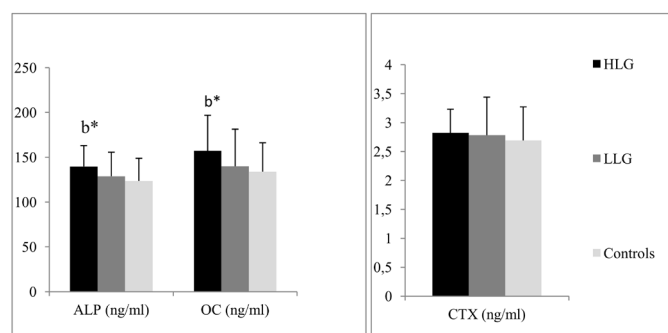
#### Biochemical data

Figure 1 shows a comparison of bone turnover markers among the three groups. No difference in CTX was observed among them. However, the HLG presented higher levels of bone formation markers (OC, BAP) compared to controls. Hormonal concentrations are summarized in Figure 2. GH, IGF-1, IGFBP-3, and cortisol were higher in HLG than in controls.

## DISCUSSION

Volleyball training exerts positive effects on the skeleton, highlighted by the increase of BMC and bone area in the most used sites [2,11]. Indeed, we studied the effect of different levels of this practice on bone turnover markers, cortisol, and the growth hormone axis (GH, IGF-1 and IGFBP-3). The results of the present study showed that the HLG had a significant increase in OC, BAP, GH, IGF-1, IGFBP-3 and cortisol compared to controls. However, CTX levels showed no significant intergroup difference. All participants in this study had different physiological and anatomical characteristics. The HLG were taller and heavier than the control group, so we adjusted results for FSH, height and weight.

The enhanced  $\dot{V}O_2\max$  and performance obtained by horizontal and vertical jumps rise in parallel with the enhanced height values of the training frequency. Therefore, there is no significant difference



**FIG. 2.** COMPARISON OF TURNOVER MARKERS BETWEEN THE THREE GROUPS

Note: b: comparison between HLG and C; \*:  $p < 0.05$

in basal physical activity and calcium intake among groups. These constraints induced by physical activities lead to endocrine and paracrine bone adaptations, which are correlated with the level of practice. Among adults, mechanical forces induced by physical exercise contribute to metabolic bone adaptation and this can be seen at the level of bone formation (BAP, OC) and resorption markers (CTX) [22]. Moderate physical activity practised by adults leads to an increase in bone formation markers (OC) [3], but among our LLG, no variations in bone formation markers were detected compared to controls; this implies that in childhood a minor physical activity level does not have an effect on bone formation markers. No significant difference in bone resorption markers was found among the three groups, whereas an increase in bone formation markers was clearly seen among the high level training group compared to controls. Few studies have investigated bone turnover markers during skeletal development. However, these results were partially in accordance with the data presented by Jurimae et al., who found an increase of OC in rowers after 6 months of highly specific training [26]. When compared to controls, Hetland et al., found that elite endurance runners had higher rates of OC [21]. As opposed to our results, some studies found no variation in OC and BAP generated by physical exercise [30,47], as soccer practice could have less impact on the skeleton, and lower intensity than the volleyball HLG. A decrease in bone formation markers seems to be associated with extreme intensity practice, as also shown by Bass et al., among prepubescent female gymnasts [10].

However, no change in the bone resorption marker CTX was observed among the three groups. These results confirm those reported by Zouch M et al., in prepubescent soccer players [47]. Nevertheless, Nickols-Richardson et al., reported a decrease in bone resorption markers after a 6-month training period for female child artistic gymnasts [36]. In the present study, this decrease in CTX has been demonstrated in girls in contrast with boys. This may reflect the earlier beginning of training and the more intensive practice among gymnasts than volleyball players. Indeed, the strain stimulus provided by gymnastic training, such as the reaction force supported by the skeleton after performing acrobatic movements based on jumps, is more important than the impact of simple jumps in volleyball.

In addition to changes in biochemical markers, the adaptations of the skeleton to physical activity induce hormonal modifications [32,35]. GH, IGF-1 and IGFBP-3 are anabolic hormones enhancing bone formation by stimulating osteoblasts and collagen synthesis, whose concentration level increases during normal puberty [29]. These hormones act both on the process of growth and on bone development [29]. The elevated IGF-1 serum may explain the significant improvements in bone formation [48]. Most of the circulating IGF-1 is bound with IGFBP-3. GH plays the role of a regulator of IGF-1 and IGFBP-3 levels in humans [7]. The secretion of GH, IGF-1 and IGFBP-3 is stimulated by intense practice [17, 44,45]. The valuable effect of physical activity on hormonal improvement is well documented in girls, whereas only a few follow-up studies have discussed this in pubescent boys [38]. However, the influence of volleyball practice on GH, IGF-1, IGFBP-3, and cortisol of prepubescent boys has never been explored to our knowledge. Schwarz et al., found that, after 10 minutes of low-intensity exercise, at first, the rate of IGF-1 and IGFBP-3 increased over the pre-exercise baseline by  $7.7 \pm 2.7\%$  and  $12.5 \pm 3.3\%$  respectively, then after 10 min of high-intensity exercise, the rate of IGF-1 and IGFBP-3 increased over the pre-exercise baseline by  $13.3 \pm 3.2\%$  and  $23 \pm 6\%$  respectively [45]. However, among adolescent volleyball players, after 1 hour of volleyball practice, there were no significant changes in IGF-1 and IGFBP-3 levels and only an increase in GH level [18]. Our results corroborate the elevated basal concentration of GH, IGF-1 and IGFBP-3 observed in HLG after 18 months of intensive training compared to controls. These results confirm those reported by Nebigh et al., who showed that, in prepubescent soccer boys, IGF-1 and IGFBP-3 increased more than in a control group [38].

Before onset of puberty, too intense practice may lead to a decrease in IGF-1 and IGFBP-3. Bouix et al. (1997) found a decrease of IGF-1 and IGFBP-3 among gymnasts aged 9-15 years compared to controls (IGF-1 decreased by 24% and IGFBP-3 decreased by 25%). They attributed this decrease to the relatively low-intake energy in the face of increased training load and energy demands; therefore, gymnasts need to maintain an adequate alimentation to optimize development during growth [5]. Calcium supplementation promotes bone remodelling [37]. The HLG has a higher rate of bone markers and hormones than the LLG and controls, though the mean calcium intake (approximately  $800 \text{ mg} \cdot \text{day}^{-1}$ ) was lower than the official Institute of Medicine (IOM) recommendations ( $1300 \text{ mg} \cdot \text{day}^{-1}$ ); this suggests that the optimal intensity of practice may compensate this lack of calcium, as there is no difference between LLG and controls in bone markers and hormones.

The IGF-1 and IGFBP-3 levels are likely to contribute to the understanding of height variability in a healthy population; short children have low IGF-1 and IGFBP-3 levels, whereas tall children have high levels of these hormones [7]. This was verified through our prepubescent HLG; they have higher IGF-1 and IGFBP-3 levels and a taller body than the LLG and controls.

As far as cortisol is concerned, we found that volleyball HLG has a significantly higher basal rate than LLG and controls. The fact that this significant difference in basal cortisol was experienced only by prepubescent high-training volleyball players is evidence of the unique aspect of our findings that were not provided elsewhere. The level of this hormone can be changed by exercise, either immediately during it [9] or after a training period [19]. During exercise, the increase in the rate of cortisol in plasma was linearly related to the  $\dot{V}O_2\text{max}$  percentage attained in the exercise [31]. By contrast, after one hour of volleyball practice among pubescent children, no significant effect on cortisol was observed [18]. Eliakim's results are in agreement with those reported by Duclos et al., who demonstrated in vitro plasticity of monocyte glucocorticoid sensitivity of endurance trained men, with training-induced decreased glucocorticoid sensitivity and acute exercise-induced return to the levels of the control untrained men [16]. Córdova et al., found an increase of post-exercise and basal cortisol levels in professional volleyball players at the end of the season compared to the beginning [13]. Such findings were also reported by Filaire et al., whose study indicated an increase of basal cortisol concentration among pubescent elite female artistic gymnasts [19]. These results were partially in accordance with the data presented by Cacciari et al., who found a significant increase in cortisol rate among 175 pubertal boys aged 10-16 years, who played football at a competitive level, compared to 224 controls, but unlike our results, they found no significant differences in cortisol level between prepubescent football players and controls [14]. This difference to our HLG results was explained by the more intense impact of volleyball practice, with an earlier training compared to football.

## CONCLUSIONS

Volleyball, which is a highly impact loading sport in adults, did not lead in our study to enhanced bone turnover markers and anabolic hormones of bone after a low training level when compared to controls. Indeed, a high training level induces enhanced bone formation markers and basal concentration of anabolic (GH, IGF-1, and IGFBP-3) and catabolic (cortisol) hormones of bone metabolism. Therefore, basal hormone concentrations and bone formation markers were directly related to the intensity and the duration of the training.

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**Conflicts of interest:** no conflicts of interest

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