Original Article

Effect of taking dietary supplement on hematological and biochemical parameters in male bodybuilders an equation model

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Abstract

Background: The improved physical action following administration of supplements to bodybuilders was supported by changes in laboratory parameters. Despite the fact that these supplements are sometimes associated both advantage and side effects, this study were conducted for the purpose of evaluating the possible effects of some commonly used supplements in bodybuilders on the hematological and biochemical parameters.

Materials and Methods: In this study, we included 40 male bodybuilders as cases and 40 controls in the age group of 20-40 years. They used different kinds of supplements for 1 year. In general, all the supplements used were classified into two groups: hormonal and non-hormonal. Laboratory tests were requested for evaluation of hematological and biochemical parameters.

Results: In an equation model, we found that weight ($P = 0.024$), duration of bodybuilding ($P < 0.001$), and duration of hormone supplement consumption ($P < 0.001$) were loaded significantly on the latent variables, demographic and dietary supplement, respectively. The relationship between dietary supplement and biochemical and hematological parameters was significant ($P = 0.01$) and some of these parameters including creatinine ($P = 0.023$), blood aspartate aminotransferase (AST) ($P < 0.001$), alanine aminotransferase (ALT) ($P < 0.001$), and red blood cell distribution (RDW) ($P = 0.046$) had a significant role than others. In a multivariate regression model, we found that WBC ($P < 0.001$), platelets ($P < 0.001$), blood urea nitrogen (BUN; $P < 0.001$), creatinine ($P < 0.001$), AST ($P = 0.005$), and ALT ($P = 0.001$) were higher in athletes than in controls.

Conclusions: It is strongly advised that there should be some concerns about possible supplement-induced changes in the laboratory exams for bodybuilders. The available supplements are unchecked and not approved by the US Food and Drug Administration (FDA). More studies should be designed for a better and precise administration of each supplement in athletes.

Key words: Bodybuilder, hematological and biochemical parameters, supplement

Introduction

There is a lot of interest in athletes due to self-prescription of the over-the-counter (OTC) medications.[1] Dietary supplements are the most important OTC drugs which are mainly effective on the performance and physical ability of the bodybuilders, but it should be a matter of concern to the athletes' health.[2,3]

They are categorized according to their principal contents into four different classes on the market (creatine, prohormones, mental enhancers, and branched chain amino acids).

Every product improves performance through different mechanisms;[4] for example, amino acids induce secretion of insulin and growth hormone in brain that help mainly in better achievement during sport, as well as decrease central

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fatigue by removal of ammonia from the urea cycle.[5] Also, creatine is a popular supplement for elevating energy during short, high-intensity exercises.[6]

Most of the athletes are using more than one product and widespread usage of anabolic androgenic steroids (AAS) by athletes at all levels has been reported.[7] AAS are used as ergogenic aids by athletes and non-athletes to enhance their muscular function and strength in sport.

The improved physical action following administration of this combination was supported by changes in laboratory parameters.[1,8] Modification in hematological parameters, for example, in RBC count, helps in faster and effective replenishment of glycogen storage in the body for better achievement during sport. On the other hand, some harmful additives are included in dietary supplements, which are not mentioned on the labels of their packages. Despite the fact that these supplements are sometimes associated both advantages and side effects, they are not generally under serious examination for their efficacy and safety.

Despite the availability of wide information about supplement utilization from different parts of the world, limited data is available from Iran. Supplement consumption was 45% among student athletes in Isfahan University of Medical Sciences.[9] This high consumption induces serious health consequences.[10] Several studies have also indicated some adverse effects of dietary supplements usage, including cardiovascular, hematological, metabolic, and neurological problems,[11,12] whereas there is little scientific data that confirms the advantageous effects of nutritional supplements in athletes.[13] Therefore, the amount of dietary supplement consumption should be restricted within the recommended dose.[14]

Among athletes, bodybuilders are more predisposed to use of these supplements as compared to other sportsmen. Nowadays, this idea has been raised in Iranian youth and adolescent bodybuilders. On the other hand, as the usage of supplements is more accepted by bodybuilders, warning them about the effects of supplement consumption must be taken seriously. Based on biochemical and physiological action of dietary supplements, this study was conducted for the purpose of evaluating the possible effects of some commonly used supplements by bodybuilders on their hematological and biochemical parameters and then comparison was made with a control group.

Materials and Methods

In this study, 40 cases who were male bodybuilders with no past medical history and 40 controls in the age group 20–40 years were enrolled. Their historywas examined including blood pressure (BP) and body mass index (BMI) and hematological and biochemical parameters following supplement administration were evaluated. Athletes had used different kinds of supplements for 1 year.

In general, all the substances used were classified into two groups: hormonal and non hormonal supplements. The latter group was classified into protein, amino acid, creatine, carbohydrate, and super pump [Table 1]. The athletes had used at least three of these supplements for 1 year. Hormonal components included human growth hormone, human chorionic gonadotropin, testosterone, or one of the derivatives. Thirty male bodybuilders had used these products at least for 6 months. All of the athletes were placed in one of the below mentioned groups based on the kind of supplements consumed:

Group A: protein and amino acid, creatine,
Group B: carbohydrate, SuperPump + A,
Group C: hormone + A,
Group D: hormone + B.

The following laboratory tests were requested for evaluation of hematological and biochemical parameters: complete blood count (CBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution (RDW), blood aspartate aminotransferase (AST; in IU/l), alanine aminotransferase (ALT; in IU/l), alkaline phosphatase (ALP; in IU/l), blood urea nitrogen (BUN; mg/dl), and creatinine (mg/dl). Serum biochemical parameters were measured on Hitachi 902 automated analyzer using commercial kits. Hematological parameters were determined by Sismex K1000 analyzer.

Statistical analysis

To describe data, mean (SD) and/or percent of the characteristics were used. For analytical results, structural equation modeling (SEM) was used to display the relationship between variables that were used in this study. SEM is mainly a confirmatory technique and is more likely to be used to determine whether a certain model is valid. By convention, when graphically representing the model, the observed variables are enclosed by rectangles or squares and the latent variables are enclosed by ovals or circles.[7,15]

We fit structural equation models to determine the most likely pathway explaining the mediating role of dietary supplements on the demographic characteristics and hematological and biochemical parameters.

Global goodness of SEM fit indices included the Chi-square statistic for evaluating overall model fit. In addition, other goodness of fit indices were also examined and included

Table 1: Ingredients of SuperPump 250

Refreshing orange		
Supplement facts		
Serving size 1 scoop (20 g)		
Servings per container 14		
Amount per serving	**% daily value**[†]	
Calories	25	-
Calories from fat	0	-
Total fat	0 g	0%
Saturated fat	0 g	0%
Trans fat	0 g	0%
Cholesterol	0 g	0%
Total carbohydrates	6 g	2%
Sugars	0 g	*
Protein	0 g	0%
Vitamin B6 (as pyridoxine HCl)	25 mg	1250%
Folate (as folic acid)	420 mcg	105%
Vitamin B12 (as cyanocobalamin)	140 mcg	2333%
Biotin	500 mg	167%
Phosphorus (as sodium phosphate, potassium phosphate, magnesium phosphate)	150 mg	15%
Magnesium (phosphate and oxide)	100 mg	25%
Sodium	135 mg	6%
Potassium	90 mg	3%
SuperPump 250™ proprietary blend	14,409 mg	*
(Gaspari Nutrition Novel Compound #250 (patent pending))		
Anabolic Signaling Complex™ (patent pending):		
Glucose polymer blend, creatine monohydrate, NO2 complex [l-arginine, l-arginine α-ketoglutarate, l-arginine ketoisocaproate], Guanipro™ (guanidino propionic acid), salicyclic acid 15%, Panax ginseng extract		
Lipolytic/Xtreme Focus Agent™ (patent pending)		
l-Tyrosine, methylxanthines (caffeine), *N*-acetyl cysteine, *N*-acetyl tyrosine, glucuronolactone, *Rhodiola rosea* root extract (standardized to 5% total rosavins), *Ginko biloba* extract		
Myogenic Transcription Factor/Agonist™ (patent pending)		
Taurine, l-leucine, l-glutamine, l-valine, l-isoleucine, l-citruline AKG, turkesterone (11,20- dihydroxyecdysone from *Ajuga turkestanica* extract), choline bitartrate		
Insulin Secretagogue Complex™ (patent pending)		
Indole-3-carbinol, 4-hydroxyisoleucine (from fenugreek seed extract), cinnamon bark extract, *Bacopa monnieri*		
Intra SORB™ Rapid Absorption - Myo-Hydration Matrix		
Potassium glycerophosphate, magnesium glycerophosphate, α-lipoic acid, Bioperine™		
[†]Percent daily values are based on a 2000 calorie diet		
*Daily values not established		
Other ingredients		
Citric acid, malic acid, natural and artificial flavor, lecithin (soybean), xanthan gum, acesulfame potassium, sucralose, Blue #2		

HCl: Hydrochloride, α-AKG: α-ketoglutarate, A-KIC: Arginine ketoisocaproate, NAC: N-acetyl cysteine

root mean square error of approximation (RMSEA), with values equal to or less than 0.05 for this index indicating good fit of the model, and also comparative fit indices such as incremental fit index (IFI) and comparative fit index (CFI), which range from 0 to 1. Values equal to or greater than 0.9 indicate good fit to the model.[16]

We started the analysis of the SEM model with three unobserved latent variables, namely demographic (age, weight, smoking, duration of bodybuilding), dietary supplement (supplement, duration of hormone supplement, and duration of non-hormone supplement), and hematological and biochemical parameters (BUN, creatinine, ALT, AST, ALP, MCV, RDW). We commenced the analysis with the SEM default model [Figure 1].

Analytical statistics of Chi-square test was used to determine the association between categorical factors; *t*-test was applied for finding mean difference between the groups of athletes and controls, and multivariate (WBC, RBC, MCH, MCHC, platelets, RDW, BUN, creatinine, AST, ALT, and ALP) regression model controlling for age, BMI, BP, and supplement use was performed using SPSS software for Windows, version 18.

Ethical considerations

After our research project was approved by ethic community, all of the participant that entered to our study filled consent form.

Results

The mean age (SD) of the participants was 25 (4.2) years, ranging from 20 to 40 years. Only 15% of them smoked one or two cigarettes per day [Table 2].

For the default model, the estimated RMSEA was 0.044. Furthermore, the comparative fit indices were as follows: 0.903 for IFI and 0.850 for CFI. All these indices represent good fit to the data.

The results showed that creatinine (P = 0.023), AST (P < 0.001), ALT (P < 0.001), and RDW (P = 0.046) were loaded satisfactorily on the latent variable of hematological and biochemical parameters. This means that in our sample, these aforementioned variables have reasonably explained our latent variables.

Table 2: Main characteristics of the participants

Characteristics	*n* (%)	Mean (SD)
Age (years)	40	25 (4.2)
BMI (kg/m^2)	40	26.9 (3.8)
Smoking (per day)	40	1.8 (0.3)
Yes	6 (15)	-
No	34 (85)	-
Duration of bodybuilding (months)	40	72.3 (44.6)
Duration of hormone supplement consumption (months)	40	4.4 (2.6)
0	10 (25)	-
3	1 (2.5)	-
6	29 (72.5)	-
Supplement	40	4.1 (1.4)
Group A	4 (10)	-
Group B	6 (15)	-
Group C	10 (25)	-
Group D	20 (50)	-
Duration of non-hormone supplement consumption (months)	40	5.8 (0.6)
3	1 (2.5)	-
4	1 (2.5)	-
6	38 (95)	-
BUN (mg/dl)	40	18.9 (3.8)
Creatinine (mg/dl)	40	1.3 (0.2)
AST* (IU/l)	39	37 (2.2)
ALT* (IU/l)	39	45.7 (24.1)
Alkaline phosphatase (IU/l)	40	208.1 (55.6)
MCV (fl)	40	83.2 (4)
RDW (%)	40	13.6 (1.3)

*The total number is different from strata because of the missing values. BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, MCV: Mean corpuscular volume, RDW: Red blood cell distribution, BMI: Body mass index, SD: Standard deviation

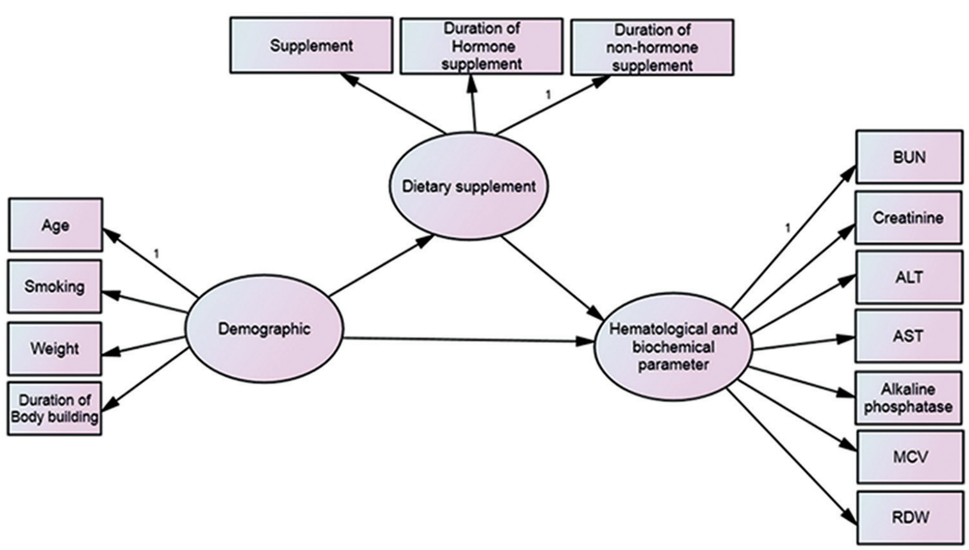

Figure 1: Structural equation modeling with three latent variables: demographic, dietary supplement, and hematological and biochemical parameters

The relationship between dietary supplement and biochemical and hematological parameters was significant ($P = 0.01$). It could be inferred that dietary supplement had a significant impact on hematological and biochemical parameters. The other relationships between latent variables were found to be statistically significant. In addition, we found that weight ($P = 0.024$), duration of bodybuilding ($P < 0.001$), and duration of hormone supplement consumption ($P < 0.001$) were loaded significantly on the latent variables, demographic and dietary supplement, respectively. The path coefficients for this model are represented in Table 3.

When the athletes group was compared with control group, BMI and BP were higher in cases than in controls (*P* values 0.045 and < 0.001, respectively). Based on fitting multivariate linear regression model, we found statistically significant relationship between the group of participants (athletes and controls) and some clinical characteristics such as WBC ($P < 0.001$), platelets ($P < 0.001$), BUN ($P < 0.001$), creatinine ($P < 0.001$), AST ($P = 0.005$), and ALT ($P = 0.001$). However, there was no significant mean difference between the two groups with regard to other variables. The model was adjusted for age, BMI, BP, and supplement usage. Further details are presented in Table 4.

Discussion

Our results showed that supplementation had a positive effect on hematological and biochemical parameters in athletes and some of these parameters that included creatinine, AST, ALT, and RDW had a prominent role than others. Furthermore, we found significant differences between athletes and controls in variables such as BMI and BP, as well as in hematological and laboratory parameters including WBC, platelets, BUN, Creatinine, AST, and ALT which were higher in cases than in controls.

Studies published during the last 40 years have shown biochemical and hematological changes in endurance athletes.[17] In some studies, elevated erythrocyte count has been found during sport and it was explained that it should be the natural consequence of hemoconcentration mechanisms.[8,18,19] In contrast, some reports mention that erythrocyte counts decrease, which is referred to as “sports anemia,”[20,21] or remain unchanged[22,23] after exercise.

On the other hand, dietary supplements such as prohormones, creatinine, and amino acids are used by athletes to gain more physical abilities.[1,24-27] However, adding these components to exercise could act as an

Table 3: Regression coefficients for the structural equation modeling

	Unstandardized estimate	Standard error	Standardized estimate	*P* value
Demographic				
Age	1.000	-	0.239	-
Weight	3.143	1.395	0.210	0.024
Smoking	0.037	0.025	0.104	0.134
Duration of bodybuilding	95.371	27.037	2.159	<0.001
Dietary supplement				
Supplement	1.000	-	0.658	-
Duration of hormone supplement	2.786	0.521	1.024	<0.001
Duration of non-hormone supplement	0.039	0.086	0.070	0.653
Hematological and biochemical parameters				
BUN	1.000	-	0.285	-
Creatinine	0.079	0.035	0.480	0.023
AST	7.245	1.856	0.701	<0.001
ALT	14.880	3.703	0.728	<0.001
Alkaline phosphatase	−15.204	8.401	−0.328	0.070
MCV	−0.108	0.608	−0.033	0.858
RDW	0.396	0.199	0.360	0.046
Demographic				
Dietary supplement	0.031	0.058	0.031	0.595
Hematolical and biochemical parameters	−0.064		−0.054	0.395
Dietary supplement				
Hematological and biochemical parameters	0.644	0.248	0.541	0.010

BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, MCV: Mean corpuscular volume, RDW: Red blood cell distribution

Table 4: Summarizing the results of descriptive and analytical statistics differences in the athletes group (n=40) and in controls (n=40)

	Type	Mean (SD)	$F^*_{(1,77)}$	*P* value	Effect size
WBC	Athletes	9080 (2263.9)	24.7	<0.001	0.243
	Control	6572.5 (1610.1)			
	Total	7826.3 (2324.2)			
RBC	Athletes	5.6 (0.4)	0.050	0.823	0.001
	Control	5.5 (0.3)			
	Total	5.5 (0.3)			
MCH	Athletes	27.5 (1.4)	0.008	0.928	0.0001
	Control	27.5 (1.2)			
	Total	27.5 (1.3)			
MCHC	Athletes	33.1 (0.9)	0.052	0.820	0.001
	Control	33.2 (0.64)			
	Total	33.1 (0.79)			
Platelets	Athletes	322,975 (76,076)	48.1	<0.001	0.385
	Control	210,625 (53,907)			
	Total	266,800 (86,529)			
RDW	Athletes	13.6 (1.3)	3.60	0.063	0.044
	Control	13.2 (0.65)			
	Total	13.4 (1.1)			
BUN	Athletes	18.9 (3.9)	38.2	<0.001	0.331
	Control	14.5 (3.2)			
	Total	16.7 (4.2)			
Creatinine	Athletes	1.3 (0.2)	41.2	<0.001	0.349
	Control	1.1 (0.1)			
	Total	1.2 (0.2)			
AST	Athletes	38.4 (13.9)	8.40	0.005	0.099
	Control	30.8 (8.1)			
	Total	34.6 (11.9)			
ALT	Athletes	47.6 (26.5)	11.7	0.001	0.132
	Control	31.1 (12.2)			
	Total	39.4 (22.1)			
ALP	Athletes	208.2 (55.7)	0.637	0.427	0.008
	Control	196.2 (45.4)			
	Total	202.2 (50.8)			

*F value using multivariate regression model controlling for age, BMI: Blood pressure, and supplement use, BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, RDW: Red blood cell distribution, WBC: White blood cell, RBC: Red blood cell, MCHC: Mean corpuscular hemoglobin concentration, SD: Standard deviation

additive factor that has an influence on hematological and biochemical parameters.

Creatine is an organic compound which is synthesized in kidneys and liver. Protein-rich foods such as meat are the main natural sources of this organic compound.[1,28] It has been considered as a component of athletes' nutritional regimen that helps in improved performance in short, high-intensity exercises.[6] These products have also been reported to increase blood creatinine,[1,29,30] which is in agreement with our results and in comparison with another marker in kidney (BUN) has been more influenced under supplement consumption. However, a large number of studies have indicated that consumption of this product is safe and has no long-term detrimental effects on kidney or liver functions.[31,32]

Another usual product consumed is amino acid that leads to larger aerobic capacity by causing changes in hematological parameters.[4,5] Testosterone and its derivatives are primarily used for myotrophic action by bodybuilders[7] and following consumption, elevation in the levels of liver enzymes (AST, ALT, ALP) has been described.[1] In our experiment, the most significant parameter influenced by supplements was liver markers. In addition, following consumption of AAS, elevation in serum creatinine, BUN, and uric acid has been also reported.[33] But most of these values often go back to normal range once the drugs are ceased.[34,35] AAS cause acute renal damage following rhabdomyolysis in bodybuilders.[36] Consumption of this compound is associated with elevated RBC, hematocrit,[33] and the tendency of thrombocytes to aggregate, which are associated with increased cardiovascular risk and total mortality.[37] There are several case reports of thrombosis in young strength sportsmen[38,39] and this may be a point of major concern. We observed increase in platelet count; therefore, administration of these products needs more exploration. In addition, administration of 200 mg testosterone per week was found to cause a slight increase in WBCs,[40] similar to the results of our study. But most of these parameters often revert to normal range once the drugs are stopped.

The combination of AAS and creatine supplement that has been currently abused by bodybuilders may cause renal damage, as we observed a significant role of creatine influencing the laboratory parameters.

It is an undeniable fact that most athletes in our design used more than one supplement at one single time, so we could not come to a clear conclusion about one single product influencing the laboratory parameters.

Although we examined the influence of dietary supplements on hematological and biochemical parameters, we observed that some of these parameters (RDW, creatinine, ALT, AST) showed significant changes compared to others in laboratory values. Elevation in the liver enzymes AST and ALT is an indicator of hepatocellular injury.[41] Anabolics are one of the most important products that are administered in bodybuilding and are metabolized by the liver; for this reason, they tend to be more hepatotoxic than other supplements.[41] But for arriving at better conclusion related to hepatocellular dysfunction, more precise examination needs to be performed.

Elevated RDW indicates a greater difference in size among RBCs, and is simply an epiphenomenon of underlying abnormal ferritin levels and/or anemia. More recently, an association between RDW and plasma inflammatory markers has been reported.[15] In recent studies, it has been reported that increased risk of mortality and morbidity is associated with elevated RDW both in patients with heart disease and in the general population, and that this biomarker could predict cardiovascular accident.[42] On the other hand, metabolic syndrome in the liver could increase the levels of both AST and ALT, and RDW.[43] In our study, we did not separate that progression level in RDW was dependent on increase in the level of liver enzymes or independent of this correlation. However, increased RDW acts as a risk in bodybuilding athletes. Some existing scientific data, which consist of case reports and clinical observations, describe serious cardiovascular adverse effects following usage of performance-enhancing substances, including sudden death, cardiac arrhythmia, BP increase, and others.[44] However, published evidence on the usage of popular supplements among athletes is scant, inconclusive, or conflicting. In another study by Ziegenfuss *et al*., it has been reported that supplement consumption including a product containing primarily β-alanine, arginine, or creatine enhances performance without negatively affecting systemic hemodynamics.[45]

Conclusion

This study highlights potential effects of diverse unchecked supplements, which may influence some laboratory parameters. It is strongly advised that there should be some concerns about possible supplement-induced liver injury in all bodybuilders or other athletes. Available supplements are unchecked and not approved by the FDA. More studies should be designed for a better and precise consumption of each supplement in athletes.

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