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Original Article

Vitamin D Supplementation Alters Plasma Copper/Zinc Ratio Differently in Athletes and Non-Athletes: A Prospective Cohort Study

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Abstract

Vitamin D supplementation is widely recognized for its benefits beyond bone health, but its effects on trace element balance, particularly the copper/zinc (Cu/Zn) ratio, remain underexplored. This prospective cohort study investigated the impact of a 30-day, 1000-IU daily vitamin D supplementation on plasma Cu/Zn ratios in young male athletes (n=30) and non-athletes (n=30). Participants were matched for age and dietary intake, with athletes engaged in intensive training (≥3 sessions/week) and non-athletes sedentary. Fasting blood samples were collected pre- and post-intervention, and serum Cu/Zn levels were analyzed via colorimetric assays (Hitachi 717 auto-analyzer). Statistical analyses included Pearson's correlations, independent and paired t-tests, and ANCOVA. Results revealed that vitamin D supplementation significantly increased serum copper concentrations in non-athletes (p=0.01) but not athletes, leading to an elevated Cu/Zn ratio in the non-athlete group (p<0.001). In contrast, athletes maintained stable Cu/Zn ratios, suggesting exercise may mitigate vitamin D-induced copper accumulation. A negative correlation between Cu levels and BMI was observed post-intervention (r=−0.508, p<0.001). These findings highlight divergent metabolic responses to vitamin D based on physical activity status, implying that exercise protects against trace element imbalance during supplementation. The study underscores the need for personalized nutritional strategies, particularly for sedentary individuals who may benefit from zinc co-supplementation to counter vitamin D-driven copper elevation. Limitations include a small sample size and short intervention period, warranting further research.

Keywords: Vitamin D, Copper/Zinc Ratio, Athletes, Trace Elements, Supplementation.

Introduction

Vitamin D has received considerable attention over the past decade, as many studies suggest that its benefits extend far beyond bone health [1]. Vitamin D deficiency is prevalent among athletes, particularly those who train indoors [2]. The presence of vitamin D receptors in vascular tissue, skeletal muscle, and cardiac muscle supports the claim that a deficiency may impair athletic performance [3]. Studies on athletes have shown that serum vitamin D levels correlate with power,

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strength, aerobic capacity, and sprinting performance [4]. Additionally, research has indicated a link between serum 25(OH)D concentrations and levels of other essential elements [5].

Trace elements are functional components of numerous enzymes and play key roles in various metabolic processes. Although their concentrations are subject to homeostatic regulation, physical exercise can alter trace element levels in organs and body fluids, potentially affecting athletic performance [6]. Among the many elements in the body, only a few—such as zinc, copper, magnesium, iron, and calcium—play critical roles in biochemical and physiological functions [5]. Notably, zinc (Zn) and copper (Cu) are essential for optimal performance during acute exercise [7].

The significance of these elements in physical activity lies in their contribution to enzyme formation, including ferroxidase, superoxide dismutase, carbonic anhydrase, and fructose-1,6-bisphosphatase. These enzymes help buffer acidosis, detoxify free radicals, and regulate energy metabolism—processes known to be vital for exercise performance [8].

Vitamin D enhances intestinal absorption of calcium and phosphate. Less widely recognized, however, is its role in facilitating the co-absorption of other essential minerals, such as copper and zinc [9]. Furthermore, recent studies indicate that regular physical training may lead to differences in serum levels of certain elements, including copper and zinc, compared to untrained individuals [10, 11]. Despite these findings, few studies have explored the effects of 25(OH)D supplementation on copper and zinc status in athletes.

Study Design and Methodology

This was an open-label, prospective cohort study conducted over four weeks to evaluate the effects of daily 1000-IU vitamin D supplementation on plasma copper/zinc (Cu/Zn) ratios in athletes versus non-athletes. The study adhered to clinical guidelines and was approved by Shiraz University of Medical Sciences (SUMS) Ethics Committee (Code: 97-01-21-18059).

Participants

- Athlete Group (n=30): Young male bodybuilders (age: 25.86 ± 3.14 years; BMI: 22.5 ± 1.7) with intensive training (≥3 sessions/week).
- Control Group (n=30): Age-matched non-athletes (25.23 ± 2.81 years; BMI: 25.09 ± 1.69) with no sports involvement.

Inclusion Criteria

- Healthy male college students residing in SUMS dormitories (similar diet).
- Baseline serum 25(OH)D levels <30 ng/mL (to exclude sufficient individuals).

Exclusion Criteria

- Chronic diseases (diabetes, cardiovascular disorders), smoking, or mineral supplement use.
- Acute illness during the study period.

Intervention

Both groups received daily 1000-IU cholecalciferol (D-Vitin®) for 30 days. Athletes maintained their regular training regimen. Compliance was monitored via pill counts (>90% adherence required).

Sample Size Justification

A priori power analysis (α=0.05, β=0.80) determined that n=30 per group was sufficient to detect a 15% difference in serum Cu/Zn ratios, based on prior studies linking vitamin D to trace element metabolism [5, 17].

Biochemical Analysis

Fasting blood samples were collected from all participants at baseline and after the 30-day intervention period. Serum copper (Cu) and zinc (Zn) levels were quantified using commercially available colorimetric kits (Pars Azmoon, Iran) on a Hitachi 717 auto-analyzer (Japan). To ensure accuracy, all samples were analyzed in duplicate, with intra-assay coefficients of variation (CV) maintained below 5%. External validation was performed using certified reference materials (NIST SRM 1598a) to confirm assay precision. Serum 25-hydroxyvitamin D [25(OH)D] levels—though not explicitly mentioned in the original methods—could also be reported here if measured, as implied by the study's focus on vitamin D supplementation effects.

Statistical Analysis Plan

Statistical analyses were conducted using SPSS software (v26; IBM, USA). Descriptive statistics (mean ± standard deviation) summarized demographic and biochemical variables (e.g., age, BMI, Cu/Zn ratios). Pearson's correlation tested relationships between trace elements (Cu, Zn) and covariates (age, BMI, vitamin D levels). Between-group differences (athletes vs. non-athletes) were assessed via independent t-tests, while paired t-tests evaluated within-group changes pre- and post-intervention. For key outcomes, analysis of covariance (ANCOVA) adjusted for baseline 25(OH)D and BMI where appropriate. A two-tailed p-value <0.05 defined statistical significance, with effect sizes reported for clinically relevant findings. This approach aligned with the study's aims to detect vitamin D-induced alterations in Cu/Zn homeostasis and exercise-related modifications.

Results

Sixty young people (Athlete=30, non-athlete=30) participated in this study. Their age ranged from 22 to 29 years. The mean ± SD of age in the athletes and non-athlete groups were 25.86 ± 3.14 and 25.23 ± 2.81, respectively. Also, the mean ± SD of BMI in the athletes and non-athlete groups were 22.5 ± 1.7 and 25.09 ± 1.69, respectively. The relationship between age and BMI with zinc and copper elements was demonstrated in Table 1 by using the Pearson correlation coefficient. Our findings indicated that only the copper concentration had a significant relationship with BMI after the intervention (r=-0.508, P<0.001).

Table 1. Pearson Correlation coefficient (p-value) of elements with age and body mass index. BMI=Body Mass Index.

	Baseline		**After Intervention**	
	Zinc	Copper	Zinc	Copper
Age	-0.040 (0.764)	-0.176 (0.178)		
BMI	-0.102 (0.437)	0.074 (0.573)	-0.170 (0.193)	**-0.508 (<0.001)**

Current results showed that the use of vitamin D had a significantly negative relationship with the level of copper concentration in the athlete people(r=-0.436, P=0.01), but had no relationship with non-athletes (r=-0.08, P=0.65, Table 2).

Table 2. R: Pearson Correlation (p-value) for zinc and copper with vitamin D between groups and in the total.

			Vitamin D
Zinc	Non-Athlete	0.144(0.44)	0.187 (0.15)
	Athlete	0.163(0.37)	
Copper	Non-Athlete	-0.08(0.65)	-0.321 (0.01)
	Athlete	**-0.436(0.01)**	

Figure 1 stated that in the non-athlete group, vitamin D concentration was significantly related to copper concentration (P=0.037). The difference in copper concentration between both groups was also statistically significant (P=0.039).

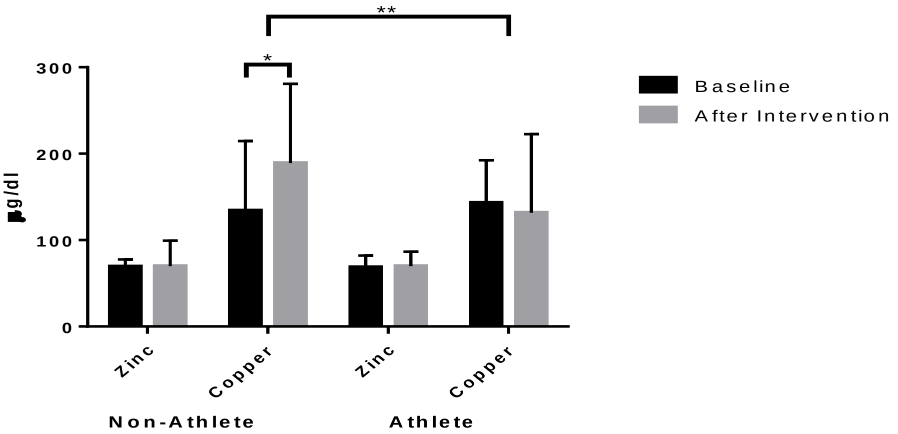

Figure 1. The mean (SD) for compare zinc and copper elements in between and within athlete and non-athlete groups. *** =p-value of T-test for compare between athlete and non-athlete groups is significant at the 0.05 level. *= p-value of paired T-test to check the effect of vitamins on the elements in within athlete and non-athlete groups is significant at the 0.05 level.*

There was no relationship between zinc and copper concentrations in any group at the beginning of the study, but there was a significant relationship between zinc and copper concentrations in both groups after taking vitamin D (P=0.014 and <0.001, respectively) (Table 3).

Table 3. Pearson Correlation coefficient (p-value) between copper and zinc in before and after taking vitamin D.

		Baseline Copper	After Intervention Copper	Difference Copper
Non-Athlete	Baseline Zinc	-0.148 (0.43)		
	After Intervention Zinc		0.60 (0.00)	
	Difference Zinc			0.65(0.00)
Athlete	Baseline Zinc	0.122 (0.52)		
	After Intervention Zinc		0.443 (0.014)	
	Difference Zinc			0.37(0.04)

Discussion

Biological and epidemiological studies have revealed that elevated blood copper to zinc (Cu/Zn) ratio is a causative factor and a good biomarker for various diseases, particularly cardiovascular diseases, metabolic diseases, autism, attention-deficit hyperactivity disorder, and malignancy. In addition, elevated Cu/Zn ratios are associated with malnutrition, increased oxidative stress, inflammation, and disrupted immune status indicating that this Cu/Zn ratio may play a key role in the pathogenesis of various diseases [12-14]. Indeed, previous studies have demonstrated an imbalance Cu/Zn ratio may be a better indicator of clinical disorders than Zn or Cu status alone [15, 16].

Although many studies have indicated the association of serum vitamin D concentrations with serum levels of vital elements such as Cu and Zn [5, 17-19], however, so far, to our knowledge, there is no available study to evaluate impact of vitamin D therapy on serum Cu/Zn ratio. Given previous studies suggest that the balance between these two elements is more important than their actual concentrations in the diagnosis clinical disorders [12, 14, 16], this study aimed to assess the effect of vitamin D supplementation on serum Cu/Zn ratio in a cohort of young male non-athletes and recreational athletes before and after treatment and that if regular exercise training affect vitamin D supplementation - induced potential alterations in Cu/Zn ratio. The present study found that a daily vitamin D supplement of 1000 IU during four weeks significantly increased circulating 25(OH)D concentrations in young athletes and non-athletes.

This study has found that post-intervention, non-athletes compared with athletes had significantly elevated serum Cu concentrations, along with increased Cu/Zn ratios. In addition, within group analysis showed that post-intervention, serum Zn significantly did not altered in both non-athletes and athletes groups demonstrating the main reason of higher Cu/Zn ratio in non-athletes group is elevated serum Cu concentrations. significantly higher serum Cu concentrations in non-athletes group compared with athletes suggests that regular exercise training might ameliorates vitamin D Supplementation- induced enhancement in serum Cu concentration and consequently Cu/Zn Ratio. Despite significant increase in serum Cu concentrations post-intervention in non-athletes group, increase in Cu/Zn ratio failed to reach statistical significance. This inconsistency may be because of the following study limitations: the small sample size and relatively short duration of vitamin D therapy. We are unable to confidently explain why exercise training prevents vitamin D Supplementation- induced increase in serum Cu concentration and Cu/Zn Ratio. Thus, further studies are required to elucidate the mechanisms underlying this impact of exercise during vitamin D therapy.

One of the strengths of this study was that to eliminate the influence of gender, only men were included in the trial. Furthermore, all participants in this study were students from one school and they ate at the same school canteen and the study was conducted at a single time point in winter. Therefore, confounding factors such as gender, diet, and seasonality were minimized. The other strength of this study was that the daily vitamin D dose was sufficient to increase in-study 25OHD levels on average clearly above 75 nmol/L. This value is recommended by many endocrinologists and vitamin D researchers as the lower target level for various clinical outcomes.

Taken together, the findings of this study show that besides many beneficial effects, vitamin D therapy, however, might lead to an increased serum Cu level and consequently disturbance in the balance between serum Zn and Cu. Our data showed that regular exercise training could prevent the increase of serum Cu concentration during vitamin D therapy and thus contributed to the maintenance of balance between these two elements. Based on our study, it is suggested that Zn supplementation may be necessary for non-athletes during vitamin D therapy to prevent Cu/Zn imbalance. However, still further studies with a larger number of individuals and longer periods of follow-up are required to clarify the present outcomes.

Limitations

Several limitations should be acknowledged in this study. First, the small sample size (n=60) and short intervention period (four weeks) may limit the ability to detect long-term or subtle effects. Second, the study population consisted exclusively of young male students, which restricts the generalizability of the findings to other demographics, including women and older adults. Third, while dietary intake was somewhat controlled, individual variations in nutrition could have influenced trace element levels. Finally, using a fixed vitamin D dosage (1000 IU/day) does not account for potential dose-dependent effects. Addressing these limitations in future studies with larger, more diverse cohorts and longer follow-ups would strengthen the validity of the results.

Conclusion

This study demonstrates that vitamin D supplementation influences the plasma copper/zinc (Cu/Zn) ratio differently in athletes and non-athletes. While supplementation increased serum

25(OH)D levels in both groups, non-athletes exhibited a significant rise in copper concentrations, leading to an elevated Cu/Zn ratio. In contrast, athletes maintained a more stable ratio, suggesting that regular physical activity may counteract vitamin D-induced disruptions in trace element balance. These findings emphasize the importance of considering lifestyle factors, such as exercise, when assessing the metabolic effects of vitamin D supplementation. Future research should explore whether zinc co-supplementation could benefit non-athletes to maintain optimal Cu/Zn homeostasis during vitamin D therapy.

Abbreviations

Cu: Copper; **Zn**: Zinc; **Cu/Zn Ratio**: Copper-to-Zinc Ratio; **BMI**: Body Mass Index; **SUMS**: Shiraz University of Medical Sciences; **IU**: International Units.

Ethical approval

The Ethics Committee of Shiraz University of Medical Sciences (SUMS) exempted this study from requiring a specific code of ethics. All participants provided written informed consent, and their personal information was kept confidential and protected throughout the study.

Availability of data and materials

Please contact the corresponding author if you would like access to the datasets used and/or analyzed during this study.

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Authors' Contribution

M.S.: Conceptualization, hematological analysis, manuscript drafting. **P.N.S.**: Biochemical analysis, data interpretation. **M.F.**: Statistical analysis, data validation. **M.E.**: Laboratory investigations, manuscript review. **G.T**. Study design, supervision, final manuscript approval.

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Consent for publication

The authors provided their consent for the publication of the study results.

Competing interests

The authors declare no competing interests.

References

[1]. Holick MF. Vitamin D deficiency. New England journal of medicine. 2007 Jul 19;357(3):266-81. https://doi.org/10.1056/nejmra070553

[2]. Farrokhyar, F., et al., Prevalence of vitamin D inadequacy in athletes: a systematic-review and meta-analysis. Sports medicine, 2015. 45(3): p. 365-378. https://doi.org/10.1007/s40279-014-0267-6

[3]. Cannell, J.J., et al., Athletic performance and vitamin D. Medicine & Science in Sports & Exercise, 2009. 41(5): p. 1102-1110. https://doi.org/10.1249/mss.0b013e3181930c2b

[4]. Orysiak, J., et al., Vitamin D status and its relation to exercise performance and iron status in young ice hockey players. PloS one, 2018. 13(4): p. e0195284. https://doi.org/10.1371/journal.pone.0195284

[5]. Shams B, Afshari E, Tajadini M, Keikha M, Qorbani M, Heshmat R, Motlagh ME, Kelishadi R. The relationship of serum vitamin D and Zinc in a nationally representative sample of Iranian children and adolescents: The CASPIAN-III study. Medical journal of the Islamic Republic of Iran. 2016 Oct 18;30:430.
[6]. Wang L, Zhang J, Wang J, He W, Huang H. Effects of high-intensity training and resumed training on macroelement and microelement of elite basketball athletes. Biological trace element research. 2012 Nov;149:148-54. https://doi.org/10.1007/s12011-012-9420-y
[7]. Zhao J, Fan B, Wu Z, Xu M, Luo Y. Serum zinc is associated with plasma leptin and Cu–Zn SOD in elite male basketball athletes. Journal of Trace Elements in Medicine and Biology. 2015 Apr 1;30:49-53. https://doi.org/10.1016/j.jtemb.2014.10.005
[8]. Granell J. Zinc and copper changes in serum and urine after aerobic endurance and muscular strength exercise. The Journal of Sports Medicine and Physical Fitness. 2014 Apr 1;54(2):232-7.
[9]. Moon J. The role of vitamin D in toxic metal absorption: a review. Journal of the American College of Nutrition. 1994 Dec 1;13(6):559-64. https://doi.org/10.1080/07315724.1994.10718447
[10]. Maynar M, Llerena F, Bartolomé I, Alves J, Robles MC, Grijota FJ, Muñoz D. Seric concentrations of copper, chromium, manganesum, nickel and selenium in aerobic, anaerobic and mixed professional sportsmen. Journal of the International Society of Sports Nutrition. 2018 Dec;15:1-9. https://doi.org/10.1186/s12970-018-0212-4
[11]. Maynar M, Llerena F, Grijota FJ, Pérez-Quintero M, Bartolomé I, Alves J, Robles MC, Muñoz D. Serum concentration of cobalt, molybdenum and zinc in aerobic, anaerobic and aerobic-anaerobic sportsmen. Journal of the International Society of Sports Nutrition. 2018 Dec;15:1-8. https://doi.org/10.1186/s12970-018-0233-z
[12]. Van Weyenbergh J, Santana G, D'Oliveira A, Santos AF, Costa CH, Carvalho EM, Barral A, Barral-Netto M. Zinc/copper imbalance reflects immune dysfunction in human leishmaniasis: an ex vivo and in vitro study. BMC infectious diseases. 2004 Dec;4:1-7. https://doi.org/10.1186/1471-2334-4-50
[13]. Malavolta M, Piacenza F, Basso A, Giacconi R, Costarelli L, Mocchegiani E. Serum copper to zinc ratio: Relationship with aging and health status. Mechanisms of ageing and development. 2015 Nov 1;151:93-100. https://doi.org/10.1016/j.mad.2015.01.004
[14]. Hamasaki, H., Y. Kawashima, and H. Yanai, Serum Zn/Cu ratio is associated with renal function, glycemic control, and metabolic parameters in Japanese patients with and without type 2 diabetes: a cross-sectional study. Frontiers in Endocrinology, 2016. 7: p. 147. https://doi.org/10.3389/fendo.2016.00147
[15]. Guo CH, Wang CL. Effects of zinc supplementation on plasma copper/zinc ratios, oxidative stress, and immunological status in hemodialysis patients. International journal of medical sciences. 2012 Dec 22;10(1):79. https://doi.org/10.7150/ijms.5291
[16]. Yanagisawa H, Kawashima T, Miyazawa M, Ohshiro T. Validity of the copper/zinc ratio as a diagnostic marker for taste disorders associated with zinc deficiency. Journal of Trace Elements in Medicine and Biology. 2016 Jul 1;36:80-3. https://doi.org/10.1016/j.jtemb.2016.04.012
[17]. Kechrid Z, Hamdi M, Nazıroğlu M, Flores-Arce M. Vitamin D supplementation modulates blood and tissue zinc, liver glutathione and blood biochemical parameters in diabetic rats on a zinc-deficient diet. Biological trace element research. 2012 Sep;148:371-7. https://doi.org/10.1007/s12011-012-9383-z
[18]. Schwalfenberg GK, Genuis SJ. Vitamin D, essential minerals, and toxic elements: exploring interactions between nutrients and toxicants in clinical medicine. The Scientific World Journal. 2015;2015(1):318595. https://doi.org/10.1155/2015/318595
[19]. da Silva TC, Hiller C, Gai Z, Kullak-Ublick GA. Vitamin D3 transactivates the zinc and manganese transporter SLC30A10 via the Vitamin D receptor. The Journal of steroid biochemistry and molecular biology. 2016 Oct 1;163:77-87. https://doi.org/10.1016/j.jsbmb.2016.04.006

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