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Taislaine Cristina Alipio
School of Physical Education and Sport of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil

Hugo Tourinho Filho
School of Physical Education and Sport of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil

Marcos Corrêa Junior
Department of Pediatrics - Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil

Larissa Ribeiro Da Silva
Institute of Mathematics, Statistics and Scientific Computing, State University of Campinas, Campinas, Sao Paulo, Brazil

Carlos Eduardo Martinelli Junior
Department of Pediatrics- Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil

Corresponding Author:
Taislaine Cristina Alipio
School of Physical Education and Sport of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil

Acute and chronic effects of cross fit training on the kinetics of the IGF-I / IGFBPs system in young adults

Taislaine Cristina Alipio, Hugo Tourinho Filho, Marcos Corrêa Junior, Larissa Ribeiro Da Silva and Carlos Eduardo Martinelli Junior

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Abstract
The components of the IGF-I/IGFBPs system constitute a group of growth factors that are directly related to somatic and tissue growth in many species, and exercise programs are related to this anabolic function. The aim of this study was to verify the kinetics of the IGF-I/IGFBPs system in young adults during a CrossFit training program. Eight young adults were evaluated before and after the execution of a standardized training session (Acute effect), during the initial, intermediate and final phases of the CrossFit training program (Chronic effect). To analyze the kinetics of the IGF-I/IGFBPs system throughout the training program, two non-parametric statistical methods were used: the Kruskall-Wallis test for one factor and the Wilcoxon test. The significance level adopted was 0.05. No significant changes were found in the concentrations of IGF-I ($p=0.73$) and IGFBP-3 ($p=0.23$) between the initial, intermediate and final phases of the training program. There were also no significant differences in the IGF-I and IGFBP-3 concentrations between the pre and post training sessions in the initial, intermediate and final phases. Even though the standard session was classified between "moderate" and "very difficult", it was not enough to modify the values of IGF-I and IGFBP-3, which can be explained by the participants' practice time and adaptation to intense training, predominant characteristic in the CrossFit modality.

Keywords: IGF-I, IGFBP-3, cross fit, biomarkers

1. Introduction
Baseline levels of IGF-I are positively correlated with muscle mass and physical fitness in children, adolescents and adults [1, 2, 3].
The components of the IGF-I/IGFBPs system constitute a group of growth factors that are directly related to somatic and tissue growth in many species, and exercise programs are related to this anabolic function. However, some studies have shown a decrease in the circulation of these elements in response to acute physical training sessions [4, 5, 6, 7, 8].
Regarding the chronic effects of physical training on the IGF-I/IGFBPs system, some authors have investigated the kinetics of IGF-I and its binding proteins in longer periods of training. Studies suggest the possibility of having a so-called catabolic phase in response to physical exercise followed by an anabolic phase [9, 10, 11, 12].
According to Nemet & Eliakin [12], there seems to be a fine line between anabolic responses and catabolic/inflammatory responses to exercise that will determine the efficiency of the training program. If the anabolic response is stronger, the training program will probably lead to an increase in muscle mass and improved physical fitness. On the other hand, a greater catabolic/inflammatory response, especially if it persists for a long time, can lead to overtraining [12].
For this reason, changes in the anabolic/catabolic balance and cytokines circulation can be used to assess the training intensity of athletes and sports teams, with special attention to training programs for young athletes [12, 13, 14, 15].
Based on this assumption, the present study aimed to verify the kinetics of IGF-I and its binding protein, IGFBP-3, in young adults during a CrossFit training program.

2. Material and Methods

2.1. Sample

Initially, the sample considered for this study consisted of 23 CrossFit practitioners of both genders, male and female. During the collection period, there was a sample loss due to the practitioners' lack of availability to adjust the collection days with the external championships that were happening in the same period. Thus, a total of 8 practitioners were exposed to training sessions over a 3-month period of the CrossFit training program (Table 1).

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and it was approved by the local Ethics Committee (n. 979.575).

Table 1: Characteristics of CrossFit practitioners. Values expressed as mean and ± SD

Variables	n = 8
Age (years)	27 ± 5,4
Time of practice (months)	15 13,7

2.2 Data collection

a) After being informed, verbally and in writing, through the Free and Informed Consent Term regarding the procedures that would be adopted in the research, the volunteers underwent a training session and blood collection at three times throughout the training program.

b) Blood collection was performed before the beginning of the training session, preceded by approximately 10 minutes of rest and 10 minutes after the end of the session, totaling two blood samples, with an interval between pre-training collection and post-training collection of approximately 1h00min (Figure 1).

c) All procedures were performed in the initial phase, intermediate phase and final phase of the practitioners' training program, which lasted approximately three months. Thereby, six blood samples (pre and post-training) were totaled throughout the training program.

Fig 1: Study design diagram: Data collection, training session and blood collection

2.3 Blood collection

The venous blood collection was performed via puncture through access to the blood vessel through the anterior surface of the forearm. The collection was carried out in a reserved place located in the participants' CrossFit training center. Before access, the region where the collection was performed was properly sanitized with 70% ethanol. 10 ml of blood were collected from each individual in tubes without the addition of anticoagulant for serum acquisition (10 ml pre-training and 10 ml post-training), totaling 60 ml throughout the training program, which were stored between 0 and 4ºC immediately after the procedure. Subsequently, the samples were centrifuged between 0 and 4ºC at 1200rpm for 12' to separate plasma and serum. Once this step was completed, plasma and serum were removed from the collection tube and stored in 1.5mL tubes for later freezing at -80ºC for the dosages described below. The remaining content of the collection tubes was disposed of in biological waste (Class A waste) and collected for final disposal by the municipal government.

2.4 24-hour diet recall

A 24-hour food recall was applied to assess the food consumption performed by the participants. For the analysis of habitual food intake, practitioners were asked to take a 24-hour recall of three days, two days during the week and one day at the weekend (Saturday or Sunday). The calculations of food records were performed with the assistance of the Dietbox® program.

Food intake was described in portion size, brand of processed foods, sugar and oil consumption. The energy intake was estimated by the consumption of macronutrients through the 24-hour recall, where 1g of protein or carbohydrate is equal to 4.1 kcal and 1g of lipids is equal to 9 kcal.

The nutritional needs of all athletes were estimated according to the Dietary Reference Intakes - DRI (Nutritional Research Council, 2006).

2.5 Standardized CrossFit training session

The standard training session used in the present study had a warm-up part consisting of educational and stretching exercises, lasting approximately 20 minutes; and a main part with a sequence of functional exercises characteristic of the modality known as “Fight gone bad”, which consists of three sets of five different exercises: wall ball, box jump/ box step-up, high-pull sumo deadlift, push-press and rowing. Participants performed each exercise for one minute, maintaining the highest possible intensity, until all five exercises were completed. At the end of the series, participants took a one-minute break before starting the next round. After the end of the three series, the participants performed stretching exercises for the “cool down”.

Participants were instructed to determine their own load for each exercise. Therefore, they had to choose a load at which they could maintain the highest intensity throughout the main part of the training session. The height of the box, for the box jump and box step-up exercises, were also determined according to the individuality of each participant.

A perceived subjective exertion (PSE) scale proposed by Foster *et al.* [16] was used to monitor the intensity of the standard training session. Shortly after the session ended, participants had to answer a single question: “How was your training session?” The answer had to refer to the training session as a whole and to answer the question, the participants scored on the PSE scale from 0 to 10 points.

2.6 Immunoassays

2.6.1 Serum IGF-I and IGFBP-3 concentrations

Serum concentrations of IGF-I and IGFBP-3 (Immulite 2000, Siemens, Los Angeles, CA) were determined by specific immunoassays using commercial kits. All study samples were analyzed in duplicate within the same. The intra-assay variations were 2.77% for IGF-I; and 2.60% for IGFBP-3.

2.7 Statistical analysis

Analysis of the kinetics of the IGF-I/IGFBP-3 system, throughout the standardized CrossFit training session, were performed by two non-parametric statistical methods. The non-parametric Kruskall-Wallis test for one factor was used to analyze the difference between the phases (initial, intermediate and final), while Wilcoxon test was used to verify the differences between the pre and post training session in each phase. The significance level adopted was 0.05.

3. Results

Through the perceived subjective exertion (PSE) scale proposed by Foster *et al.* [16] to monitor the intensity of the training session, CrossFit practitioners reported an intensity between 3 (Moderate) and 9 (Very difficult) after the standardized training session, used for the measurement of IGF-I and its binding protein, IGFBP-3.

In Figure 2, it is possible to verify the IGF-I kinetics of all participants during the initial, intermediate and final phases of the CrossFit training period that lasted three months.

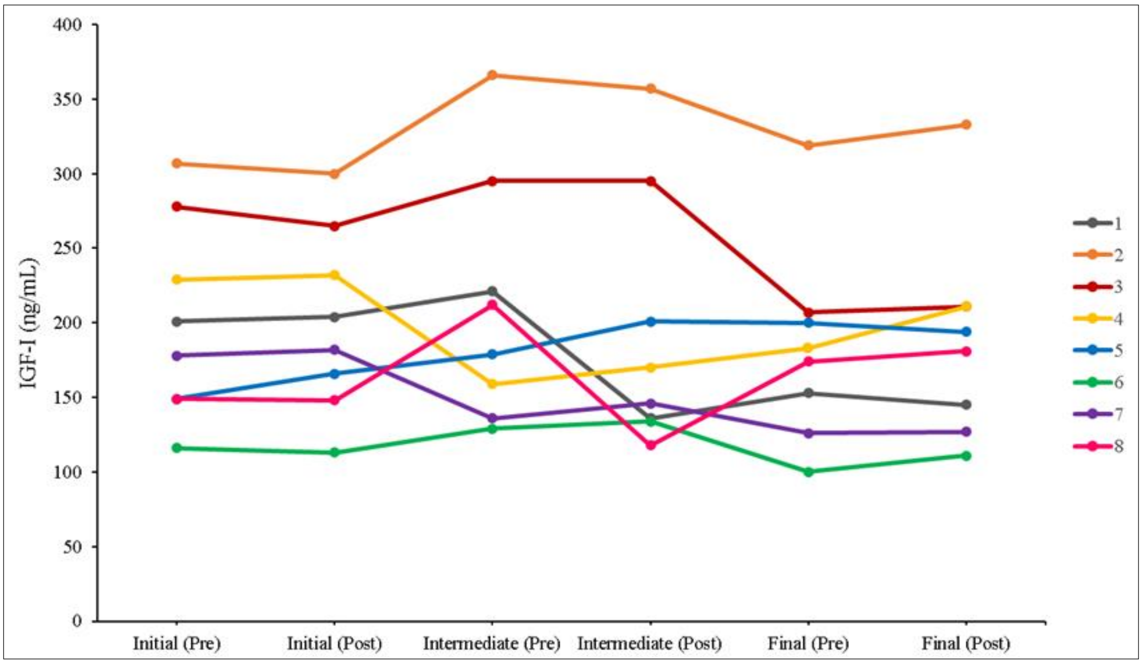

Fig 2: IGF-I kinetics between initial, intermediate and final phases of all participants in the CrossFit training program

No significant differences were found in IGF-I (p=0.73) and IGFBP-3 (p=0.23) concentrations between the initial, intermediate and final phases of the training program. (Figures 3 and 4)

Additionally, there were no significant differences in IGF-I concentrations between the moments before and after the training session in the initial (p=1.0), intermediate (p=0.49) and final (p=0.71) phases. The concentrations of IGFBP-3 also did not show significant differences between the moments before and after the training session in the initial (p=1.0), intermediate (p=1.0) and final (p=0.79) phases.

Throughout the training program, there were no significant changes in the participants' caloric intake that could be related to the levels of IGF-I and IGFBP-3 found in this present study.

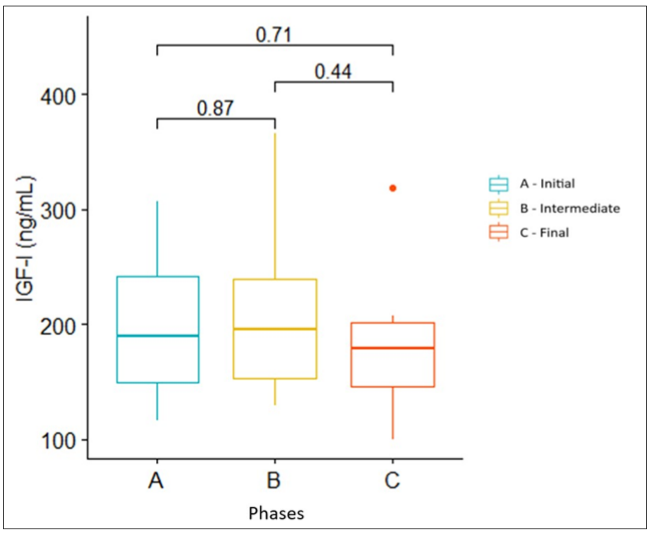

Fig 3: Serum IGF-I concentrations in the initial, intermediate and final phases of the CrossFit training program

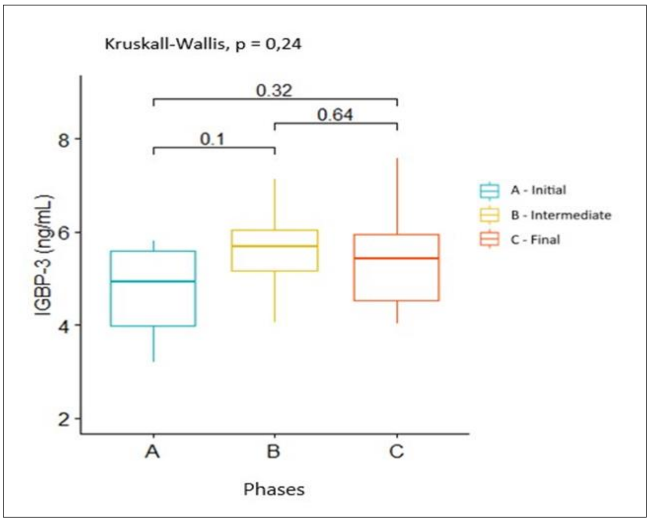

Fig 4: Serum IGFBP-3 concentrations in the initial, intermediate and final phases of the CrossFit training program

4. Discussion

The main finding of the present study was to verify that, despite the CrossFit modality being known for its intense training sessions, it was not possible to find any significant change in the serum concentrations of IGF-I and IGFBP-3 during the training program that lasted three months.

In contrast, previous studies that aimed to evaluate the acute effects of a training session on the kinetics of the IGF-I/IGFBPs system, demonstrated predominantly catabolic responses of the system after an intense physical training session [4, 7, 8]. Nemet *et al.* [4] conducted a study whose objective was to determine the behavior of the GH/IGF-I axis in response to a typical Greco-Roman wrestling training. The authors found a significant decrease in IGF-I and insulin levels. However, there was also a significant increase in the concentrations of IL-6, TNFα, IL-1β and IGFBP-1, a binding protein known for inhibiting the effects of IGF-I.

Furthermore, Pilz-Burstein *et al*. [7] simulated a Taekwondo match with young elite athletes of both genders, in order to assess the behavior of anabolic and catabolic hormones in response to intense training. Since the anabolic components (IGF-I, LH and FSH) levels have suffered a significant decrease, the authors concluded that the hormonal behavior evaluated in response to a Taekwondo training session, has a response characterized as catabolic.

Tourinho *et al*. [17] evaluated 9 fighters from the Brazilian Jiu-Jitsu team, during a standard training session, and did not find significant changes in IGF-I and IGFBP-3 concentrations, which can be explained by the high level of training of the athletes. The results found by Tourinho *et al*. [17] are similar to those found in the present study with CrossFit practitioners.

In order to analyze the behavior of the GH/IGF-I axis during longer periods of training, some authors have speculated that there may be a so-called catabolic phase of the GH/IGF-I axis in response to physical exercise, followed by an anabolic phase [9, 10, 11, 12]. Such behavior could be explained by the "Two-phases hypothesis" proposed by Eliakim & Nemet [14].

In the two-phases hypothesis, the authors suggest that the increase in pro-inflammatory cytokines leads to a decrease in IGF-I levels, however, if adaptation to training is successful over a long period of training, the pro-inflammatory cytokines decrease, therewith the suppression of IGF-I ceases, being able to return to the basal values. Possibly, an anabolic rebound of the axis may occur, and may even exceed the levels observed pre-training.

Rosendal *et al*. [10] submitted trained (n=12) and sedentary (n=7) young subjects, to a 11-week intense physical training program. The data obtained in the study showed that the intense training program resulted in a considerable influence on the IGF-I system and its binding proteins with more pronounced changes in the group of sedentary young subjects, indicating that intense training affects the behavior of the GH/IGF-I axis of trained and sedentary individuals differently.

Nine male swimmers aged between 16 and 19 years were evaluated by Tourinho *et al*. [15], in order to measure the IGF-I, IGFBP-3 and ALS concentrations during a training season. Concentrations of IGF-I and IGFBP-3, in this study, were shown to be sensitive to the acute and chronic effects of training. Furthermore, Tourinho *et al*. [15] found the presence of a catabolic phase characterized by a reduction in IGF-I concentrations in the intermediate phase of the training season, where training intensity was high, followed by an anabolic phase, where concentrations of IGF-I had a significant increase during the final phase of the season.

Similarly, Fornel *et al*. [18] assessed the levels of GH, IGF-I, IGFBP-3, CK and LDH in young male soccer athletes for a total period of 7 months. Blood samples were collected before and after a standard training session at three different times of the season (initial, intermediate and final). IGF-I levels were significantly higher in the intermediate phase when compared to the final phase of the training season, which indicates that IGF-I is a sensitive biomarker of the acute and chronic effects of training, then showing a biphasic behavior of the axis, with a catabolic phase followed by an anabolic phase.

Even though recent studies have shown the existence of a biphasic behavior of the GH/IGF-I axis in response to training, there are still controversies regarding to this behavior when observed during a training season of some sports.

Merji *et al*. [19] did not observe any changes in IGF-I levels when analyzed soccer players during a training season. The researchers found higher levels of GH at the beginning of the season compared to the middle and final moments of the season. According to the authors, as the athletes were not training for a specific competition, the training intensity was kept relatively constant during the training period in which the collections were performed.

The hypothesis that the hormonal concentrations of the IGF-I/IGFBPs system present biphasic behavior during a training season was also not observed by Pisa *et al*. [20]. The authors found no significant differences in hormone concentrations, which could indicate a chronic adaptation to training. The authors believe that the concentrations of the components of the IGF-I/IGFBPs system can vary during a training period, and these changes can also be related to the intensity in the different training phases of the season and not simply to the length of time used to evaluate the subjects.

Correa Jr *et al*. [21] analyzed the kinetics of IGF-I and IGFBP-3 in adolescents undergoing a standard 10-week hypertrophy training program. The authors found that IGF-I levels increased during the training session in the 1st assessment ($p=0.03$) and also during the 10 weeks of training ($p<0.003$). No change in IGFBP-3 levels was observed during a training session or during the 10 weeks of training. Correa Jr *et al*. [21] concluded that no catabolic phase was detected in adolescents during hypertrophy training and IGF-I was sensitive to the acute and chronic effects of resistance training.

In order to verify the relationship between changes in major metabolic hormones and measures of energy balance, Geesmann *et al*. [22] evaluated 14 well-trained cyclists who completed a 1,230 km ultra-endurance cycling event. Changes in IGF-I concentrations were positively correlated with energy balance throughout the event ($r = 0.65$, $p = 0.037$), which ranged from a deficit of 11,859 kcal to a surplus of 3,593 kcal. IGF-I suppression was strongly associated with the magnitude of energy deficit, indicating that athletes who achieved a greater energy deficit exhibited a more pronounced drop in IGF-I.

Unlike the study performed with ultra-endurance cyclists, in the present study, the standard CrossFit training session did not cause significant changes in IGF-I and IGFBP-3 concentrations over the training period.

A possible explanation for the results found in this study would be the participants' training status and familiarity with the modality, since Crossfit is a form of training where there is a predominance of high-intensity interval exercises. Therefore, it is inferred that the practitioners who participated in the study were already adapted to high-intensity training and for this reason, the levels of IGF-I and IGFBP-3 did not show significant changes.

5. Conclusion

Although the standard CrossFit training session was rated between "moderate" and "very difficult", no significant changes were observed in IGF-I and IGFBP3 levels before and after the training sessions (acute effect), nor in the different times of preparation throughout the training program (chronic effect). There was also no relationship with the nutritional status of the participants.

It is believed that the biphasic behavior can be observed, probably, in sports with increased load in the specific phase of the season, within their training periodization, as occurs in modalities such as swimming, handball and soccer. In modalities with progressive load distribution such as weight training and CrossFit itself, it does not seem possible to evidence the two-phase hypothesis.

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