



EVALUATION OF LEAD, CADMIUM AND MERCURY CONTENT IN SPORTS SUPPLEMENTS IN THE MARKET OF IRAN

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ABSTRACT

This study was aimed to determine the Lead, Cadmium and Mercury content in the Sports supplements in the market of Iran. This cross-sectional study was to determine the Lead, Cadmium and Mercury level in the 12 available Sports supplements brands in Iran. The total of 60 samples was used for this purpose. HNO₃ 67% and HF40% were used to extract the metals. The heavy metal levels in samples were determined using atomic absorption spectrometry method. The obtained results were finally analyzed by ANOVA and descriptive statistics using software spss16. The results showed that highest level of lead was detected in brands of S12 (2.31±0.01) and S11 (1.95± 0.003) while the lowest average level was detected in S1 brand (0.003± 0.0007). The highest level of Cadmium was in brands of S9 (0.412±0.007) and S11 (0.704± 0.003) and lowest average level of Cadmium was detected in S5 brand (0.012± 0.002). The maximum level of mercury was determined in S8 brand (0.55± 0.025) and S9 brand (0.50± 0.020) while the minimum level was observed in S1 brand (0.11± 0.011). It can be concluded that there are various amount of heavy metals in the sports supplements in the Iran and the monitoring of these products is important to protect the athletes' health.

Key words: Sport supplements, Athlete, Mercury, Lead, Cadmium

INTRODUCTION

Today, the athletes utilize several approaches to achieve the sporting triumphs through the use of medicinal or nutritional products (Sports supplements). There are various reasons to athletes uses these products include: increasing athletic performance and ability, accelerating of rehabilitation and reduction of muscle damage¹. Nutritional supplements are used to increase muscle growth for decades. The damages caused by some of these products have been well documented². Young and teen athletes are the consumer population group of these compounds to increase strength, muscle mass or improve physical function^{3,4}. Despite the fact that these supplements are sometimes associated with serious adverse effects or death, they have not seriously investigated for safety and their effects. Even healthy supplements such as vitamins, creatine, protein powders can be toxic, especially, if they are

consumed in high doses for a long time⁵. It is possible that various harmful additives have presented in food supplements and have not listed on their package label^{6, 7}. Five different groups of supplements exist on the market according to their original content. The term of Nutritional supplement is used for a variety of products including vitamins, minerals, plant compounds and many other effective compounds⁸. Creatine is one of these supplements. Creatine is a derivative of amino acids with the highest amounts in skeletal muscles and it is endogenously synthesized and made of the arginine, glycine and methionine of S-methyl but it can also provided through diet (mainly from meat and fish)⁹. Several studies have been conducted on the benefits of creatine supplements in appropriate amounts¹⁰⁻¹³. Other researchers have also shown that the excessive uptake of creatine causes problems¹⁴⁻¹⁷. The contamination resources can dependence to various factors such as raw materials, reagents and solvents

used in their manufacture, tubular packaging, tools and equipment (containers reaction, electrodes, etc.) that can be in contact with the product (e.g., lead is used in metal alloys) and containers that is used to store or package of the products (e.g., mercury and cadmium used in the plastics industry)^{18, 19}. The allowable daily intake for some important elements has been considered as following: Chrome (120 mg), copper (2 mg), iron (18 mg), molybdenum (75 μ g), selenium (70 μ g), zinc (15 mg) and The supplement manufacturers are responsible for ensuring the health of supplements and accuracy of the products label²⁰. The maximum limit of heavy metal amounts are 10 mg/kg for arsenic, cadmium, lead, and 1 mg/kg for mercury^{18, 19 and 21}. Mort et al has only detected mercury in measurable amounts (less than 1 mg/kg) in their study²². Hyatt et al have observed 36 nutrients and toxic elements in 42 nutritional supplements. They reported that some products had lead, zinc, manganese, molybdenum, copper and iron higher than accepted safe level²³. The aim of this study was to determine the quality of heavy metals such as lead, cadmium and mercury in different brands available sport supplements in the Iranian market due to the toxicity of the heavy metals.

Experimental Study design

This is a cross-sectional study and sports supplement brands available in the markets of Iran are the studied population. The total numbers of 12 sports supplement brands available including S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11 and S12 were provided from markets in Iran. The number of 5 samples was collected from each brand; thus, 60 samples were totally applied to detect and compare the heavy metal level among the various brands.

Material and instruments

Standard solutions of nickel, lead, cadmium and mercury at concentrations of 1 mg/ml were prepared from Merck CO., Germany. Atomic absorption spectrometry (SO Scan VARIAN), and method was used to measure heavy metals. Also, the microwave device was used for digestion and mineralization.

Experimental methods

HNO_3 67% and HF40% were used to extract the metals. The standard samples of each metal with

deionized water were applied for calibration. The samples were transferred to clean containers. 1 gr of sports supplements was carefully weighed and were transferred into the containers washed with deionized water and 5 ml of nitric acid 67% was added to each sample. For sample digestion and extraction of heavy metals (mineralization), microwave devices were used in a five-stage batch system at 280watt microwave for 3 min. The maximum pressure and temperature during extraction or mineralization should be 45 atm and 155 ° C, respectively. The samples obtained were brought to the desired volume.

Determination of the parameters

To measure the heavy metals, the samples were injected to atomic absorption spectrometry. For mercury, the dissolved mercury in the solution was reduced by $SnCl_2$ and converted to metallic mercury. Mercury vapor in the analyzer devices was directed into the sorbent cell and it was absorbed in atomic form at wavelength of 253.7 nm emitted by the mercury lamps. Thus, the concentration of mercury released in each of samples was achieved (30).

Data analysis

The obtained data were analyzed using software spss16 and descriptive statistics and the ANOVA were used to compare the average of each heavy metal in the samples.

RESULTS

The highest average level of lead was observed in brands of S12 (2.31 ± 0.01) and S11 (1.95 ± 0.003) while its lowest average level was determined in S1 (0.003 ± 0.0007). The results are shown in table 1. The highest average level of Cadmium was determined in brands of S9 (0.412 ± 0.007) and S11 (0.704 ± 0.003) and lowest average level of Cadmium was detected in S5 (0.012 ± 0.002). The results are shown in table 2. The maximum average level of mercury was determined in S8 (0.55 ± 0.025) and S9 (0.50 ± 0.020) while the minimum average level was observed in S1 (0.11 ± 0.011). The one way ANOVA test demonstrated that there is a significant difference between the mercury concentration levels in the samples. Table 3 reveals the results of mercury level in the different brands.

Table 1
The comparison of the lead average level in various studied products

product Code	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
S1	5	.00300					
S2	5	.01220					
S3	5	.01400					
S4	5	.02300	.02300				
S5	5	.03000	.03000				
S6	5		.08540				
S7	5			.30080			
S8	5			.33480			
S9	5			.34000			
S10	5				.49500		
S11	5					1.95360	
S12	5						2.31800
Sig.		.455	.067	.248	1.000	1.000	1.000

Table 2
comparison of the cadmium concentration level among the various studied products

product Code	N	Subset for alpha = 0.05									
		1	2	3	4	5	6	7	8	9	10
S5	5	.01240									
S2	5	.01400									
S1	5		.02020								
S10	5			.04600							
S6	5				.08880						
S4	5					.12620					
S7	5						.16380				
S8	5							.21480			
S12	5								.21500		
S3	5									.37420	
S9	5										.41280
S11	5										.70440
Sig.		.480	1.000	1.000	1.000	1.000	1.000	.929	1.000	1.000	1.000

Table 3
*comparison of the mercury concentration level
among the various studied products*

product N	Subset for alpha = 0.05									
	1	2	3	4	5	6	7	8	9	10
S1	.11400									
S2	.14000									
S6		.20200								
S5			.23600							
S3				.26000						
S7					.30000					
S4						.35400				
S11							.39000			
S10								.42000		
S12									.45600	
S9									.50600	
S8										.55800
Sig.	.085	1.000	.111	1.000	1.000	1.000	1.000	1.000	1.000	1.000

DISCUSSION

As it is observed in table 1, the maximum lead level was found in the S12 and S11. The One way ANOVA test showed that there is a significant difference between the average concentration of lead in the samples (P.Value<0.001). Based on the Duncan homogeneous subsets test, (Table 1), the average concentration of lead in products with code of S1 to S5 is similar but other subgroups have different concentration of lead. Also, the average concentration of S4, S5 and S6 as a subset has a similar average which it is different with other groups. Similarly, the S7, S8 and S9 are placed in a subgroup and they have different concentration rather than other groups. Dolan et al has been studied the arsenic, cadmium, mercury and lead content of 95 supplement product using microwave digestion and ICP-MS. They have found that the concentration of lead in some of these products is 48600 μ g/kg²⁷. The highest average level of Cadmium was related to S9 and S11 while lowest average level of Cadmium was detected in S5. The One way ANOVA test indicated that there is a significant difference between the average concentration of cadmium in the samples (P.Value<0.001). The Duncan homogeneous subsets test (Table 2) revealed that S2 and S5 have similar average concentration of Cadmium but the Cadmium concentration is different in other

subgroups. In addition, the average concentration of cadmium in S8 and S12 is similar but it was significantly different among all groups. The cadmium concentration was obtained to be 16800 μ g/kg in the study of Dolan et al. The maximum average level of mercury was determined in S8 and S9 while the minimum average level was observed in S1. The one way ANOVA test demonstrated that there is a significant difference between the mercury concentration levels in the samples. The Duncan homogeneous subsets test (Table3) showed that the average concentration of mercury in S1 and S2 was similar and it was different in other groups. Furthermore, its concentration is similar in S3 and S5 but it is significantly different among all groups, generally. Mort et al have investigated the creatine levels, organic pollutants and heavy metals in supplements. In this study, only mercury was observed in measurable levels (less than 1 mg per kg)²².

CONCLUSION

The results showed that the all type of studied brands of Sports supplements have different levels of heavy metal. The maximum level of heavy metal is related to lead and the cadmium and mercury are placed in next step. Since the heavy metals are

dangerous group of elements and they can have adverse effect on human health; therefore, the determination of these elements in these products is necessary and the authorities should be survey and monitor these products.

REFERENCES

1. Hozoori M, Ehteshami M, Haghvavian S, Azarpira A. Prevalence, Reasons and Information about Dietary Supplement Consumption in Athletes in Tabriz (2012). *Journal of Sport Biosciences*. 2012;4(12):77-91.
2. Timcheh-Hariri A, Balali-Mood M, Aryan E, Sadeghi M, Riahi-Zanjani B. Toxic hepatitis in a group of 20 male body-builders taking dietary supplements. *Food and Chemical Toxicology*. 2012;50(10):3826-32.
3. Avelar-Escobar G, Méndez-Navarro J, Ortiz-Olvera NX, Castellanos G, Ramos R, Gallardo-Cabrera VE, et al. Hepatotoxicity associated with dietary energy supplements: use and abuse by young athletes. *Ann Hepatol*. 2012;11(4):564-9.
4. DesJardins M. Supplement use in the adolescent athlete. *Curr Sports Med Rep*. 2002;1(6):369-73.
5. Bizzarini E, De Angelis L. Is the use of oral creatine supplementation safe? *The Journal of sports medicine and physical fitness*. 2004;44(4):411-6.
6. King DS, Sharp RL, Vukovich MD, Brown GA, Reifenrath TA, Uhl NL, et al. Effect of oral androstenedione on serum testosterone and adaptations to resistance training in young men: a randomized controlled trial. *Jama*. 1999;281(21):2020-8.
7. Haller CA, Benowitz NL. Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. *New England journal of medicine*. 2000;343(25):1833-8.
8. Petróczi A, Naughton DP, Pearce G, Bailey R, Bloodworth A, McNamee M. Nutritional supplement use by elite young UK athletes: fallacies of advice regarding efficacy. *Journal of the International Society of Sports Nutrition*. 2008;5(1):1-8.
9. Harris, R. C. (2001). Effects and safety of dietary and supplementary creatine. In R. Paoletti, A. Poli, & A. S. Jackson (Eds.), *Creatine from basic science to clinical application* (pp. 33-39). Dordrecht, Netherlands: Kluwer Academic Publishers.
10. SCF (2000). European Commission, Opinion of the Scientific Committee on Food on safety aspects of creatine supplementation, Adopted on 7 September 2000. Available from: <http://ec.europa.eu/food/fs/sc/scf/out70_en.pdf<.
11. Flisinska-Bojanowska A. Effects of oral creatine administration on skeletal muscle protein and creatine levels. *Biology of Sport*. 1996;13:39-46.
12. Fry DM, & Morales, M. A re-examination of the effects of creatine on muscle protein synthesis in tissue culture. *Acta Physiologica Scandinavica*. 1995;153: 207-9.
13. Poortmans JR, Francaux M. Adverse effects of creatine supplementation. *Sports Medicine*. 2000;30(3):155-70.
14. Benzi G. Is there a rationale for the use of creatine either as nutritional supplementation or drug administration in humans participating in a sport? *Pharmacological Research*. 2000;41(3):255-64.
15. Brudnak MA. Creatine: are the benefits worth the risk? *Toxicology letters*. 2004;150(1):123-30.
16. Clark JF. Creatine and phosphocreatine a review of their use in exercise and sport. *Journal of athletic training*. 1997;32(1):45.
17. Tarnopolsky M, Martin J. Creatine monohydrate increases strength in patients with neuromuscular disease. *Neurology*. 1999;52(4):854.
18. Brink, W. D. (1998). What's in your creatine? Available at: <<http://www.brinkzone.com/articledetails.php?acatid=3&aid=89><.
19. Brink, W. D. (1999). What's Really in Your Supplements? An Update on Creatine. *Mesomorphosis* [online], Vol. 2, No. 13. Available at:

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<<http://www.mesomorphosis.com/articles/bri nk/creatine-impurities.htm><

20. Krejčová A, Kahoun D, Černohorský T, Pouzar M. Determination of macro and trace element in multivitamins preparations by inductively coupled plasma optical emission spectrometry with slurry sample introduction. *Food chemistry*. 2006;98(1):171-8.

21. EFSA (2004). Opinion of the scientific panel on food additives, flavouring, processing aids and materials in contact with food on a request from the commission related to creatine monohydrate for use in foods of particular nutritional uses. *The EFSA Journal* 36, 1–6. Adopted on 17 February 2004. Available at: <http://www.efsa.europa.eu/en/scdocs/doc/opinion_afc_09_en1,3.pdf<

22. Moret S, Prevarin A, Tubaro F. Levels of creatine, organic contaminants and heavy metals in creatine dietary supplements. *Food chemistry*. 2011;126(3):1232-8.

23. Hight SC, Anderson DL, Cunningham WC, Capar SG, Lamont WH, Sinex SA. Analysis of dietary supplements for nutritional, toxic, and other elements. *Journal of Food Composition and Analysis*. 1993;6(2):121-39.

24. Davodi m., eivasszadeh o., soveisi m.r., karimi k. Measurement and evaluation of heavy metals in canned tuna collected from various sources in 1390. *Journal of food technology and nutrition* 2014 , 11 (2) ; 31 - 36.

25. Sołtyk K, Łozak A, Ostapczuk P, Fijałek Z. Determination of chromium and selected elements in multimineral and multivitamin preparations and in pharmaceutical raw material. *Journal of pharmaceutical and biomedical analysis*. 2003;32(3):425-32.

26. Kasraie S, Assary M, Abdosamadi H, Mani Kashani K, Arabzadeh S. Evaluation of the effect of 16% carbamide peroxide gel (Nite White) on mercury release from Iranian and foreign spherical and admixed amalgams by cold vapor atomic absorption method. *Journal of Dental Medicine. [Research]*. 2007;20(4):268-75

27. Dolan SP, Capar SG. Multi-element analysis of food by microwave digestion and inductively coupled plasma-atomic emission spectrometry. *Journal of Food Composition and Analysis*. 2002;15(5):593-615.