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ORIGINAL ARTICLE

Vitamin D receptor gene *Fok*I polymorphisms influence bone mass in adolescent football (soccer) players

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Abstract The genetic influence on bone mineralization during adolescence is unclear possibly due to modifying factors such as skeletal maturation and lifestyle. We evaluated the influence of polymorphisms of the vitamin D receptor (VDR) gene on longitudinal changes in bone mass, bone- and calcium-related hormones in 46 adolescent soccer players (11.8–14.2 years). Total body bone mineral content (TBMC) and density (TBMD) were measured at baseline and after 6 months. Insulin-like growth factor-I (IGF-1), testosterone, intact parathyroid hormone, and activity of plasma bone alkaline phosphatase were measured at baseline and after 3 months. The influence of *Fok*I or *Taq*I VDR genotypes on changes in the outcome variables were analyzed by univariate ANOVA with adjustment for chronological age, skeletal age and body weight at baseline. At baseline, boys with Ff genotype had higher TBMC, TBMD, TBMD *Z*-score compared to those with FF genotype ($P < 0.05$). After 3 months, Ff boys also had higher increment in plasma IGF-1 ($P < 0.05$). *Fok*I polymorphism did not influence changes in bone mass measurements after 6 months, although differences detected at baseline remained significant after 6 months. There were no differences in the outcome variables according to *Taq*I genotypes. This study demonstrates that *Fok*I polymorphisms affect bone mass in Brazilian adolescent soccer players and suggests that the *Fok*I effect on bone mineralization occurs during bone maturation, possibly at the initial pubertal stages.

Keywords Vitamin D receptor gene · Polymorphisms · Soccer · Bone acquisition · Adolescent boys

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Introduction

Biological maturation during puberty results in a marked increase in linear growth and body mass with consequent adjustments in bone growth and bone mineralization. Skeletal mass approximately doubles by the end of adolescence, with about 40% of peak bone mass being achieved during the overall pubertal development (Yilmaz et al. 2005). The rate of bone development and mineralization varies with the stage of puberty, being particularly high at Tanner stage IV in boys (Yilmaz et al. 2005; Van Coeverden et al. 2002; Silva et al. 2007; Pomerants et al. 2007). Bone mineralization during adolescence may also vary according to nutrition (Whiting et al. 2004; Vatanparast et al. 2005; Cashman

2007), physical activity (Nordström et al. 2008; Calbet et al. 2001 Bailey et al. 1999; Forwood et al. 2006) and genetics (Abrams et al. 2005; Laaksonen et al. 2004; Strandberg et al. 2003).

Several gene polymorphisms have been suggested to influence bone mass in different populations, being the vitamin D receptor (VDR) gene the most investigated (Wood and Fleet 1998; Eisman 1999). The VDR gene mediates $1,25(OH)_2D$ biological actions in several cellular systems, including those involved in the regulation of calcium and bone metabolism (Thakkinstian et al. 2004). Over 20 polymorphisms have been identified in the VDR gene including those recognized by *Fok*I, located at the 5′ end containing the start codon in exon II, and by *Taq*I located at the 3′ end in exon IX (Zmuda et al. 2000). Several studies have found evidence of a relationship between *Fok*I or *Taq*I polymorphisms and bone measurements, although these associations were inconsistent across population groups (Abrams et al. 2005; Macdonald et al. 2006; Falchetti et al. 2007; Bezerra et al. 2008).

The genetic effects on bone mass may be more evident in early life stages (Wood and Fleet 1998) although only few studies have been conducted in children and adolescents (Abrams et al. 2005; Laaksonen et al. 2004; Strandberg et al. 2003; Lorentzon et al. 2000), with conflicting results. In adolescents, some studies have found higher bone mineral content in those with FF genotype (Abrams et al. 2005; Strandberg et al. 2003), but another study found a higher content in those with ff genotype (Terpstra et al. 2006). The influence of *Taq*I genotypes on lumbar spine BMD of adolescents was also described, with higher (Sainz et al. 1997) or lower (Lorentzon et al. 2001) values for those with TT genotype, and with other studies reporting no effect (Abrams et al. 2005; Cusack et al. 2006). These conflicting results may be due in part to complex interactions between genetic factors, skeletal maturation (Strandberg et al. 2003; Terpstra et al. 2006) and physical activity (Nakamura et al. 2002; Rabon-Stith et al. 2005) on bone mineralization during adolescence.

Studying adolescents in a narrow range of skeletal maturation and similar physical activity habits may contribute to better investigation of the influence of VDR polymorphisms on bone metabolism. Moreover, the probability of detecting changes in bone mass over time increases in physically active subjects as shown by several studies evaluating the impact of exercise on bone mass and metabolism (Nordström et al. 2008; Calbet et al. 2001; Bailey et al. 1999; Forwood et al. 2006). The aim of the present study was to evaluate the influence of VDR gene polymorphisms recognized by *Fok*I and *Taq*I, on longitudinal changes in bone mass and bone- and calcium-related hormones in mid- and late-puberty adolescent soccer players.

Materials and methods

Subjects and sample collection

Forty-six physically active adolescent boys (11.8–14.2 years) of mixed white and black ethnicity were studied during a 6-month period. For all the adolescents, the baseline measurements were done during the same season, between April and May 2006. The adolescents were soccer players recruited from the under-13 and under-15 teams of Botafogo Soccer Club in Rio de Janeiro. Most of adolescents were at pubertal stage III or IV, and only six boys were at pubertal stage V, according to the results of self-assessment of genitalia and pubic hair stage, using an illustrated questionnaire according to the Tanner classification (Tanner 1992). All subjects enrolled in this study were on the same training schedule and have been practicing this sport modality for at least 5 years. To be enrolled, subjects had to be healthy, nonsmokers, nonusers of vitamin or minerals supplements and with no history of bone or renal disorders affecting bone mass. The habitual diet of the adolescents was assessed at enrollment using 3-day dietary intake records. However, since only 3 of the 46 adolescents studied completed successfully these records, it was not possible to obtain reliable information on habitual dietary intake. Informed written consent was obtained from each subject and the parent or legal guardian. The study protocol was approved by the Ethical Committee of Pedro Ernesto University Hospital (Rio de Janeiro, Brazil). Morning blood samples (10 mL) were obtained from each subject after overnight fast. Aliquots of total blood and serum were stored at −20°C until analyzed.

Anthropometric and skeletal maturity measurement

Standing height was measured using a portable direct reading stadiometer (Sanny®) and body weight was determined in the adolescents without shoes or coats using a calibrated electronic scale (Filizola®), at baseline and after 6 months. Skeletal maturity was assessed at baseline, through skeletal age (SA) measurement, based on a radiograph of the left hand and wrist, by an experienced observer using the Tanner–Whitehouse 3 (TW3) method and the radius–ulna–short bones (RUS) system option (Tanner et al. 2001).

Bone measurement

Total body bone mineral content (TBMC) and total body bone mineral density (TBMD) were assessed by dual-energy X-ray absorptiometry using the Lunar Prodigy Advance densitometer (GE, Healthcare), at baseline and after 6 months. *Z*-score for TBMD was obtained by comparison with an age-, gender- and race-matched reference

according to the manufacturer database. Total body calcium content was calculated as 32.2% of TBMC (Ellis et al. 1996). Bone calcium retention rate was calculated as the ratio between change in total body calcium content and days elapsed between measurements.

Laboratory analysis

All bone- and calcium-related hormones and bone marker measurements were evaluated at baseline and after 3 months. Plasma testosterone was determined by immunoradiometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA). Serum intact parathyroid hormone and plasma insulin-like growth factor-I were assessed by chemiluminescent enzyme-labeled immunometric assays, using IMMULITE 2000 (Diagnostic Products Corporation, Los Angeles, CA, USA). Activity of plasma bone alkaline phosphatase (ALP) was assessed by colorimetric method according to Farley et al. (1981).

VDR genotyping

DNA was extracted from whole blood using GFX Genomic Blood Purification kit (Amersham Biosciences, NJ, USA). VDR genotypes for two polymorphisms were determined by polymerase chain reaction (PCR) amplification and enzymatic digestion of the products with *Fok*I (New England, Biolabs, Beverly, MA, USA) and *Taq*I (Jena Bioscience, Jena, Germany). Identification of *Fok*I polymorphism was conducted as previously described (Morrison et al. 1994), except for the primers sequences. The primers used were 5′- GGC CTG CTT GCT GTT CTT AC -3′ (forward) and 5′- TGC TTC TTC TCC CTC CCT TT -3′ (reverse) and generated a fragment with 221 pb. Identification of *Taq*I polymorphism was conducted as previously described (Bezerra et al. 2008). Alleles having the respective sites for *Fok*I and *Taq*I were denoted by f or t and alleles lacking the respective sites were denoted by F or T.

Statistical analysis

Differences in general characteristic between genotypes within each VDR polymorphism were analyzed by one-way ANOVA. The effect of *Fok*I and *Taq*I genotype on bone mass measurements, and bone-and calcium-related hormones were evaluated using a generalized linear model (univariate, ANOVA) with adjustments for chronological age and significant confounding variables affecting bone mineral density at baseline, that were identified after stepwise multiple regression analysis and used as covariates. Body weight and skeletal age were significant determinants in the multiple regression analysis. Body height, body fat percentage, and Tanner stage did not contribute significantly to the regression model. If ANOVA was significant, post hoc differences between genotype subgroups were tested using the Fisher's least significant difference test (LSD). Associations between variables were evaluated by Pearson correlation analysis. Values of $P \leq 0.05$ were considered significant. The statistical analyses were performed using SPSS 12.0 for Windows (Chicago, IL, USA). Values were reported as means ± SD.

Results

Adolescent boys entered the study with 13.3 ± 0.6 years of chronological age and 13.4 ± 1.8 years of skeletal age. When grouped by VDR genotype there were no significant differences in chronological and skeletal age between groups, except for chronological age in *Taq*I genotype that was higher in adolescent boys with TT genotype compared to those with Tt ($P = 0.034$) and those with tt genotype ($P = 0.037$) (Table 1). In all subjects, the mean height (160.2 ± 9.3 cm) and body mass index (19.2 ± 2.0 kg/m^2) were between the 50th and 75th percentile for chronological age and sex (NCHS/CDC 2000). Height and body weight at baseline (Table 1) and after 6 months (data not shown) did not differ among adolescents with different genotypes for *Fok*I and *Taq*I.

In the whole group studied ($n = 46$), the baseline *Z*-score for TBMD was on average 0.6 ± 0.9, with only two adolescents having TBMD *Z*-scores ≤ -1.1, and none having TBMD *Z*-scores ≤ -2.0. During the 6 months of the study, the average bone calcium retention rate was 332 mg/day. Skeletal age was significantly correlated with TBMD ($r = 0.74$; $P < 0.001$) and TBMD *Z*-score ($r = 0.56$; $P < 0.001$) at baseline and with the bone calcium retention rate ($r = 0.43$; $P < 0.01$) during 6 months of the study.

After adjustment for chronological age, skeletal age, and baseline body weight, adolescent boys with Ff genotype had higher TBMD ($P = 0.01$), TBMD *Z*-score ($P = 0.03$) and TBMC ($P = 0.02$) than those with FF genotype at baseline (Table 2). Total bone area did not significantly differ between *Fok*I genotypes (Table 2). There were no differences according to *Taq*I genotypes on bone mass measurements in the adolescents studied (Table 3). After 6 months, changes on TBMC, TBMD *Z*-score and total bone area did not differ among adolescents with different genotypes for *Fok*I (Table 2) and *Taq*I (Table 3). Similarly, bone calcium retention rate during 6 months did not differ between groups (Tables 2, 3). However, when absolute values of bone measurements after 6 months were compared according to *Fok*I genotype, those with Ff continued to have higher TBMD ($P = 0.02$), TBMD *Z*-score ($P = 0.01$) and TBMC ($P = 0.01$) than those with FF genotype (data not shown).

Table 1 General characteristics in the adolescent soccer players studied according VDR genotype

Genotype	*n* (%)	Chronological age (years)	Skeletal age (years)	Height (cm)	Body weight (kg)
FokI					
FF	19 (41.3)	13.3 ± 0.6	13.5 ± 1.3	160.1 ± 7.5	49.9 ± 6.4
Ff	22 (47.8)	13.3 ± 0.6	13.1 ± 2.2	159.6 ± 10.9	48.0 ± 10.1
ff	5 (10.9)	13.3 ± 0.8	14.3 ± 1.4	163.2 ± 8.8	56.4 ± 10.7
TaqI					
TT	13 (28.3)	13.6 ± 0.4[a]	14.0 ± 2.3	161.0 ± 10.9	52.3 ± 11.5
Tt	30 (65.2)	13.2 ± 0.7[b]	13.1 ± 1.6	159.8 ± 9.0	48.8 ± 7.9
tt	3 (6.5)	12.8 ± 0.3[b]	13.5 ± 1.2	160.8 ± 6.3	48.1 ± 7.0

Values are presented as mean ± SD

For each genotype, means in the same column with superscripts without a common letter indicates a significant difference between subgroups (ANOVA followed by Fisher's LSD test, $P < 0.05$)

Table 2 Longitudinal bone measurements, plasma and serum hormones, and bone marker by *Fok*I genotypes in the adolescent soccer players

	*Fok*I genotype		
	FF	Ff	ff
n	19	22	5
Bone measurements			
TBMD (g/cm²)			
Baseline	1.030 ± 0.054[a]	1.056 ± 0.098[b]	1.116 ± 0.085[ab]
Change after 6 months	0.034 ± 0.012	0.030 ± 0.020	0.031 ± 0.026
Z-score of TBMD			
Baseline	0.4 ± 0.7[a]	0.7 ± 1.1[b]	1.1 ± 0.5[ab]
Change after 6 months	0.0 ± 0.3	0.1 ± 0.2	0.5 ± 1.0
TBMC (g)			
Baseline	1986 ± 297[a]	2023 ± 502[b]	2377 ± 513[ab]
Change after 6 months	171 ± 62	165 ± 64	148 ± 183
Total body calcium (g)			
Baseline	640 ± 95[a]	651 ± 162[b]	746 ± 155[ab]
Change after 6 months	55 ± 20	53 ± 21	72 ± 24
Bone area (cm^2)			
Baseline	1920 ± 204	1894 ± 318	2063 ± 312
Change after 6 months	98 ± 53	98 ± 39	129 ± 48
Bone calcium retention rate (mg Ca/day)	326 ± 118	316 ± 121	447 ± 176
Hormones and bone marker			
Plasma testosterone (ng/dL)			
Baseline	342 ± 245	298 ± 187	294 ± 256
Change after 3 months	46 ± 132	80 ± 135	139 ± 137
Serum iPTH (pg/dL)			
Baseline	67.9 ± 31.6	59.4 ± 26.8	66.9 ± 28.1
Change after 3 months	−26.6 ± 26.9	−20.4 ± 17.0	−15.2 ± 25.6
Plasma IGF-1 (ng/mL)			
Baseline	328 ± 128	290 ± 104	249 ± 117
Change after 3 months	32 ± 131[a]	115 ± 97[b]	96 ± 65[ab]
Plasma bone ALP (U/L)			
Baseline	82.0 ± 33.2	78.8 ± 37.2	77.3 ± 16.6
Change after 3 months	6.6 ± 32.4	0.6 ± 35.9	11.4 ± 53.6

Values are presented as mean ± SD. Means in the same row with superscripts without a common letter indicates a significant difference between subgroups (ANOVA followed by Fisher′s LSD test, using chronological age, skeletal age, and body weight at baseline as covariates, $P < 0.05$)

Table 3 Longitudinal bone measurements, plasma and serum hormones, and bone marker by *Taq*I genotypes in the adolescent soccer players

	TaqI genotype		
	TT	Tt	tt
n	13	30	3
Bone measurements			
TBMD (g/cm²)			
Baseline	1.075 ± 0.105	1.045 ± 0.075	1.017 ± 0.033
Change after 6 mo	0.030 ± 0.018	0.033 ± 0.018	0.031 ± 0.010
Z-score of TBMD			
Baseline	0.7 ± 1.2	0.6 ± 0.9	0.5 ± 0.9
Change after 6 mo	0.1 ± 0.2	0.2 ± 0.4	−0.1 ± 0.5
TBMC (g)			
Baseline	2164 ± 538	2004 ± 402	1954 ± 275
Change after 6 mo	160 ± 58	168 ± 87	174 ± 52
Total body calcium (g)			
Baseline	697 ± 173	642 ± 125	629 ± 89
Change after 6 mo	52 ± 19	58 ± 22	56 ± 17
Bone area (cm^2)			
Baseline	1988 ± 337	1895 ± 253	1918 ± 208
Change after 6 mo	89 ± 42	105 ± 48	109 ± 51
Bone calcium retention rate (mg Ca/day)	306 ± 111	347 ± 138	301 ± 62
Hormones and bone marker			
Plasma testosterone (ng/dL)			
Baseline	354 ± 165	305 ± 228	289 ± 354
Change after 3 mo	75 ± 127	78 ± 138	−33 ± 139
Serum iPTH (pg/dL)			
Baseline	64.8 ± 31.0	64.4 ± 29.0	52.6 ± 19.7
Change after 3 mo	−25.6 ± 24.4	−21.2 ± 22.9	−25.0 ± 7.8
Plasma IGF-1 (ng/mL)			
Baseline	337 ± 154	293 ± 99	226 ± 46
Change after 3 mo	85 ± 139	76 ± 111	27 ± 74
Plasma bone ALP (U/L)			
Baseline	81.7 ± 26.9	81.5 ± 33.9	63.8 ± 51.5
Change after 3 mo	3.8 ± 37.4	0.9 ± 33.6	36.4 ± 39.4

Values are presented as mean ± SD. Means in the same row with superscripts without a common letter indicates a significant difference between subgroups (ANOVA followed by Fisher's LSD test, using chronological age, skeletal age, and body weight at baseline as covariates, $P < 0.05$)

In the entire group of adolescent boys studied, plasma testosterone was adequate (mean 321 ± 217 ng/dL) for their pubertal stage according to Tanner (Ankarberg-Lindgren and Norjavaara 2004; Hiort 2002). At baseline, the levels of plasma testosterone, serum iPTH, plasma IGF-1 and activity of plasma ALP were not different according to *Fok*I and *TaqI* genotypes (Tables 2, 3). Changes in plasma testosterone, serum iPTH and activity of plasma BAP after 3 months were also not influenced by the different VDR genotypes (Table 2 and 3). However, the *Fok*I genotypes influenced significantly the increment in plasma IGF-1 after 3 months, with the boys with Ff genotype having higher increment than those with FF genotype ($P = 0.03$) (Table 2).

Discussion

In the few studies performed in adolescents, the association between VDR polymorphisms defined by *Fok*I and bone indices is controversial (Abrams et al. 2005; Laaksonen et al. 2004; Strandberg et al. 2003; Terpstra et al. 2006). In a previous longitudinal study conducted in adolescent boys and girls, higher TBMC and TBMD were found for those with FF genotype (Abrams et al. 2005). In contrast, higher BMD at cortical sites was observed in adolescent boys with Ff genotype (Laaksonen et al. 2004). Similar to this finding, in our study the adolescent soccer players (physically active adolescent boys) with Ff genotype showed higher TBMC and TBMD at baseline than those with FF genotype, resulting

in better adequacy of TBMD. We did not find an influence of *Fok*I polymorphism on total bone area, in contrast with a previous study also in adolescent boys (Strandberg et al. 2003). The contrasting results between studies could be explained by differences in ethnicity and also by factors that may modify the genetic influence on bone mass such as pubertal stage, physical activity and nutrition (Abrams et al. 2005; Laaksonen et al. 2004; Strandberg et al. 2003; Wood and Fleet 1998; Nakamura et al. 2002). In our study, the adolescent boys were all of mixed white and black ancestry, at medium or advanced stages of puberty (Tanner stage III or higher), regularly practicing an intense physical sport activity (soccer), and possibly on marginal calcium diets.

Although information on dietary habits of the adolescents studied could not be obtained, previous studies in Brazil indicate that adolescents habitually consume between 500 and 800 mg Ca/day (Peters et al. 2009; Silva et al. 2007; Lerner et al. 2000), that represents 40–60% of the internationally recommended calcium intake for adolescents (IOM 1997). In spite of their possibly low calcium diets, it is interesting to note that independent of *Fok*I genotype, the adolescents studied had, on average, adequate TBMD at baseline. Also, the rate of bone calcium retention (332 mg/day) during 6 months was similar to the reported values for Canadian boys (359 mg/day) with similar chronologic age but higher dietary calcium intake (1,140 mg Ca/day) (Bailey et al. 2000), and within predicted calcium retention values for adolescent American boys (Hill et al. 2008). Assuming that the dietary calcium intake in the adolescents in present study was as reported in previous studies in Brazilian adolescents (500–800 mg/day), the observed bone calcium retention rate would require 42–66% efficiency in intestinal calcium absorption, higher than usually observed in adolescents (Abrams et al. 2009). However, the regularly intense physical activity of the adolescents in the present study could have favored increased calcium absorption, as previously reported in exercise-trained young men (Zitterman et al. 2000).

Vitamin D status could in theory also influence calcium retention rate in adolescents. Although serum 25 (OH) D was not measured in the present study, vitamin D status was presumably marginal, based on the few studies evaluating vitamin D status in Brazilian adolescents reporting that insufficiency [serum 25 (OH) D < 75 nmol/L] is commonly observed (Peters et al. 2009; Bezerra et al. 2008). However, since calcium retention rate was adequate, our results are consistent with recent studies showing that calcium absorption and retention are not directly related to vitamin D status in children and adolescents (Weaver et al. 2008; Abrams et al. 2009).

The well-described rapid increase in TBMC and TBMD during adolescence (Yilmaz et al. 2005; Van Coeverden et al. 2002) is strongly stimulated by steroid hormones, in conjunction with growth hormone and IGF-1 (Van Coeverden et al. 2002; Silva et al. 2007; Pomerants et al. 2007). However, bone mineralization is a complex process that besides hormones, genetics and diet, is also influenced by exercise (Yilmaz et al. 2005; Cusack et al. 2006). Moreover, it has been suggested that changes in bone mineral density in response to exercise training may be influenced by VDR gene polymorphisms (Nakamura et al. 2002; Rabon-Stith et al. 2005). In our study, the higher bone mass observed in the adolescents with Ff genotype at baseline could not be explained by differences in the hormones evaluated, since there were no differences according to *Fok*I genotypes on plasma testosterone and IGF-1 and serum iPTH. One possible explanation for our results is that the *f* allele, that is frequently associated with low BMD (Abrams et al. 2005; Strandberg et al. 2003; Katsumata et al. 2002), may be more sensitive to the influence of regular long-term exercise training on bone mineralization in adolescent boys. Consistent with this hypothesis, we observed that those with ff genotype had the highest (although not significant) bone mass indices. However, this hypothesis should be considered with caution since there were only five subjects with ff genotype and our study was not designed to examine if bone mass response to exercise training is different according to *Fok*I genotype.

It is well described that an increase in levels of IGF-1 occurs with advancing of pubertal stages, with a stimulatory effect on somatic growth, including bone formation and bone mineral apposition (Van Coeverden et al. 2002; Kanbur et al. 2005). In our study, the adolescent soccer players with Ff genotype showed higher increment on levels of IGF-1 in plasma after 3 months than those with FF genotype. However, this higher increment was not reflected in higher activity of plasma bone alkaline phosphatase after 3 months, nor higher bone mass indices after 6 months. It is possible that the stimulatory effect of IGF-1 on somatic growth in the adolescents studied occurred in another body compartment, such as muscle mass (Garnett et al. 2004; Arslanian and Kalhan 1996).

Only few studies evaluated the influence of *Fok*I polymorphism on longitudinal changes in bone mass in adolescents (Abrams et al. 2005; Strandberg et al. 2003). In a study conducted in adolescent boys and girls at Tanner stages II or III (Abrams et al. 2005), those with FF genotype showed the highest increase on TBMC and rate of bone calcium retention after 1 year. However, no difference according to *Fok*I genotype in the increase in TBMD and TBMC after 1 year was observed in a study in adolescent boys at Tanner stage IV or V (Strandberg et al. 2003). In agreement with this latter finding, we did not observe an influence of *Fok*I polymorphism on the increment of bone mass measurements after 6 months in adolescent boys that were at least at Tanner stage III. However, differences

detected at baseline remained significant after 6 months, suggesting that the effect of *Fok*I polymorphism on bone mass probably occurred before Tanner stage III. Taken together these results suggest that the influence of *Fok*I polymorphisms on BMD in adolescents may be more evident at the initial pubertal stages.

The association between VDR gene polymorphisms at the *Taq*I site and bone mass measurements was also investigated in adolescents (Abrams et al. 2005; Lorentzon et al. 2000; Sainz et al. 1997; Kitagawa et al. 1998) with most studies finding no relationship (Abrams et al. 2005; Sainz et al. 1997; Kitagawa et al. 1998). In agreement with these studies, we did not found any association between *Taq*I polymorphism with bone mass measurements and bone-and calcium-related hormones at baseline and after 6 months. These results suggest that *Taq*I polymorphisms do not affect bone mineralization in Brazilian adolescent soccer players.

In conclusion the present study demonstrated, in spite of its small sample size, that *Fok*I *VDR* gene polymorphisms affect bone mass indices in Brazilian adolescent soccer players with higher TBMC, TBMD and *Z*-score for TBMD observed in those with Ff genotype. Although there was no influence of *Fok*I polymorphism on longitudinal changes in bone mass, differences detected at baseline remained significant after 6 months. These results suggest that the effect of *Fok*I polymorphisms on bone mineralization occurs during bone maturation process, possibly at the initial pubertal stages.

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Conflict of interest statement All authors have no conflicts of interest.

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