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Copper, iron, zinc and magnesium status of physically active young Indian males

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I. Introduction

Many of the nutritionally important elements present in traces (copper, iron and zinc) or in large quantities (sodium, potassium, calcium, phosphorous and magnesium) participate in a plethora of biological processes in human body. Copper, iron, zinc and magnesium act as cofactors for several enzymes in energy metabolism therefore it is important to study the interactions between exercise and nutritionally important minerals [1]. Copper, iron, zinc and magnesium play important roles in facilitating the conversion of chemical energy of food into potential energy for work and thereby integrate physiological functions to enhance physical performance.

Individuals involved in some kind of physical activity require adequate amount of these micronutrients in their diet to ensure the capacity for increased energy expenditure and enhanced work performance. Some of the studies carried out in the past years have shown exercise influences the status of minerals in the human body. Physical activity results in short term increase of mineral loss in both urine and sweat, which leads to alteration and perturbation of mineral status [2]. Decreased zinc and magnesium concentrations in serum have been reported in athletes participating in some kind of physical activity [3, 4].

Dietary intake and biochemical profile is generally used to determine the nutritional status of minerals. Dietary intake patterns and biochemical profiles of copper, iron, zinc and magnesium have not been measured concurrently in active healthy male population. Although copper, iron, zinc and magnesium are considered nutritionally important minerals and are generally non-toxic; some of the studies have indicated that the excessive intake or deficiency of metals can lead to serious health effects. Trace elements are generally considered as the missing link in many of the common human diseases like osteoporosis, atherosclerosis, hypertension, ischemic heart disease and arthritis.

Iron is a mineral which is found in almost every cell of the human body and is vital for various physiological functions. It is a component of various important enzymes like catalase, cytochrome, peroxidases and plays a crucial role in maintaining healthy immune system, carrying oxygen from lungs to tissues and in aiding energy production. However, the deficiency of iron in the body can lead to fatigue, lethargy, impaired learning etc and is the most common nutritional deficiency affecting almost 15 % of the world's population [5]. Copper (about 1.5-2.0 ppm) is present in adult human body as a constituent of some of the metalloenzymes like cytochrome c oxidase of the mitochondrial electron transport and cytosolic Superoxide dismutase and is also required for haemoglobin synthesis [6]. Copper deficiency in humans leads to demineralization of bones, depressed growth, gastrointestinal disturbances etc. while excess of copper causes liver cirrhosis, neurological disorders, dermatitis etc [6]. Zinc which makes up approximately 33 ppm of the normal adult human body is an important constituent of several enzymes involved in large number of physiological functions such as protein synthesis and energy metabolism. It also acts as a cofactor for the important antioxidant enzyme superoxide dismutase (SOD). The deficiency of zinc leads to dwarfism, dermatitis, and hypogonadism while toxicity resulting from excessive intake leads to anaemia, lethargy and electrolyte imbalance [7, 8]. Magnesium is an important intracellular cation in human cells and is a cofactor of nearly 300 enzymes. It is involved in all the major cellular processes such as energy metabolism, DNA transcription & protein synthesis [9]. Magnesium deficiency has been implicated in a number of diseases of cardiovascular & neuromuscular function, malabsorption syndrome, diabetes mellitus, renal wasting, and alcoholism [10].

The level of nutritionally important minerals needs to be determined or evaluated in physically active individuals so as to prevent their toxicity and deficiency. There have been very few attempts to curb mineral deficiencies and toxicities in humans through large scale intervention programmes and the possible explanation for the failure to confront mineral deficiencies and toxicities is the lack of specific data on the status of nutritionally important minerals. A large number of nutrition surveys have been conducted so far to evaluate the mineral status of men but there is paucity of data on the status of nutritionally important minerals for physically active healthy population [11, 12]. Also, a large amount of variability has been found in the levels of nutritionally important minerals for healthy male individuals belonging to different geographical regions [13, 14].

Keeping in view the pivotal role played by elements like copper, iron, zinc and magnesium, we tried to determine the status of nutritionally important elements for healthy active male Indian population using flame Atomic Absorption Spectrometry (FAAS) which is one of the preferred techniques due to its rapidness, specificity and affordability. Also, we aimed to study if there is any effect of vegetarian vs. non-vegetarian diets on nutritional status of minerals.

II. Materials and Methods

01. Subjects

This study was conducted on healthy young male participants age (19±0.9 y; x ±SD) based at INS Chilka, a training institution of Indian Navy. The study subjects represented to all states of the country. Participants were informed in detail about the nature of study and procedures approved by Institutional Ethics Committee. The trainees willing to participate in the study were randomly selected (n = 108). All participants were non-smokers and that they were not taking any kind of mineral or vitamin supplement. All the participants were consuming food at common mess. The food intake was ad libitum and any food item provided extra to mess was also recorded. In the study, out of 108 participants, 69 were non-vegetarians and 39 were vegetarians.

02. Measurement of total energy expenditure (TEE)

Two different methods were used to assess physical activity and for the evaluation of TEE:

1. Activity records: The time spent in different activities was recorded and energy cost of different activities on the basis of oxygen consumption was used to calculate TEE.
2. Accelerometry based actical system: Actical devices (Mini Mitter Company, Inc. Bend, OR 97701) were worn by a group of 20 subjects on their wrist and their minute by minute TEE was recorded over a period of seven days [15].

03. Body composition and haematological variables

Measurement of body weight, stature and circumference (waist and hip) using standard measuring devices was carried out [Seca human weighing scale (least count 0.1 kg) for body weight, Martin’s anthropometer for height (least count 0.1 cm)]. Body composition variables like body fat, body mass index (BMI), body water, lean body mass were measured by bioelectrical impedance based body composition analyzer (BF 906, Matron International Ltd., Ryleigh,Essex, England) using disposable electrodes.

Automatic Haematology Analyzer (MS4, Melet Scholoesing laboratories, Pontoise, Cedex – France) was used to study haemoglobin levels, blood cell counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Physiological variables like resting blood pressure and heart rate were recorded in morning hours after overnight rest.

04. Dietary analysis

The daily dietary intake of Cu, Fe, Zn and Mg by vegetarians and non-vegetarians was measured using food inventory as well as analysis of plate samples collected during study using Atomic Absorption Spectrophotometer and then compared to the recommended dietary allowances (RDA) for the Indians [16].

05. Blood sample collection

Samples of blood (5 ml) were drawn by venipuncture into mineral free heparinised tubes from an antecubital vein after overnight fast at 0700-0800 hrs. Samples were centrifuged at 1000 X g for 15 min for the separation of plasma. Red blood cells were washed three times with 150 mM KCl. Aliquots of both plasma and RBCs were brought in frozen conditions and preserved at -80º C till the time of analysis.

All the glassware’s and plastic wares used were checked for contamination by trace elements.

06. Analytical method for Flame Atomic absorption spectrophotometer (FAAS)

Atomic absorption spectrophotometer (Thermo Electron Corporation, UK M6 Spectro with integrated software SOLAAR AA) was used for analyzing Cu, Fe, Zn and Mg concentration in plasma and red blood cells. Commercially available standard solutions (magnesium, iron, copper and zinc; Merck Private limited; concentration 1000 ppm) were used to prepare suitable calibration curve for calculating mineral concentration in plasma and red blood cells. All the working standard solutions are prepared in 10 mmol/L Nitric acid.

Preparation of plasma sample solution and analysis of copper, iron, zinc and magnesium

Plasma samples were diluted 100 fold for magnesium, 10 fold for copper, iron and zinc in 10mmol/L Nitric acid and then aspirated directly in FAAS. A blank was used for setting of zero absorbance of spectrophotometer.

Preparation of Red blood cells samples and analysis of magnesium

RBC lysates (10% v/v) were prepared using distilled water, vortexed and centrifuged at 1000 X g for 15 mins. A 150 fold diluted red blood cell lysates were directly aspirated in flame of atomic absorption spectrophotometer for the measurement of magnesium concentration. All the readings for the plasma and red blood cell samples were taken in duplicates.

07. Statistical Analysis

Upper and lower limits were recorded for magnesium, copper, iron and zinc concentration in plasma and for magnesium concentration in red blood cells. All the results are expressed as mean ± SD. The Student't' - test was used for comparison of mean values of plasma mineral concentration and dietary mineral intake for vegetarians and non-vegetarians. All the statistical analysis including study of correlation between the two variables was performed using Graph pad Prism.

III. Results

The age range of participants of the present study was 18-20 years. The height and weight of subjects was found to be 1.70±0.05 m and 59.19±6.45 kg respectively. The body mass index of the subjects was 20.4±1.8. The values of other important anthropometric and demographic variables were in normal healthy range (Table I).

All the hematological variables (haemoglobin, differential leukocytes count, red blood cell, platelet counts, hematocrit, MCV, MCH and MCHC), blood pressure (systolic and diastolic) and heart rates of all the subjects were in normal range indicating that the participants were in good health (Table II, III).

01. Energy expenditure using different methods

Average TEE of the study group was measured using Actical device on a subgroup of 20 participants only. Analysis of time spent in different grades of activities was made on the basis of metabolic equivalents (METs) in order to determine EE using Actical device (Table IV). The EE obtained using Actical devices for different days during training varied from 2561-4760 kcal. The mean TEE was found to be 3431 ± 443 kcal/day.

The average TEE based on other method i.e. time spent in different activities and energy cost of particular activities on different days was found to be 3771 ± 304 kcal (3286- 4339 kcal). The average of calculated BMR of subjects was 1615 kcal and multiplying this value with 2.3 (PAL used for calculating RDA for heavy activity group by ICMR), the energy requirement comes to be 3715 kcal.

02. Dietary intake analysis

The mean daily dietary intake of copper, iron, zinc and magnesium was found to be statistically different between the vegetarians and non-vegetarians ($P < 0.001$). The daily intakes of copper, iron, zinc and magnesium by the trainees in the present study were found to be greater than the RDA values for Indians (Table V).

03. Plasma and red blood cell mineral concentration

The mean value of plasma zinc concentration of healthy male subjects in the present study was 0.725±0.144 ppm and was similar in both the vegetarians and non-vegetarians (Table VI).

The mean value of plasma copper concentration of healthy male subjects in the present study was 1.177±0.197 ppm (range: 0.775-1.589 ppm) and was found to be nearly same in vegetarians and non-vegetarians (Table VI).

There was a significant difference in the mean value of plasma iron concentration between vegetarians and non-vegetarians ($P<0.01$). The mean plasma iron concentration for vegetarians and non-vegetarians was 39.25 µg/dL and 49.39 µg/dL respectively (Table VI).

The magnesium concentration in plasma was found to be 1.11±0.13 mmol/L or 26.86±3.28 ppm and no difference was observed between the magnesium levels of vegetarians and non-vegetarians (Table VI). The Mg concentrations in plasma were in a range of 0.73-1.45 mmol/L or 17.54 ppm – 34.85 ppm (Table VI). The Mg concentration in red blood cells was 2.24±0.80 mmol/L (range 1.20-3.37 mmol/L) with no difference between the vegetarians and non-vegetarians (Table VI).

A significant positive correlation was observed between BMI and zinc plasma levels ($r = 0.404$, $n= 72$, $P < 0.001$) in the present study (Fig I). However, no significant correlation was observed between BMI and plasma copper, iron and magnesium levels. There was a non-significant positive correlation between zinc and

TEE (r = 0.31, n=18, p=0.210). Although not statistically significant, plasma magnesium and total daily EE were negatively correlated to one another (r = -0.36435, n= 20, P = 0.114). No significant correlation was observed among the plasma levels of metal element studied.

IV. Discussion

There is a limited community based data on the plasma copper, iron, zinc and magnesium concentration with respect to factors affecting their levels and dietary intake of active Indians. The selection of healthy trainees allowed the assessment of micronutrient status and helped in examining relationship between the micronutrients without any confounding health factors. The mean intake of copper, iron, zinc and magnesium for the active participants exceeded the RDA value for Indians [16]. Similar findings have been reported in a study which employed the use of one day diet record for calculating mean daily mineral intake for US Navy SEAL trainees [1]. The mean intakes of magnesium, zinc and copper exceeded the RDA value or the estimated safe and adequate intake. Similarly, in another study, involving male and female skiers, the magnesium intake was found to be 170-185% of RDA [17]. In a study involving university students from Universiti Pertahanan Nasional Malaysia (UPNM), Sungai Besi, Kuala Lumpur and Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor, enrolled in a military training, the mean daily intake of iron (54.8± 17.4) was greater than the recommended nutrient intake of Malaysia (RNI) value [18].

Copper, iron, zinc and magnesium despite their relative paucity in the diet and the body, perform key roles in regulating metabolism of the body including energy utilization and work performance. Some of the earlier animal experiments have shown that zinc is required to improve skeletal muscle performance and in providing resistance to fatigue [19, 20]. Zinc containing enzymes like lactate dehydrogenase, aldolase and carbonic anhydrase play a key role in regulating energy metabolism of the body [21]. The wide array of zinc dependent functions indicates that zinc plays a vital role in regulating work performance. However, the daily dietary zinc intake and plasma zinc concentration for healthy active Indian men have not been adequately studied before. The mean daily dietary intake of zinc by healthy active trainees in the present study was found to be lower for vegetarians in comparison to non-vegetarians. The dietary zinc intake for vegetarians was almost near to the RDA value of 12mg/day for Indians but for the nonvegetarians the daily intake was higher than the RDA value. These values indicate that the dietary intake of zinc was adequate for both vegetarians and nonvegeterians. Vegetarian diets are typically low in zinc when compared to non-vegetarian diets like meat, eggs etc. However, when the mean plasma zinc concentration of vegetarians was compared to the non-vegetarians, the values were found to be similar (Table 6). Since, the mean daily intake of both vegetarians and non-vegetarians met or exceeded the RDA value and was adequate for both the groups; there was no difference in their mean plasma zinc concentration. This implies that zinc homeostasis was maintained whereby when the dietary intake of zinc increased, its absorption decreased thereby maintaining constant levels in the body. The mean plasma zinc concentration in the present study was found to be 0.72 ppm (11 µmol/L). It was also found that 25 % of the study participants had plasma zinc concentration lower than 10µmol/L. These results are consistent with the findings of Dresendorfer and Sockolov [4] who reported that 23% of the male runners had plasma zinc concentration < 10 µmol/L. Studies carried out by Haralambie [22] and Singh *et al* [1] have also reported that 24% of the trainees had plasma zinc concentration lower than 11.5 µmol/L and it was inversely related to training mileage. Serum zinc concentration was also found to be low for 22 % female marathon runners [23]. The results of the study involving physically active individuals when compared to general population in the second National Health and Nutrition Examination Survey revealed that only 0.8±0.19 % of men aged 20-44 years had lower serum zinc concentration [11]. Since, physical activity causes loss of minerals from the body, it may be speculated that physically active individuals have lower levels of circulating zinc in comparison to general population. A significant increase in urinary zinc (about 1 mg/day) was observed on the day of strenuous exercise in comparison to preceding nonexercise day [24]. The surface loss of zinc during exercise may reach around 1.5mg/day which approaches 10% of recommended dietary intake of zinc whereas sweat loss of zinc without any exercise is only 0.8 mg/day [25]. In addition to increased urinary and sweat losses, the low plasma zinc concentration in physically active individuals may be attributed to the redistribution of zinc to tissues that are involved during exercise. The rapid post exercise decrease in plasma zinc concentration may be associated with an increased urinary excretion coupled with redistribution from the plasma into the liver [26] which may be a consequence of the acute-phase response modulated by cytokines [27]. In one of the study, military personnel engaged in 5-day training course had lower plasma zinc value because of increased plasma concentration of IL-6 which stimulates the synthesis of metallothionein [28]. In the present study we have measured only the intake and plasma zinc level which is a limitation of the study. However, with our data it is clearly indicated that RDA of 12 mg/day is sufficient for the intake of active individuals. In case of vegetarians intake of 11.49 mg/day is able to maintain plasma zinc levels similar to non-vegetarians who had higher intake levels.

Copper is a key component of haemoglobin and is associated with cytochrome c oxidase, which is involved in electron transport chain concerned with oxidative phosphorylation [24]. It is needed for proper utilization of iron and protects the cells against oxidative damage. It can be postulated that copper plays a major role in large number of physiological processes concerned with energy metabolism and regulates physical performance of individuals. However, there are a very few studies which have measured the plasma copper levels for physically active individuals taking into account their mean daily dietary intake. The mean daily dietary intake of vegetarians (2.35±0.3 mg/day) and non-vegetarians (2.7±0.3 mg/day) in the present study exceeded the RDA value [16] for Indians. Although the daily intake of non vegetarian was significantly (P<0.001) greater than the vegetarians, the mean value of plasma copper concentration calculated for vegetarian and non-vegetarian group was found to be nearly the same (1.12 mg/L and 1.19 mg/L or 17.6 µmol/L and 18.7µmol/L). The mean value of plasma copper concentration calculated for healthy active male population in the present study was found to be 1.17±0.20 mg/L (18.4 µmol/L) and the range of plasma copper concentration was found between 12µmol/L to 24µmol/L. None of the individuals in the study had values < 11µmol/L which is the lower limit of normal range of copper concentration in plasma. Only 3% of the trainees in the present study had values less than 13.6 µmol/L and about 16% of the trainees had values > 22µmol/L. Similar findings have been reported in a study involving US Navy SEAL trainees where only 1 % of the trainees had mean plasma copper concentration < 11 µmol/L [1]. In another study involving cross sectional comparison of athletes and control subjects, it was found that the plasma copper concentration of athletes was slightly higher than the control subjects though it was not statistically different [29]. Although it is considered that trained men generally have higher concentration of plasma copper concentration in comparison to control subjects not involved in any kind of physical activity, no large scale nutritional studies to measure plasma copper concentration in general population have been carried out [30].

Magnesium is a ubiquitous element, which is involved in more than 300 different enzymatic reactions of the body including glycolytic pathway, fat and protein metabolism as well as hydrolysis of ATP. Magnesium is known to induce the production of 2, 3-diphosphoglycerate in red blood cells thereby ensuring the delivery of oxygen to tissues involved in physical activity [29] and is required for the activity of enzymes which are concerned with utilization of ATP [21]. However, there is very little information on the dietary intake and magnesium status of physically active individuals. The mean daily intake of magnesium for vegetarians and non-vegetarians in the present study was near to the safe limit of magnesium intake for Indians (750 mg/day). However, the values of mean daily intakes for both vegetarians and non-vegetarians exceeded the RDA value (340 mg) for Indians. The dietary surveys of male and female athletes carried out in the past years have shown different patterns of magnesium intake. While some of the studies confirmed inadequate magnesium intake by the athletes, there are studies which have indicated increased magnesium intake by the physically active individuals usually more than the RDA. When the magnesium intake for both male and female cross country skiers was measured, it was found to be around 170-185% of RDA [17]. In another study involving both male and female athletes; the magnesium intake for male athletes met or exceeded the RDA value of 350 mg. However, the magnesium intake for female athletes was just about 60-65% of the RDA value of 280mg. Athletes engaged in wrestling or in other sports requiring weight restriction consumed only 30-35% of RDA value [31]. The results of the present study have also indicated increased magnesium intake by the male athletes (about 171% of RDA value). The mean plasma magnesium concentration for the physically active individuals was found to be 1.11mmol/L. Around 21% of the individuals had mean plasma magnesium concentration below 1.0 mmol/L but no plasma magnesium deficiency was reported in any of the individuals of the present study. These results are consistent with the findings of Fogelholm [32] who reported that there was no effect of physical training on plasma magnesium levels. Contrary to this, there are studies which have indicated an increased magnesium loss and redistribution of magnesium in the body during or immediately following exercise leading to decreased plasma magnesium concentration. The urinary excretion of magnesium increased to around 21% on the day of exercise but returned to control levels on the day following exercise [33].

The mean daily intake of iron in the present study for both vegetarians (44.90±5.4 mg/day) and non-vegetarians (50.7±5.4 mg/day) exceeded the RDA value of 17mg/day for Indians. A similar result of increased iron intake by Army trainees in public institutions of higher education in Malaysia has been found [18]. The mean daily dietary intake of iron was found to be 54.8±17.4 mg/day which exceeded the recommended nutrient intake of Malaysia (RNI) value of 14 mg/day. Since, the mean daily dietary intake of non-vegetarians was found to be higher than the vegetarian group (P< 0.001) in the present study, the mean plasma iron concentration in the non-vegetarians (49.39µg/dL) was found to be statistically greater (P<0.01) than the vegetarians (39.25µg/dL). It may be because of the fact that heme iron, a main constituent of non-vegetarian diet is more bioavailable than the nonheme iron which is present in vegetarian diet. The mean plasma iron concentration in the present study was found to be 45.84±16.9 µg/dl which is lower than the normal range of plasma iron concentration (47-161µg/dL) for physically active healthy individuals. Fe plays an important role in regulating energy metabolism during physical activity [34]. The requirement of iron is higher for the athletes in comparison to individuals who are not involved in any kind of physical activity because of increased iron losses resulting from gastrointestinal bleeding, sweat etc [35]. Also significant haemolysis of red blood cells in swimmers, runners and military recruits after prolonged marches has been observed which lead to increased iron loss from individuals involved in physical activity.

In general, the mean plasma levels of metal elements were within the normal physiological range (except Fe) in spite of the fact that the intakes exceeded the RDA values. This may be due to greater intake of cereal based diet containing phytates which complexes with minerals like iron, zinc, magnesium, calcium, thereby reducing their absorption [36].

A significant positive correlation was observed between BMI and serum zinc levels for healthy male individuals in the present study. Similar findings have been reported previously in various studies [37, 38]. However, in some of the studies [39, 40] authors have reported a negative correlation between BMI and serum Zinc level.

V. Conclusion

This study indicates sufficient nutritional status of minerals studied and may be due to higher intake from diet. This study also indicates that both vegetarian and non-vegetarian Indian diets are able to provide adequate level of nutritionally important minerals. However, the limitation of the study is that the excretion levels of minerals were not measured.

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Conflict Of Interest:

The authors declare that there are no conflicts of interest.

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TABLES AND FIGURES:

***Table I*: Demographic and anthropometric features of subjects involved in the study**

Characteristics of subjects	Values
Age (y)	19±0.9
Height(m)	1.70±0.05
Weight (kg)	59.19±6.45
Body mass index (kg/m^2)	20.4±1.8
Body fat (%)	11.4±2.3
Body fat (kg)	6.84±1.9
Lean Body Mass (kg)	52.43±5.66
Body water (L)	38.93±6.80
WHR	0.860±0.036

Values for all the parameters are represented as mean±SD. WHR:Waist hip ratio

***Table II*: Hematological profile of trainees**

Variables	Mean ± SD	Normal Range
WBC (m/mm^3)	6.8 ± 1.8	3.0-12.0
Lymphocytes (%)	37.0 ± 2.5	15.0-40.0
Monocytes (%)	8.3 ± 0.8	3.0-10.0
Granulocytes (%)	49.2 ± 11.4	30.0-70.0
RBC (M/mm^3)	5.2 ± 0.5	4.0-5.9
MCV (fl)	102.5 ± 6.1	83.0-98.0
Hct (%)	52.5 ± 3.2	35.0-54.0
MCH (pg)	26.2 ± 2.4	25.0-33.0
MCHC (pg)	25.3 ± 1.5	28.0-40.0
Hemoglobin (g/dL)	13.6 ± 0.8	12.0-18.0
Thrombocytes (m/mm^3)	309 ± 94	150-450

Values for all parameters expressed as mean±SD. RBC: red blood cells, MCV: mean corpuscular volume, Hct (%): hematocrit, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration.

***Table III*: Physiological Variables (Blood pressure, Resting heart rate)**

Variable	Mean ± SD
Blood Pressure (mm Hg)	
Systolic	110±10
Diastolic	64±7
Heart rate (beats/min)	60±8

Values expressed as mean±SD.

***Table IV*: Time spent in different activity grades and energy expenditure of trainees at INS Chilka**

Time spent in min				Total energy Expenditure
SED	LIGHT	MOD	VIG	(TEE) kcal/day
452 ± 144 (31.4%)	648±105 (53.3%)	298 ±103 (20.7%)	42 ±21 (2.9%)	3431 ±443 (2561-4760)

Values are Mean ± SD (n=35). Light/Moderate Cut- point 0.31 kcal/min/kg or 3.0 Metabolic Equivalents (METs), Moderate/ Vigorous Cut-point 0.83 kcal/min/kg or 6.0 METs (Abbreviation: SED,Sedentary; MOD,Moderate; VIG,Vigorous).

***Table V*: Daily Dietary intakes of copper, zinc and magnesium**

Mineral elements	Daily dietary intake (mg/day)		RDA
	Non-vegetarian	vegetarian	
Copper	2.7±0.3	2.35±0.3	2
Zinc	17±2.8	11.49±2.8	12
Magnesium	753.25	774.25	340
iron	50.7±5.4	44.90±5.4	17

Values expressed as mg/day. RDA: recommended Dietary Allowance

***Table VI*: Estimation of Copper, Zinc, Iron and Magnesium concentration in plasma and estimation of magnesium concentration in red blood cell of normal healthy active individuals.**

Parameters	Non-vegetarian			vegetarian		
	Range	Average	Std deviation	Range	Average	Std deviation
Mg concentration in plasma(mmol/L)	0.83-1.42	1.11	0.12	0.73-1.45	1.13	0.16
Mg concentration in red blood cells (mmol/L)	1.20-3.37	2.24	0.80	1.20-3.37	2.24	0.80
Zinc concentration in plasma (mg/L)	0.26-1.22	0.72	0.14	0.31-0.99	0.72	0.15
Copper concentration in plasma (mg/L)	0.87-1.59	1.19	0.19	0.77-1.48	1.12	0.20
Iron concentration in plasma (ug/dL)	27.36-86.31	49.39	17.94	17.89-61.05	39.25	13.58

Legend to figures:

Figure 1---- correlation between BMI and plasma Zinc concentration