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Henrique Santa Capita Cerqueira
Post-Graduate Program in Child and Adolescent Health, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Brazil

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

Marcos Corrêa Junior
Post-Graduate Program in Child and Adolescent Health, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Brazil

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

Corresponding Author:
Henrique Santa Capita Cerqueira
Post-Graduate Program in Child and Adolescent Health, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Brazil

ZMA supplementation does not impact IGF-IGFBPs system or body composition of young athletes

Henrique Santa Capita Cerqueira, Hugo Tourinho Filho, Marcos Corrêa Junior and Carlos Eduardo Martinelli Junior

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Abstract
IGF-IGFBPs system hormones are known for their anabolic role. ZMA is a supplement consisting of zinc, magnesium and vitamin B6, whose premise is to provide an increase in levels of GH, IGF-1 and testosterone. This hypothetical increase could cause significant changes in body composition. However, studies on its effectiveness are divergent in addition do not evaluate the IGF binding proteins (IGFBPs), which are essential in IGF-1 metabolism. Therefore, this study aimed to analyze the effects of ZMA in IGF-1, IGFBP-3 and testosterone levels. Eighteen male amateur athletes were divided into two groups: ZMA and placebo. Hormone levels and anthropometric characteristics were assessed before and after the 8-week intervention. After the intervention, there were no differences between groups in hormonal levels and anthropometric characteristics**.** Thus, the results suggest that ZMA do not offer any additional benefits for individuals that has an appropriate diet, either in body composition or in hormone levels.

Keywords: physical education and training, dietary supplements, insulin-like growth factor I; testosterone

Introduction
IGF-1 (Insulin-Like Growth Factor-1) has significant functions in metabolism, as well as IGF binding proteins (IGFBPs). The same way testosterone, which also has a power anabolic action, in addition to being related to several processes such as increase in muscle mass and consequent strength gains, which have a great significance to sports performance. Among IGFBPs, we highlight IGFBP-3 (Insulin-like growth factor-binding protein-3), one of the 6 IGF-1 binding proteins, known to enhance the effects of hormone [1-4]
A popular supplement, well known worldwide is ZMA. It consists of zinc, magnesium and vitamin B6 (in the form of pyridoxine), whose premise is to increase the levels both in hormones that compose the IGF-BPs system and testosterone [5]
However, the studies on ZMA are still limited, despite of having one of them presenting positive results [5]; while other studies have not proved to be effective [6-8]. However, none of studies explored the effects of ZMA on IGFBPs. This process is significant since there are proteins that inhibit the effect of IGF-1, while others potentialize it. Thus, the effects of the hormone may be more or less pronounced for the same concentration of IGF-1, depending on the IGFBPs levels in that situation [1, 2]. Thus, the current study aimed to analyze the effect of supplementation containing ZMA in levels of total testosterone (Total T), IGF-1 and IGFBP-3 in young amateur athletes.

Materials and Methods
This is an 8 weeks double-blind placebo-controlled trial. This study was approved by the Ethics Committee in Research of the Hospital of the Ribeirão Preto Medical School, University of São Paulo - HCFMRP - USP, under number 3.089.224.

Participants
Twenty amateur football players aged 18- 25 years were chosen to participate the study. The sample size was determined for convenience, according to the approval and availability of the subjects. Among the 20 individuals chosen, 18 completed the intervention due to their absence in the first assessment.
Inclusion criteria: males aged 18 and 25 years with at least one year of experience in the

sport. Exclusion criteria: Use any type of supplementation, use or have used anabolic steroids. The sample characteristics are presented in table 1.

Table 1: Age, height, body mass e fat mass (%) of the subjects, Values expressed as mean ± standard deviation (SD).

Features	Mean ± SD
(n=18)	
Age (years-old)	21.61 ± 2.66
Height (cm)	176.00 ± 5.08
Body mass (Kg)	85.46 ± 15.23
Fat mass (%)	23.15 ± 5.29

cm = centimeters. Kg = kilograms.% = percentage value.

The individuals have received and signed a detailed, explained consent form, in in which they were informed of the structure of the study, as well as possible risks. Assessments were carried out at the team's training location.

Design
The participants were evaluated during pre and post the 8 weeks intervention. The assessments incorporated blood collection, in order to consider hormone levels, and anthropometric measurements, so that it is determined the body composition.
After the first collection, the individuals were randomly selected and allocated in two groups: ZMA, which it received the supplementation; and placebo, which it took a capsule of equal size and weight containing maltodextrin. The individuals were instructed to ingest a capsule before going to sleep and avoiding its consumption near the mealtimes.

Blood collection
The venous blood collection was made by puncturing a blood vessel in the anterior face of the forearm. 5 ml of blood were collected from each individual into test-tubes without containing anticoagulant. The samples were promptly stored at 0-4ºC, centrifuged between 0 and 4ºC a 1200 rpm per 12 minutes, and the serum was stored at -80ºC for the hormonal dosages.

Determination of IGF-1 and IGFBP-3 levels
Serum levels of IGF-1, IGFBP-3 (Immulite 2000, Siemens, Los Angeles, CA, USA) (chemiluminescence) were established by specific immunoassays from a commercial kit and expressed in ng/mL. For IGF-1 dosages, the samples were subjected to an extraction process from the IGFs of their carrier proteins using hydrochloric acid and ethanol. All samples were analyzed in duplicate within the same assay. Intra-assay variations were 2.4% for IGF-I and 2.3% for IGFBP-3. The accuracy of the assay was 5 ng/ml for IGF-I and 0,1 mg/l for IGFBP-3.

Determination of testosterone levels
Total testosterone measurements were performed in blood serum by the electrochemiluminescence method, following the specifications of the Bio System Kit. The technique was performed on the Elecsys 2010 device (Roche Diagnostica).

Anthropometric measurements
Body mass and height were assessed in a digital scale with coupled stadiometer. The stadiometer accuracy was 0.1cm, and the scale accuracy 0.1 kg ((Sanny - BL201PP; Sanny, São Paulo, Brazil). The following skin folds were also measured: pectoral, abdominal, thigh, suprailiac, subscapular, triceps and axillary middle from a scientific adipometer (Prime Med A10; Prime Med, São Paulo, Brazil). For the calculations of lean mass and fat, it was considered the Jackson & Pollock protocol, in conjunction with the formula developed by Siri [9,10]

Training Program
The training program consisted of physical preparation (2 sessions/week, involving strength training and aerobic conditioning, representing around 90 minutes each session), besides a technical-tactical preparation, in which the athletes performed physical, technical and tactical activities typical of the sport.

Diet and total energy expenditure
Total energy expenditure (TEE) has been estimated from the equations prescribed by Dietary Reference Intakes – DRIs [11]. The mean TEE of the individuals are present in Table 2, as well as the amount of dietary macronutrients.

Table 2: Total energy expenditure (TEE) per day (Kcal) and composition of the subjects' diet. Values expressed as mean ± standard deviation (SD).

TEE and macronutrients (n=18)	Mean ± SD
Total energy expenditure (Kcal)	3319 ± 410
Carbohydrates (%)	60%
Lipids (%)	15%
Proteins (%)	25%
kcal = kilocalories.% = percentage of the calories intake.	

Statistical treatment
The linear model of mixed effects was used for the analysis, in which it was adopted a significance level of 0.05. The value variables are presented as mean and standard deviation. The data were analyzed from the SAS Statistical Software (version 9, 3; SAS Institute, Inc. Cary, NC).

Results
Our study aimed to analyze the effects of ZMA supplementation on the hormone levels, in addition to body composition of young amateur athletes. The main results are described below.

Anthropometric measurements
By considering the analysis of the data, it were not found any statistically significant differences among groups related to body composition over the 8-week intervention (Table 3). The variations between the pre and post times (Δ) were calculated by the equation: post time value - pre time value.

Table 3: Body composition pre and post supplementation

Variables	ZMA			Placebo		
	Pre	Post	Δ	Pre	Post	Δ
Body mass (kg)	87,87 (±17,09)	88,22 ± (16,89)	0,35 (±1,59)	83,04 (±13,69)	83,49 (±13,37)	0,45 (±0,95)
Lean mass (kg)	66,75 (±14,68)	68,54 (±10,38)	1,79 (±0,77)	63,56 (±8,47)	65,36 ±(±8,67)	1,8 (±0,55)
Fat mass (kg)	20,30 (±8,29)	19,68 (±7,87)	-0,62 (±1,09)	19,48 (±6,84)	18,13 (±6,51)	-1,35 (±0,97)

cm = centimeters. Kg = kilograms.% = percentage value. Δ = Post-Pre

Hormone levels

We found intra-group differences in pre and post times for levels of IGF-1, IGFBP-3 and Total T in both groups. But there were no differences among groups of each parameters analyzed.

Table 4: IGF-1, IGFBP-3 and Testosterone levels before and after supplementation.

Variables	ZMA			Placebo		
	Pré	Pós	Δ	Pré	Pós	Δ
IGF-1 (ng/ml)	200,89 (±82,10)	212* (±86,41)	11,11 (±5,49)	285,11 (±88,17)	303,76* (±94,98)	18,65 (±7,83)
IGFBP-3 (ug/ml)	4,29 (±0,27)	4,36* ± (0,27)	0,07 (±0,02)	4,23 (±0,27)	4,30* (±0,26)	0,07 ± (0,01)
Testosterone (ng/ml)	7,48 (±2,53)	9,02* (±3,22)	1,54 (±0,87)	8,45 (±0,97)	10,21* ± (0,98)	1,76 (±0,44)

ng/ml = nanograms per milliliter
ug/ml = micrograms per milliliter
* significant difference pre x post

Discussion

Despite of being very popular worldwide, ZMA still does not show consistent results about its effectiveness [2,6-8]. In the current study, ZMA has not influenced hormone levels, nor body composition of the athletes.

Regarding the body composition, we have noticed that there was an increase in body mass and lean mass, accompanied by a decrease in fat mass in both groups. No differences were found between the groups in any of the parameters: body mass (p=0.52); lean mass (p=0.49); fat mass (p=0.66). There was also an increase in arm and thigh circumference, as well as a decrease in waist circumference in both groups, but without differences among them. Our findings support the results presented by Wilborn *et al*. (2004), in which authors also found an increase in lean mass and a decrease in body fat in both ZMA and placebo groups, non presenting differences among them as well. The characteristics of our sample are in line with the ones presented in studies that evaluated Brazilian amateur football teams [12-14].

Concerning hormone levels, there was increase in both groups, compared with themselves. However, there were no differences among them in all parameters analyzed. Such increase occurred within the physiological levels. These oscillations are common and, at this magnitude of increase, they would not be able to promote additional benefits regarding mass and strength gain [2, 15]. These oscillations can be modulated both by nutrition and the total volume of the training program [2, 16]

The current study analyzed the total T levels, but not free testosterone. However, previous studies have demonstrated that ZMA also had no effect on free testosterone levels. Koehler *et al*. (2009) considered the effects of ZMA on series levels of total and free testosterone, in addition to its excretion in urine of young men. There was no difference between the supplemented and the placebo group. The studies conducted by Wilborn *et al*. (2004) and Moëzzi *et al*. (2013) also considered the effects of ZMA on total and free testosterone, and also found no differences with the use of supplementation. Only the study of Brilla & Conte (2000) demonstrated positive effects of ZMA supplementation on both free and total testosterone levels. In the current study there was no difference between groups regarding total T (p=0.26).

Regarding IGF-1, the only studies that analyzed the influence of ZMA on this parameter were those made by Brilla & Conte (2000) and Wilborn *et al*. (2004). In the Brilla & Conte study, ZMA promoted increases in IGF-1 levels, while there was no difference among the groups in the Wilborn *et al*. study. Our findings support those of Wilborn *et al*., as we also found no differences between the two groups after 8 weeks of supplementation (p=0.06)

This is the first study made in order to investigate the effects of ZMA on IGFBP-3 for our knowledge. This is the most abundant circulating IGFBP, which is known to enhance the action of IGF-1 [1, 17]. Nevertheless, supplementation containing ZMA had no effect on the levels of this binding protein (p=0.64).

Enthusiasts of ZMA commonly argue that supplementation would increase GH levels and physical performance. However, again only one study demonstrated these positive effects: the study conducted by Brilla & Conte (2000), while other studies investigating these issues found no benefits of ZMA supplementation [7, 8].

The premise of ZMA is to increase the levels of IGF-1 and testosterone. This premise is based on the fact that zinc and magnesium are related to IGF-1 and testosterone production. It is already well established in animal model that the deficiency of these two minerals leads to an abrupt drop in IGF-1 levels [18, 19]. Vitamin B6 is present in the formulation under the claim of increasing absorption of zinc and magnesium [5]. However, we could not observe it in practice, either in this study or other previously made [6-8]. Therefore, ZMA does not seem to be able to provide any kind of benefits, both in hormone levels and body composition. An essential issue to be discussed is that some individuals in the supplemented group reported improvement in sleep quality. This is a very common effect on ZMA users, however not yet investigated by the literature [20]. As the present study also did not evaluate the quality of the individuals' sleep, we cannot assure that ZMA is effective from this purpose. We also highlight that subjects in our study have reported no adverse effect on supplementation.

Conclusion

Thus, considering our results, we understand that ZMA was not able to promote any benefits, in either body composition, or promoting increases in of IGF-1, IGFBP-3 and testosterone levels. In conjunction with data from other studies previously carried out, we can assume that ZMA is not effective for what it proposes. We also highlight that oscillations in hormone levels occur naturally, being influenced, among other factors, by training and nutrition. Therefore, additional doses of the micronutrients investigated here do not seem to guarantee any type of further benefit.

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Authors' contributions
HC and CM conceived and designed the study. CM were responsible for coordination the study. HC and MC collected data. HC, CM, MC and HT analyzed data and reported results. HC and HT constructed the first manuscript. All authors edited the manuscript and approved the final version of manuscript.

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Availability of data and materials
Data and publication materials are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This study was approved by the Ethics Committee in Research of the Hospital of the Ribeirão Preto Medical School, University of São Paulo - HCFMRP - USP, under number 3.089.224. according to ethical standards in the research of the Brazilian National Health Council.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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