*Original Research Article*

# Retrospective study on health impact of dietary protein, vitamin and electrolyte supplementation: a pilot study




## ABSTRACT

**Aims:** Daily use of commercially available protein, multivitamin and electrolyte dietary supplements (MS) is a common practice among athletes. The aim of this study was to identify the effects of MS on athlete's health.

**Study design:** A retrospective cohort, pilot study

**Place and Duration of Study:** Study was carried out at the Sri Lanka Anti-Doping Agency, Lanka Hospital Sri Lanka between June 2019 and February 2020.

**Methodology:** This Study was conducted with 45 healthy athletes who were using MS (n=25) and not using MS (n=20). In this study, MS users were consuming protein, multivitamin and electrolyte dietary supplements daily for more than 2 months continuously. Health effects of the study subjects were evaluated by biochemical tests and physical examination by a physician.

**Results:** Negative effect was observed in the Lipid profile and weight of the MS users. MS users had shown higher relative risk (RR) to have increased total cholesterol (RR=8.7; 95%CI: x-y) and LDL levels (RR=2.9; 95%CI: x-y) compared to non-MS users. This risk is more prominent among MS ~~using~~ users males than females. MS using males had a higher RR to increase their weight compared to non-MS using males. RR to increase weight had varied with type of sport and period on MS.

**Conclusion:** Consuming MS more than 2 months, has an increasing effect on the lipid profile and weight of athletes and it is more pronounced in males. The clinical implication of this finding needs further study.


**Comment [Rev1]:** This information should be also in the Methodology section, study description.

**Comment [Rev2]:** I believe this sentence is more suitable in the conclusion

**Comment [Rev3]:** In my opinion would be more clear if the authors write the main results as RR and 95% CI in the abstract

**Comment [Rev4]:** These two sentences are redundant... they mean exacly the same and none has a value that we can comprove the sentence. I suggest something like... «The RR of overweight was 2.4 higher for MS users males than females.»

*Keywords: dietary supplements, sports, lipid, whey protein, vitamin, health effects*

**Comment [Rev5]:** I don't undersatan why the authors choose these words. The purpose of the study is to investigate dietary supplements in general, not some specific ingredients. I recommend to delete them.

## 1. INTRODUCTION

Consumption of more than one supplement per day is found to be a common practice among athletes around the world [1-5] and similar pattern is observed in Sri Lanka as well. It was demonstrated by the studies which were done in 2006 and 2018. A study in 2006, showed that some of the Sri Lankan national level athletes have consumed four supplements/day (29%) and six supplements/day (10%) [6]. Following the same pattern, a study in 2018 has shown that 56.8% of national level athletes in Sri Lanka were consuming three supplements/day [7]. In addition to that, those have discovered MS is one of the prevalent supplement consumption patterns among those athletes [7]. Furth some athletes are on MS without medical advice in order to enhance their performance in sports. Therefore, safet is at a question.

**Comment [Rev6]:** Althoug it is in the abstract, in may opinion, the abbreviation should be in parenthesis after the first time it appears in the sentence, in the main text of the manuscript.

Human liver and kidney play major roles in metabolism and excretion of dietary ingredients in dietary supple Therefore, scientists have been conducting studies to discover the effects of dietary supplements on liver and functions over the past decades. Animal studies also showed that use of whey protein in a uniform manner

**Comment [Rev7]:** This sentence lacks references. Instead, the next sentence should be placed after the description of human studies.

exercise, cause adverse effects on the liver [8]. A recent study has discovered that 20% of hepatotoxicity in United States was induced by herbal or dietary supplements and it identified multi-ingredient nutritional supplements as one of the major implicative agents [9]. Furthermore, dietary supplements which used to improve physical performance, endurance and appearance were thought to induced liver injury [10]. A case report has described the development of cholestasis in humans attributed to dietary supplements [11]. Recent reviews have shown that there is a potential to promote renal diseases by increasing intake of dietary protein [12] and dietary supplements [13]. Drug induced liver injury network has studied that dietary supplement increases liver injuries [9]. However, prior researches had little evidence about the influences of dietary supplements on athlete's health. Especially, there is no evidence available in the literature about how MS effects on athlete's health. Therefore, this retrospective study was conducted to find out the effect of MS on athlete's health and its variation with gender, MS usage period and type of sport played.

## 2. METHODOLOGY

### 2.1 Study Design

This is a retrospective cohort, pilot study. Clinical variables of athletes in both experimental and control groups were compared in this study to identify the risk for developing adverse health effects by MS. Hepatic, renal and hematological profiles, anthropometry and general health conditions were assessed to evaluate the health status of each study subject. In addition to that, their dietary intake was also recorded to determine their nutritional adequacy.

### 2.2 Study Group

Total of 45 healthy athletes playing wushu, kabaddi or rugby participated in this study. They were categorized in to two groups as experimental group (MS users, n=25) and control group (non-supplement users, n = 20). All MS using athletes had consumed a combination of commercially available protein, multivitamin and electrolyte dietary supplements daily for two to six months.

**Comment [Rev8]:** Is there a explanation for authors to choose these type of sports? Are they the most popular sports in Sri Lanka?

The study subjects were recruited to this study by a screening. Initially volunteers were invited from sports teams and sports clubs for a screening. All the volunteers were informed about the study protocol by explaining both verbally and in writing, in their native language and written consents were obtained from them. They were provided with an interview administered questionnaire to retrieve their demographic characteristics, sports history, medical history, dietary and supplementation habits. Study subjects were selected for this study based on the data in the questionnaire. Participants aged between 20-30 years, who were engaged in physical training for at least 1 hour per day for more than two years, healthy according to their medical history were recruited for this study. Following participants were excluded from this study; those who have liver disease, renal disease or nutritional deficiency, contraceptive, testosterone, western or traditional medicine consumers within past three months, pregnant or lactating mothers, alcoholics, those who were following strict diets to lose or gain weight. Out of 108 athletes participated for the screening, 45 athletes were elected for the study. According to their usage of MS habit they were assigned in to two groups as experimental group and control group. Ethic approval for this study was taken from the Ethic Review committee of University of Kelaniya in Sri Lanka ( FWA00013225).

### 2.3 Biochemical and Physical examination

Venous blood was collected from each subject between 7 and 8 a.m. after an overnight fast for biochemical and hematological tests. Urine sample was also collected from them for urine full report and urine protein/creatinine ratio test. The tested variables are given in table 1. These tests were conducted at a medical laboratory with ISO 15189:2012 scope of accreditation, following the standard testing methods.

**Table 1. Biochemical and hematological variables measured from blood and urine analysis.**

| Name of the test | Measured health variables |
|---|---|
| Liver profile | Total bilirubin, bilirubin direct, bilirubin indirect, total protein, albumin, globulin, albumin/globulin ratio, AST, ALT, alkaline phosphatase, gamma-glutamyl transferase |
| Kidney profile | Blood urea and urea nitrogen, serum creatinine, serum uric acid, serum calcium, serum phosphorus, serum sodium, serum potassium, |

| | |
|---|---|
| | serum chloride |
| Lipid profile | Total cholesterol, HDL, LDL, VLDL, triglycerides, total cholesterol/HDL ratio |
| Fasting glucose | Serum glucose |
| Total testosterone | Serum testosterone |
| Full blood count | Neutrophil, eosinophil, basophil, monocytes, lymphocytes, RBC, hemoglobin |
| Urine full report | Urinary protein, glucose, ketones, bile salts, bilirubin, nitrite, urobilinogen, pus cells, red blood cells, epithelial cells, casts, crystals |
| Urinary protein /creatinine ratio | Urinary protein, urinary creatine, protein/ creatinine ratio |

*AST, aspartame aminotransferase; ALT, alanine aminotransferase; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; RBC, red blood cell.

Anthropometric variables including height, weight, neck circumference, waist circumference, hip circumference of the study subjects was measured at the hospital by a trained medical staff. Their body mass index was also calculated. General physical examination of body temperature, blood pressure (systolic / diastolic), pulse rate, ~~Jaundice~~jaundice, liver, anemia, spleen was conducted by a physician.

### 2.4 Assessment of dietary intake

Food frequency questionnaire was filled from all the study subjects recalling their three days dietary history through an interview. Data about varieties of food taken and their quantities and frequencies taken were collected through this food frequency questionnaire. The portion sizes of different food items used by study subjects were measured following the standard measuring cups, spoons and exemplary portions defined in the nutritive value of food data base published by agriculture department of United States [14]. Nutrient content and energy intake of the study subjects were estimated using the data collected from food frequency questionnaire following the same data base [14].

**Comment [Rev9]:** Was that FFQ validated for the population of Sri Lanka or Asia?

### 2.5 Statistical analysis

Collected data were fed in to a database created using statistical software of IBM©SPSS© Statistics, version 22 (IBM Corporation, New York). Pearson's chi squired test was used to compare the difference in occurrence of adverse health events between the experimental group and control group. The significance of the statistical difference between the experimental group and control group was evaluated compared to the 95% confidence level (P = 0.05). RR for developing harmful health events in experimental group were calculated compared to the control group.

**Comment [Rev10]:** It is the fist time this abbreviation appears in the text (even if it is in the abstract). Please indicate what it means.

## 3. RESULTS AND DISCUSSION

### 3.1 Population

Selected sociodemographic and sports data of the study participants are presented in table 2.

**Table 2. Selected sociodemographic and sports data of the study participants**

| Characteristic | Total | MS users | Non-supplement users |
|---|---|---|---|
| Age range (years) | 22– 30 | 24–30 | 22–30 |

| | | | |
|---|---|---|---|
| Gender (n*) | | | |
| Male | 23 | 12 | 11 |
| Female | 22 | 13 | 9 |
| Sport type (n) | | | |
| Wushu | 14 | 8 | 6 |
| Kabaddi | 16 | 9 | 7 |
| Rugby | 15 | 8 | 7 |
| Training (hours/day) | 3.4±1.4 | 3.9±1.4 | 2.9±1.0 |

*n, number of athletes*

Anthropometric characteristics of the athletes participated for this study was shown in the table 3.

**Table 3. Anthropometric characteristics of the athletes participated for this study.**

| Anthropometric parameter | MS users | | Non-MS users | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| Height (cm) | 171.7±6.1 | 160.5±5.3 | 169.7±6.1 | 162.1±5.0 |
| Weight (Kg) | 74.1±11.8 | 56.5±9.1 | 70.3±12.1 | 60.1±10.6 |
| Body-mass index (Kg/m$^2$) | 24.9±3.0 | 22.0±3.1 | 24.1±3.5 | 23.0±4.1 |
| Neck circumference (cm) | 38.8±2.7 | 32.3±2.2 | 37.4±2.2 | 32.0±2.3 |
| Waist circumference (cm) | 85.3±7.3 | 83.5±8.9 | 86.3±9.4 | 79.6±11.3 |
| Hip circumference (cm) | 93.1±6.7 | 88.5±9.7 | 91.4±10.6 | 91.1±13.3 |

Approximately 77.8%, (n=35) of the study participants were not consuming alcohol while 20.0%, (n=9) were consuming alcohol monthly and 2.2%, (n=1) consuming alcohol weekly.

### 3.2 Dietary intake

Daily nutrients consumption of all study participants through their food only were 442.8±58.7 g of carbohydrates, 137.4±38.4 g of proteins and 84± 23 g of fat. Daily caloric intake of the participants through food only was 3129 ± 700 Kcal and gender wise it was 2995±635 Kcal in female 3252± 764 Kcal in male. Daily protein intake of participants through the food was 2.2± 0.9 g/kg of their body weight. Daily carbohydrate intake of participants through the food was 7.3± 3.2 g/kg of their body weight.

### 3.3 Supplement usage

Supplement using athletes in the study had used combination of commercially available protein supplement, multivitamin product and oral rehydration fluid daily. Daily intake of dietary ingredients through different dietary supplements were given in table 4.

**Table 4. Daily dietary ingredients intake of the multiple supplements using athletes in the study.**

| Dietary supplement | Daily dietary ingredients intake of athletes |
|---|---|
| Protein supplement | 50 - 51 g of protein, 200 – 233 Kcal and 2.4 g of Fat or 10 mg of cholesterol with additives in the supplement |

**Comment [Rev11]:** It should be in the legend

**Comment [Rev12]:** It should be clarify in the legend what it is (standard deviation?)

**Comment [Rev13]:** If the authors performed the analysis by MS users/ non users I recommend to include these results in the Table 2. It would be better to aggregate all results in the tables used, and including in the written text the most important results and on which will focus on the discussion.

**Comment [Rev14]:** Do the authors refer to the nutrient intake from dietary supplements?It is not clear ...

**Comment [Rev15]:** Are these values the minimum and maximum of nutrients daily intake from supplements?

| | |
|---|---|
| Oral rehydration fluid | 100 ml of fluid contained sodium chloride (2.6 g), sodium citrate (2.9 g), potassium chloride (1.5 g), anhydrous glucose (1.4 g). Athletes consumed 200 ml daily. |
| Multivitamin | Vitamin A (750 µg), vitamin C (60 mg), vitamin D (10 µg), vitamin E (10 -30 mg), calcium (600 mg), iron (12 g), zinc(15 mg), copper (1 -2 mg), magnesium (60 -30 mg), manganese (2.5 -3 mg), selenium (50 -150 µg), chromium (50 -200 µg), vitamin B complex (thiamine, riboflavin, nicotinamide, pantothenic acid, pyridoxine, cyanocobalamin, biotin, folic acid). |

The study subjects were influenced to use dietary supplement by their coaches, sports doctors or by themselves. None of them had taken advise from a specialized dietician. The source of influence to use MS was given in the figure 1.

**Comment [Rev16]:** These results appears without being refered in the Methodologic section. Please include this variable in the Method section.

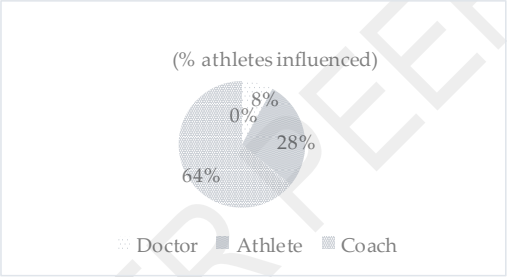


**Figure 1. Source of influence for the MS use**

**Comment [Rev17]:** The figure shows 4 frequencies (0%, 8%, 28% and 64%) but legend only has 3 sources of influence. Please clarify.

### 3.4 Lipid profile

RR in increasing total cholesterol between 200-240 mg/dL was 8.7 MS in users compared to non-MS users. RR in increasing LDL levels between 160-171 mg/dL was 2.9 MS users compared to non-MS users. Increase in total cholesterol and LDL levels was significantly higher among MS users than non-MS users (P<0.05). Male athletes showed high RR for elevate their total cholesterol and LDL levels than female athletes. Supplement usage period was shown to have an effect on lipid profile of athletes. RR of increasing both total cholesterol and LDL levels were elevated when athletes used MS for 3 – 6 months than 2–3 months. RR of increasing both total cholesterol and LDL levels were highest among kabaddi players compared to wushu and rugby. There was no remarkable effect of MS use on triglyceride, and HDL and VLDL levels.

**Comment [Rev18]:** I believe the authors mean «for the MS-users».

**Comment [Rev19]:** It is confusing, because the authors present the same results in Table 5, Discussion section. I believe that would be more understandable if all the results appear in the same table, and then the authors emphasize the main findings.

### 3.5 Build

Male MS users showed 2.4 RR for being overweight compared to non-MS using males. They showed significantly high overweight than female MS users (P< 0.05). Compared to male wushu players, male kabaddi players had shown significantly high overweight (P < 0.05; RR, 4.9). Increased RR was observed in building overweight with the longer MS

usage. RR was for building overweight was 1.8 among 2–3 months MS users while it was 3.1among 3 – 6 months MS users.

According to the dietary nutrient assessment of study subjects, percentage caloric consumption of carbohydrate from food was 62±5.2 and percentage caloric consumption of fat from food was below 26% in overweight athletes.

### 3.6 Hematological profile

RR in increasing basophil in blood was 2.1 and eosinophil in blood was 3.2 were observed in MS users compared to non-MS users. Some MS using athletes (20%) had shown elevated lymphocyte level in blood along with increased total cholesterol and LDL levels. But there was no statistical difference was observed in the serum testosterone level or Blood glucose level between MS users and non-MS users.

### 3.7 Other health parameters

There was no statistical difference or significant RR that could be observed in the other health parameters studied in MS users compared to the non-MS users.

### 3.8 Discussion

According to this pilot study, MS had adversely affected the lipid profile and body weight of the athletes. In addition to those changes were observed in basophil and eosinophil counts of the MS users. No statistical difference was observed in other health parameters measured during this study. This study is not sufficient to conclude increasing effect on lipid profile and body weight were induced solely by MS. Therefore, future prospective large sale study should be conducted to confirm the observations of this pilot study. However, these findings suggest that athletes who consume MS should assess their lipid profile in every six months while on MS and consult a sports dietician before initiating MS.

The risk for increased total cholesterol and LDL levels in blood had changed with gender, duration on MS and type of sport (Table 5).

**Table 5. Variation of relative risk of increasing total cholesterol and LDL levels in MS users against MS usage period, gender and sport.**

| Factor affect to the MS-induced health effects | | RR for increasing Total Cholesterol | RR for increasing LDL level |
|---|---|---|---|
| MS usage period | 2-3 months | 7.0 | 2.7 |
| | 3-6 months | 12.6 | 3.3 |
| Gender | Female | 2.9 | 2.3 |
| | Male | 7.1 | 4.0 |
| Sport | Kabaddi | 13.4 | 13.4 |
| | Rugby | 5.0 | 5.0 |
| | Wushu | 5.8 | 5.8 |

Similarly, risk for increased weight in MS using athletes had varied with gender, period on MS and type of sport. Therefore, gender, duration on MS and type of sport played were confounding factors for MS-induced changes. However, this study is not sufficient to confirm those lipid profile and weight changes were induced by only MS. There might be other factors influenced to them such as carbohydrate and fat intake from food. Nutritional assessment data of this study

**Comment [Rev20]:** This information should be in the Results section.

**Comment [Rev21]:** It would be important to show the Confidence intervals

shows that the study subjects did not gain weight due to over consumption of carbohydrate and fat from food. Because, they had consumed carbohydrate and fat from food below the recommended daily allowance for athletes. Recommended daily carbohydrate and fat intake for an athlete is 55-65% of total calories from carbohydrates and 25% of total calories from fat [15]. Furthermore, kabaddi players showed higher risk for increased total cholesterol level, LDL level, and weight than wushu players. Although Wushu and Kabaddi are endurance sports, they differ in nature, duration of the game, number of participants, energy expenditure, intensity and frequency of the exercises performed during the training. Therefore, these dissimilarities might cause variation in MS-induced adverse effects in kabaddi and wushu. However, further research is necessary to confirm changes in lipid profile and weight has ~~arose~~arisen due to MS.

Some literature has shown that dietary supplements adversely affect liver [8,9,11] and kidney [12,13]. However, conversely to that some studies had shown the dietary supplements improve the function of the diseased liver [16] or kidney [17,18]. In agreement with the second finding, healthy MS users participated in this study had not shown significant change in their liver or kidney profiles. But those past studies and the present study has monitored healthy athletes consuming dietary supplements beyond 6 months. Athletes participated in this study had initiate MS 3-6 months prior to a competition and discontinued it after the competition following a void period. High price of the supplements and the financial limitations was the reason for this consumption pattern. This implies that the, short term use MS dose not seems to be harmful for the liver or kidney. However, a future study is necessary to assess its long-term effect.

This study has identified the negative effects induced by MS, but it is not sufficient to explain which supplement or dietary ingredient was responsible for them. Previous six-month randomized controlled study showed that consumption of a multivitamin, fish oil and some herbal supplements had no effect on human metabolic and cardio-vascular health [18]. Few reviews has pointed out that high protein intake promote liver and kidney diseases [9,12], especially from whey protein supplements [8,10,11]. According to that, whey protein supplement might be the main responsible agent for negative effects observed in this study. Furthermore, label of some commercially available whey protein and multivitamin supplements indicated that they contain additives such as concentrated herbal extracts. The effect of these additives was not well defined by scientific evidence. According to the product description given by the manufacturers, they were meant for improve the sports performance and gain energy. Therefore, it is challenging to identify the responsible constituent in MS for developing adverse effect.

This study has observed some weaknesses in the diet plan of Sri Lankan athletes. Sports nutritionists recommend daily allowance of 3800 Kcal/day for athletes engaged in intensive-exercise training [15]. Endurance athletes are recommended to consume 7-8 g/kg/day of carbohydrate per day [15]. According to the study 16 % athletes were in this recommended range while 24% of athletes were above and 60% of athletes were below the recommended range. Endurance athletes are recommended to consume 1.2- 1.4 g/kg/day of protein per day [15]. This study has observed athletes consume 2.2± 0.9 g/kg/day of protein per day through food only. ~~e~~Energy and nutrition assessment of these athletes showed that they need further education about the dietary nutrients and energy requirement. In the same time, they need assistance of the dietician to prepare a diet plan according to their weight of the body, duration of training per day, type of sports, gender etc.

According to this study 64% of athletes were influenced to consume supplements by their coaches (figure 1). Earlier research carried out in Sri Lanka also showed the influences of coaches to use supplements. It was 48% for national level athletes [6]. This shows the attitudes and knowledge of coaches towards the diet. Therefore, the education programs for coaches needs reformatting in order to cultivate the values of sports in the coaches and build confidence to depend on the diet for nutritional requirements. In the same time, it is necessary to create opportunity for athletes to meet dieticians to take guidance about their diet.

There are few draw backs in the retrospective research methodology and food frequency method followed in this study. Data collected in both methods were depended on responder's memory. In addition to that, retrospective research methodology has restricted the control of potential factors for adverse health effects. Therefore, it is recommended to carry out large scale prospective interventional study following diet diary method to collect dietary intake.

## 4. CONCLUSION

MS use is a health concern in terms of promoting unhealthy changes in the lipid profile of athletes and resulting in overweight in athletes as well. The supplement usage period, gender and sports discipline should be considered as~~are~~ some of the confounding factors ~~of~~ MS-induced health effects. It is advisable for MS using athletes to check their lipid profile ~~every 6 months~~regularly. ~~This retrospective pilot study has proven the necessity of a~~Further large-scale prospective intervention studies are needed ~~y~~ for further conclusions.

**Comment [Rev22]:** And what about alcohol? Did the authors adjusted statistical analysis for alcohol consumption? Because alcohol intake was refered in the results, it should be intesting include it in the discussion.

**Comment [Rev23]:** It should also be included the strengths of the study

**Comment [Rev24]:** Health status and even supplement use are correlated with socioeconomic, education and cigarrete smoking. These variables were not used as confunders in the present study. In my opinion this information should be included as a limitation of the study.

**Comment [Rev25]:** The authors must be cautious with this statement. The findings of the study show a association between MS use and unhealthy changes in the lipid profile and weight. However the authors are not able to say that MS consumption is resulting in overweight.

**Comment [Rev26]:** Check lipid profile every 6 months was not a conclusion of this paper.

## CONSENT

All authors declare that 'written informed consent was obtained from the athletes who participated in this study for taking their data for research purpose. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal

## ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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