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IGF-I and IGFBP-3 are modulated in adolescent during a competitive soccer season

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Abstract
Purpose: The aim of this study was to verify the kinetics of the concentrations of different components of the GH/IGF-I axis and to monitor the motor performance of adolescent soccer players during a competitive season.
Methods: Eleven U-15 male athletes from a Brazilian soccer team participated in this study. The participants were evaluated in 3 moments during seven months of season: before (M1), during (M2), and after the season (M3). In all assessment moments, the following field tests were applied: Léger, RAST, 30m velocity, six jump, and agility T-tests. In addition, before and after a standard training session (STS), the kinetics of the GH, IGF-I, and IGFBP-3 were analyzed via venous blood samples.
Results: IGF-I levels were reduced post-STS in M3 (p=0.04). The variation in IGF-I concentrations post-STS was higher in M2 compared to M3. IGFBP-3 concentrations were higher pre-STS (p=0.03) and post-STS in M2 when compared to M3 (p=0.04). GH did not change during the competition. Anaerobic power values were higher in M2 compared to the other moments (p=0.007). Peak velocity in the 30m velocity test also reached its highest value in M2, being significantly higher than in M1 (p=0.03).
Conclusions: IGF-I as well as IGFBP-3 showed variations during the competition, suggesting they were sensitive to both acute and chronic stimuli effects caused by the competition. Regarding motor performance, it should be stated that in predominantly anaerobic tests there was a tendency for the best results, with higher IGF-I and IGFBP-3 concentrations.

Keywords: GH/IGF-I axis, maturation, adolescents, sports science

1. Introduction
The component hormones of the GH/IGF-I axis (growth hormone – insulin growth factor), along with genetic inheritance, constitute the group of factors which directly influence human growth [1]. During childhood and youth, regular physical exercise is another determinant variable associated with the development of muscle and bone mass. With the onset of puberty, the GH/IGF axis is up-regulated, thus a close interaction between the gonadal steroids and the peak of growth velocity (PHV) is observed [2].
In this phase of intense physical, psychological, and morphological changes, the follow-up of biological maturation is critical, since this process interferes in the motor adaptations of young people [1]. The maturation monitoring can be done using PHV, an essential tool for coaches, since it allows for the training stimuli to be adjusted to respect the interaction of factors such as growth, development, and maturation [3].
Exercise programs are closely related to the anabolic function triggered by the action of the GH/IGF-I axis, while basal IGF-I levels are positively correlated with muscle mass and physical fitness in children, adolescents, and adults [1, 2].
Despite this fact, nowadays there is information about IGF or GH/IGF-I axis kinetics after intense exercise series, but these studies concern acute series, and focus less on long-term approaches [3, 4, 5]. Therefore, it is not known whether the responses of IGF-I and IGFBPs levels to acute physical exercise may differ from those observed in response to a training program or to chronic changes in physical activity levels, besides the possibility of being able to vary between the various types of sports (individual or collective) and their different forms of planning [2, 4].

The GH/IGF-I axis is expected to exhibit two-phase kinetics. At first, a catabolic period is characterized by hormone suppression and lower performance in motor tests. If the competitive season offers ideal and potent stimuli, then an anabolic phase should occur, revealing axis optimization and evolution in motor performance, which would highlight these hormones as possible training state markers [4-6]. We hypothesized that there would be this biphasic kinetics during the competition and this could influence the motor tests.

This study chose soccer as a topic due to the increasing attention given to it over the years, as well as the number of professional players and the exponential increase in the consumption of soccer-related services. However, there is still an observed lack of investigation regarding the effects of the GH/IGF-I axis on young soccer athletes and its importance during their early training phases [3].

The literature is quite rich concerning the characterization of physiological aspects of the game [7, 8], as well as the optimization of physical fitness [9]. Nevertheless, more complete evaluations are needed, to provide a better understanding of the relationship between the fast maturation process, the spontaneous increase of anabolic growth factors (GH/IGF-I axis hormones), and the high (not to mention excessive) levels and amount of physical exercise which adolescent athletes experience simultaneously [10, 11].

Briefly, soccer is characterized by high-intensity actions that define the match outcome (sprints and quick movement activities, characterized by accelerations and decelerations with sudden direction changes; shots to goal; passes) and aerobic actions that last most of the match [11, 12].

In summary soccer described strength as being a prominent variable for the physical performance of soccer athletes, mainly in the lower limbs, acting fundamentally in the production of power [13]. Like many other characteristics, this motor capacity is strongly influenced by maturation and training processes. Both were investigated in this study.

Based on these assumptions, the objective of this study was to verify the kinetics of the concentrations of different components of the GH/IGF-I axis and to monitor the motor performance of adolescent soccer players during a competitive season.

2. Materials and Methods

2.1 Participants

The sample was composed of 11 adolescent soccer players with the post-occurrence of PHV (matured), belonging to the U-15 category. The athletes had these values represented in mean and standard deviation of height (174.76±7.59 cm), body mass (62.05±7.84 kg), age (14.93±0.12 years old), and PHV (0.43±0.58 years). They were informed verbally and in writing, through the Informed Consent Form (signed by the parents or guardians and signed by the adolescents), of the procedures that would be adopted during the research.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and it was approved by local Ethics Committee.

The athletes underwent an anthropometric evaluation, motor performance tests, and blood collection for hormonal dosage.

2.2 Design

Five milliliters (5 mL) of blood were collected from each athlete from an antecubital vein using vacuum tubes with anti-coagulant (EDTA) to obtain plasma, and 5 mL were collected in vacuum tubes without the addition of anticoagulant for serum acquisition (10mL pre-workout and 10 mL post-workout), totaling 60 mL during the season, which were stored at between 0 and 4 °C immediately after blood collection. Subsequently, the samples were centrifuged at 0 to 4 °C at 1200 rpm for 15 minutes to separate the plasma and serum. Once this step was completed, the plasma and serum were withdrawn from the collection tubes and stored in 1.5mL tubes for further freezing at -80 °C for the dosages described below. This procedure was performed at the beginning and the end of the standard training session (STS).

The battery of motor tests and anthropometric measurements was performed and, after an interval of 48 hours, the STS and the blood sample collection were completed. It is important to emphasize that on the days when the test and measurement batteries occurred no other training stimulus was given. In order to minimize the effects of possible dehydration, the athletes were instructed several times to ingest water during the STS and physical performance tests.

All procedures were performed during the initial phase (M1 - 1st month), intermediate phase (M2 - 3rd month), and final phase (M3 - 7th month) of the athletes' competition (U-15 Championship), which lasted approximately seven months.

2.3 Training Program

The adopted STS was composed of a warmup (running drill sequences followed by a sequence of jumps over an elastic cord), pause for hydration, and explanation of the next activity. The main part of the session consisted of a match involving reduced field dimensions in which the athletes were instructed to execute only 2 contacts with the ball.

The field was divided into 2 quadrants totaling an area of 800 square meters (20 m x 40 m) and the athletes performed the proposed task twice with a duration of 20 minutes each and interval of 10 minutes. In the final part of the session they jogged for 10 minutes.

2.4 Physical Performance Tests

In order to measure the aerobic power (VO_2max) of the soccer players, the multistage 20 meters shuttle run test (Léger test) [14] was used, and for anaerobic power the running-based anaerobic sprint test (RAST) was performed. The T-test was used to assess agility [12], for an explosive strength evaluation the alternate six jumps test was implemented [11], and linear cyclic velocity was measured using the 30m run test [12]. A 4-hour rest period was given between the explosive strength, agility, and aerobic power tests and the assessments of linear cyclic velocity and anaerobic power.

2.5 Evaluation of Somatic Maturation

The PHV was obtained through the following equation, which determines the age at which the maturational event occurs. Furthermore, this mathematical inference takes the following into account: body mass, height, trunk cephalic height, leg length, and chronological age (15).

PHV =

- 9.236 + 0.0002708 x (LL x TCH) – 0.001663 x (A x LL) + 0.007216 x (A x TCH) + 0.02292 (BM ÷H)

Note: LL = leg length; TCH = trunk cephalic height; A = age; BM = body mass; H = height.

2.6 Immunoassays

Determinations of GH, IGF-I, and IGFBP-3 serum concentrations were performed using specific immuno-chromatometric assays (Immulite 2000, Siemens, Los Angeles, CA, USA) and the data are expressed as ng/mL.

For the IGF-I concentration determinations, the samples were

subjected to IGF extraction from their carrier proteins using a solution containing 12.5% 2N hydrochloric acid and 87.5% absolute ethanol. All the dosages described above were performed in the same assay at the Laboratory Specialized in Scientific Analyses.

2.7 Statistical Analysis
The motor tests data presented no normality violations, following analysis using the Shapiro-Wilk normality test. For these values, the comparison between the three moments of data collection (M1, M2, and M3) was performed using the repeated measures ANOVA, followed, when necessary, by the Bonferroni post-hoc test. The hormone values showed no data normality and were analyzed using the Wilcoxon test; and, to identify the differences between moments, the Friedman test for non-parametric values was used. In order to verify if there was a correlation between the performance results and the hormonal concentrations, the non-parametric Spearman test was used. The SPSS (Statistical Package for Social Science for Windows®) software version 20.0 was used in all statistical analyses. Significance was accepted at $p \leq 0.05$.

3. Results
3.1 Hormones and Binding Protein
There was a significant difference in IGF-I concentrations between moments Pre-STS M2 and Post-STS M2 (459.9±67.52 vs. 519±115.39, p = 0.05) and Pre-STS M3 and Post-STS M3 (460.72±94.65 vs. 428.72±87.33, p = 0.04); in M2 there was an increase in concentrations after the STS (59.09±95ng/mL, p = 0. 05), while in M3 the values decreased after the STS (-32±49ng/mL, p = 0.04) (Table 1). The IGFBP-3 concentrations presented differences when comparing the values of Pre-STS M2 and Pre-STS M3 (4.91±1.04 vs. 4.6±10 ng/mL, p = 0.03) and Post-STS M2 vs. Post-STS M3 (5.53±1.7 vs. 4.5±0.8 ng/mL, p = 0.04). No significant difference was observed in intra (pre vs. post) or inter-phase (extensive vs. intensive vs. tapering) GH concentrations.

Table 1: Mean and standard deviation values of the GH/IGF-I axis pre- and post-STS throughout the competitive season ($p \leq 0.05$)

Moments	GH Pre-STS	GH Post -STS	GH Δ Post/PreM1	IGF-I Pre-STS	IGF-I Post -STS	IGF-I Δ Post/PreM2	IGFBP3 Pre-STS	IGFBP3 Post-STS	IGFBP3 Δ Post/PreM3
M1	2.85±2.94	1.75±2.31	-1.1±0.63	445.54±91.1	455.09±87.75	9.54±3.36	4.46±0.63	4.61±0.79	0.14±0.16
M2	4.29±4.48	4.11±6.15	-0.18±1.67	459.9±67.52	519±115.39	59.09±95*	4.91±1.04●	5.53±1.66●	0.62±0.65
M3	7.35±7.36	2.5±2.57	-4.85±4.79	460.72±94.65	428.72±87.33	-32±49*	4.6±0.97†	4.5±0.79†	-0.01±0.17

Note: STP = Standard Training Session. *Differences between Pre- and Post-STS (M2 p=0.05) (M3 p=0.04); ●Differences between Pre-STS M2 vs. Pre-STS M3 p=0.03; †Differences between Post-STS M2 vs. Post-STS M3 p=0.04. Values in ng/mL

3.2 Motor Tests
Differences were observed in the following tests: 30m linear cyclic velocity (M1 4.47±0.14 vs. M2 4.29±0.17, p = 0.03); agility T-test (M2 8.91±0.33 vs. M3 9.25±0.30, p = 0.004); and for RAST variables (mean power = M1 409.1±87.3 vs. M2 458±100, p = 0.007; peak power = M1 507.6±26.7 vs. M2 562.8±34.7, p = 0.03; minimum power = M1 305.5±28.9 vs. M2 376.75±25.01, p = 0.007). The alternate six jumps test and Léger test (VO_2max) did not present significant differences between moments (Table 2).

Table 2: Mean and standard deviation values obtained in motor tests throughout the competitive season ($p \leq 0.05$).

Moments	V30m (s)	Test-T (s)	Six Jumps (m)	$\dot{V}_{O_{2max}}$ (ml/Kg/min)	Mean Power (W)	Peak_Power (W)	Min_Power (W)
M1	4.47±0.17	9.33±0.28	13.99±0.40	50.82±2.79	409.08±87.27	507.58±92.42	305.50±100.12
Δ M1/M2	-0.18	-0.42	0.11	0.25	48.82	55.25	71.25
M2	4.29±0.14*	8.91±0.33†	14.10±0.82	51.07±3.67	457.90±100.93*	562.83±120.21*	376.75±86.63*
Δ M2/M3	0.14	0.34	-0.15	-2.14	-33.32	-13	-43.66
M3	4.43±0.2	9.25±0.3	13.95±0.72	48.93±3.55	424.58±50.68	549.83±83.39	333.09±39.58
Δ M3/M1	0.04	0.08	0.04	1.89	-15.5	-42.25	-27.59

Note: M1 = Initial phase assessment; M2 = Intermediate phase assessment (third month of the season); M3 = Final phase assessment (seventh month of the season). Mean_Power = Result of the average power obtained in RAST. Peak_Power = Result of peak power obtained in RAST. Min_Power = Result of the minimum power obtained in RAST. *Difference between moments M1 vs. M2. (V30m p=0.03) (Mean Power p=0.007) (Peak Power p=0.03) (Minimum Power p=0.007) †Difference between moments M2 vs. M3 (Test-T p=0.004)

3.3 Maturation
No differences in maturational stage were observed between any of the moments of the evaluation.

Table 3: Mean and standard deviation values of maturation evaluation throughout the competitive season

	PVC (years)
M1	0,43±0,58
M2	0,48±0,59
M3	0,85±0,47

4. Discussion
The main findings of this study were: a) the serum IGF-I and IGFBP-3 concentration kinetics were altered during the competition, although there were no changes in GH; b) the motor tests in which there was a predominance of anaerobic metabolism seemed to have their best performances following the results of the IGF-I/IGFBPs; and c) the athletes' maturation status did not present any variation throughout the competition.

It is noted that, in response to the stimuli provoked by the U-15 Championship, the IGF-I hormonal kinetics are characterized by acute alterations, since the hormone was altered within the same session (Pre-STS vs. Post-STS in M2), and also in response to chronic sessions (M2 vs. M3). This study corroborates the theory of a two-phase behavior of the GH/IGF-I axis [16, 17]. Recently, was demonstrated this biphasic behavior, with a significant reduction in IGF-I values during the specific training phase and an increase of this hormone during the polishing phase throughout a swimming

training season for adolescents [18]. Although GH concentrations do not differ significantly during the season, IGF-I result changes may have indicated an anabolic phase evidenced by the increase in the post-STS concentration in M2.

The results of this study differ from what has been demonstrated in part of the literature, since some research has found no increase in serum IGF-I levels for any of the typical training sessions for volleyball, cross-country, water polo, and wrestling. It is noteworthy that the authors mention increased IGF-I levels only in studies performed in a laboratorial environment with series of short efforts with supramaximal intensity (three consecutive Wingate tests) and intense aerobic exercise (10min cycle ergometer at an intensity above the anaerobic threshold). Thus, it is possible to hypothesize that the training sessions applied in the athletes' routine do not represent enough physiological stress to generate increases in IGF-I values [5, 19].

In another study, the effects of a soccer training program on GH and IGF-I levels were verified. Analyzing the parameters of the young soccer players, GH levels increased with exercise and IGF-I levels remained stable throughout the season. However, significantly higher responses were observed at the beginning of the season when compared to those obtained in the middle and at the end of the training program [20].

These data differed from the findings of the present study, since the GH concentrations did not show any variations. The aforementioned study was not able to demonstrate the biphasic behavior of the GH-IGF-I axis. The lack of concordance between the results previously presented and those presented in this paper may be due to methodological differences, as their evaluation was based on a cycle-ergometer assessment, and, in this work, it was performed through simulated-game situations [20].

In another study that aimed to determine if a training program conducted for 5 months with experienced swimmers, aged 18-22 years old and of both sexes, would cause changes in serum levels of total IGF-I, free IGF-I, and its proteins (IGFBP-1 and 3). A positive effect of training on serum IGF-I levels was identified. However, it should be noted that, after two months of training, only a modest increase in IGF-I levels had occurred, which suggests that a relatively long time is required to verify an increase in IGF-I levels with this method of training. The authors concluded that levels of free and total IGF-I, as well as IGFBP-3, can be increased with intense training [21], although the IGF-I changes were limited and, after a period of time, no increases were observed.

An important binding protein, IGFBP-3, was also analyzed in the present study and it was noted that this protein was sensitive to chronic effects of the competitive season.

In summary, there are current data suggesting that the GH/IGF-I axis hormonal alterations, in response to training sessions or training protocols, can be used as a really useful tool to monitor training load or to plan training cycles over the competitive seasons of athletes [6, 16].

The athletes in this study, when evaluated, presented a maturation status close to the PHV, since this parameter's values were always positive and showed no significant difference between the assessment moments. There are several classifications for the pubertal period, interestingly our data corroborated with a recently meta-analysis that presented a classification very closely for PHV, height and age [22]. The literature suggests that young athletes of differing maturity status could respond to equal training program in different magnitudes [22].

The results of this study demonstrated that there was no change in the maturational status of the athletes throughout the season, so the verified adaptations seemed to be attributed to the competition stimuli. Specially for the motor tests performance, it was possible to verify that the explosive strength capacities and aerobic power did not present any differences between the observed moments, while the results of tests in which there is a predominance of anaerobic metabolism (RAST, V30m, and T-Test) presented a performance peak during the competition.

Youth categories (e.g. U-13) should presented lower results when compared to those found in the U-15 and U-17 categories. Indeed, the lower train-ability in pre pubertal stage could be attributed to the fact of adolescents has lack of coordination is temporarily disrupted due to rapid growth of the limbs and trunk [23]. This seemed to be explained by the fact that the under-13s category still does not present fully developed anaerobic capacity [24]. Similarly, another data compared the RAST results of four categories: professional, junior, U-17, and U-15. The results demonstrated that with advancing age there is better anaerobic performance [25]. However, the age at which the highest muscular power is expressed (15-16 years old) should not be confused with training state [26].

Whereas IGF-I has been implicated in many anabolic pathways in skeletal muscle beside other physiologic mechanisms (endocrine as well as paracrine/autocrine) literature showed IGF-I is an important metabolic biomarker but it can't performance predict [27, 28]. Interestingly in our study, IGF-I appears be relevant training status biomarker, especially for anaerobic performance, thus providing an implement for coaches and athletes or other researchers in sport physiology and sport performance.

It is also suggested that new field studies be carried out, in order to follow up on training programs for young athletes and identify the possible physiological effects, including an analysis of cytokines, testosterone, cortisol, glycemic levels, and other IGF-I binding proteins (IGFBP1), as well as practical and applied assessments, to effectively evaluate the athletes within their modalities.

5. Conclusion

It was concluded that serum GH concentration kinetics did not change in any of the moments collected. In parallel to this result, IGF-I as well as IGFBP-3 showed variations during the competition, suggesting they were sensitive to both acute and chronic stimuli effects caused by the competition.

Regarding motor performance, it should be stated that in predominantly anaerobic tests there was a tendency for the best results, with higher IGF-I and IGFBP-3 concentrations.

Due to the fact that there is no change in maturation status, the observed alteration can be attributed to an improvement in the training state caused by competition stimuli.

It seems reasonable to suggest that some components of the axis, especially IGF-I and IGFBP-3, can serve as important training status markers for young soccer players within a competitive season.

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