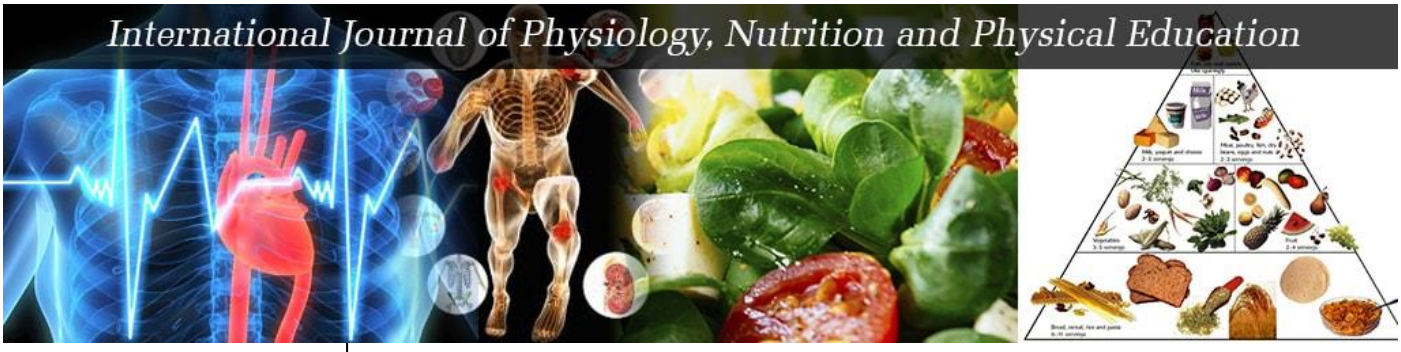

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Adequacy of diet with respect to vitamin status of Indian paramilitary soldiers

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Abstract
Paramilitary forces of India are involved in country's internal and border security during peace time. Nutritional adequacy is important for security personnel to maintain optimal health. Increased physical activity increases requirement of protective nutrients like vitamins and minerals. The present study was conducted on 100 participants from a BSF unit deployed in hot humid riverine frontier for a period of three months. Energy intake was 3500 kcal/d calculated from their 24-hour dietary recall and energy expenditure was 3348 kcal/d recorded using Actical® activity monitors. Vitamin concentrations in food, plasma and urine samples were analyzed using Reverse phase-HPLC (RP-HPLC). Vitamin concentrations in plasma samples of moderately active participants was changed significantly (p<0.05) during three months follow-up. Dietary vitamin intake of participants was more than the RDA. Percentage of urinary excretion of vitamin did not change in pre and post samples. To conclude our study, although the vitamin levels changed significantly with moderate physical activities, the levels were upheld in normal physiological range due to the sufficient dietary intake.

Keywords: Physical activity, dietary intake, energy expenditure, body composition

1. Introduction
Border security force (BSF) is the paramilitary force and act as a first wall of defence for ensuring the security of the borders of India during peace time and helps in preventing transnational crime. They are majorly involved in various physical activities from moderate to strenuous even during peace time for maintaining their ability to endure, to bear up, to withstand stress, to carry on in war or warlike circumstances. Due to the emerging awareness of the synergistic relationship between diet and physical activity; the vital role of micronutrient (both vitamins and minerals) in improving physical performance came into the limelight [1]. Vitamins help in facilitating energy metabolism rather than acting as a direct source of energy by catalyzing numerous biochemical reactions [2]. During physical activity, rates of these biochemical reactions increases; so an adequate supply of vitamins is required to promote optimal physical performance [3]. There are a number of ways in which physical activity could alter the vitamin requirements: decreased absorption from the gastrointestinal tract; increased loss via sweat or urine; increased utilization to meet the energy demands during exercise; or increased need for skeletal tissue maintenance and repair [4].
Intake of some vitamins (thiamin, riboflavin, niacin, pyridoxine, folate, cobalamin, ascorbic acid, and vitamins A and E) shows a great impact on performance during physical activity. Various previously reported studies showing that vitamins requirement increases with an increase in levels of physical activity because of the major role of B vitamins in energy metabolism [4]. A previously reported study states that consumption of thiamine for 12 weeks did not affect performance during treadmill exercise [5]. Urinary thiamine excretion, expressed as a percentage of total daily intakes, decreased when dietary thiamine was reduced. Although physical performance does not found to affect by short-duration thiamine restriction, but brief thiamine insufficiency can cause pyruvate accumulation and increase circulating lactate during work, which may promote fatigue, impair training, and thus reduce performance [6]. Initiation of increased physical activity has an adverse effect on riboflavin status. Sedentary young women consuming 2.15 mg/d of riboflavin experienced a significant increase in the

glutathione reductase receptor activity coefficient (e.g., decreased riboflavin status) within 4 d of starting an endurance exercise program with a concomitant increase in urinary riboflavin excretion [7]. The rationale to evaluate the role of niacin on performance is based on the potentially adverse effect of Vitamin B3 (niacin) on fatty acid use and its indirect effect on accelerating depletion of glycogen stores and performance measures. Murray *et al.* determined the effect of supplemental nicotinic acid (330 and 521 mg for women and men, respectively) and a glucose-electrolyte beverage on performance [8]. During exercise, pyridoxal phosphate is needed for gluconeogenesis and for glycogenolysis in which it serves as a cofactor for glycogen phosphorylase. Few studies have examined the vitamin B6 status of physically active people. Various surveys reported that 40% to 60% of athletes have reduced vitamin B6 based on the enzyme stimulation test [9]. Athletes on energy-restrictive diets and strict vegetarians are at risk for vitamin B12 depletion. Vitamin B12 plays an important role in erythropoiesis making it a potential variable for physically active individual [9]. Vitamin C is a potent antioxidant that serves to regenerate vitamin E from its oxidized byproduct. Vitamin C may indirectly benefit physical performance by enhancing physiologic functions [9]. On the other hand, amongst the fat soluble vitamins; vitamin A intakes of athletes are variable. Surveys of endurance runners, professional ballet dancers, female collegiate athletes, male collegiate athletes, female collegiate heavy weight rowers, and male cross-country runners indicate adequate dietary vitamin A intakes. Adolescents and young adults participating in wrestling, ballet, and gymnastics tend to consume less than 70% of the RDA for vitamin A. A survey of elite German athletes found plasma retinol concentrations within the range of normal values (49 to 93 µg/dL) [10]. Vitamin E serves a key nutrient in supporting physical performance due to its presence in type I muscle fibers [11]. Vitamin C and E supplementation has been shown to interfere with training adaptations, it did not affect acute stress responses or long-term training adaptations in the HSPs or antioxidant enzymes in a previously reported study [12]. Thus, generalized supplementation with vitamin E apparently does not enhance performance. Supplemental vitamin E may be beneficial against free radical assault during work like decreasing leakage of enzymes, lower malonaldehyde concentration.

Previous literature reports the effect of vitamin supplementation on individuals involved in different physical activities. But limited studies have been conducted to show the exact vitamin status of individuals involved in moderate physical activity without any vitamin supplementation. The question which arises our hypothesis is: Is there any effect of moderate exercise on the dietary vitamin intake, plasma concentration and urinary excretion of vitamins? So to answer this query, the present study has been planned to assess the dietary intake, plasma levels and urinary excretion of vitamins in the Indian males deployed as border security force who were involved in moderate physical activity.

2. Material and Methods

Methodology adopted in the present study is discussed under the following headings.

2.1 Selection of subjects

A 3 months follow-up study was conducted to assess the effect of adequate dietary intake on vitamin status of randomly selected 100 moderately active participants (mean age 35.3 $\pm$ 1.1 years) from Indian Border Security Force (BSF) deployed at riverine border of West Bengal. Out of these 100 participants 29 were lacto vegetarian and rest were non-vegetarian. Climatic conditions were hot and humid during study months May-July with rains (temperature ranged 30-36˚C with RH 60-85%). Participants were briefed about study protocol approved by the Institutional Ethics Committee and consent of the participants was obtained. Assessment of body composition, energy expenditure and blood samples collection was made both pre and during post 3-months follow-up.

2.2 Assessment of body composition, energy expenditure and nutrient intake

Measurement of height was done using standard measuring devices (Seca 216, least count 0.1cm). Body composition variables like body fat (fat mass and fat %), total body water, fat free mass, were measured by bioelectrical impedance based body composition analyzer (BC-420MA Body composition analyzer, Tanita Corporation, Tokyo, Japan). The body composition parameters were studied both pre and post 3 months follow-up on the same day of blood sample collection.

Measurement of total energy expenditure (TEE) and physical activity level (PAL) was done by using accelerometry-based wearable Actical® devices (Respironics, mini meter co. Inc Bend OR, USA) to assess the minute by minute energy expenditure of participants.

All the participants were consuming food from a common mess. Plate samples were collected per day for 7 days from mess to cover the entire menu. The weight of the food sample was recorded and then homogenized in a grinder. The homogenized food samples were brought frozen in sterile containers containing 0.1% (w/w) thymol as preservative. Preparation of food samples for vitamin estimation by RP-HPLC is a very crucial step involving liquid-liquid phase extraction. Five grams of the preserved food sample were weighed in a 50ml falcon tube and 22 M KOH solution was added to it. This mixture was kept at 70°C for 45 minutes for saponification and then cooled immediately. To this 10 ml of cold ethanol (Merck 99.9% purity) was added and mixed vigorously. 10 ml hexane (HPLC grade) was added to the mixture and each sample was vortexed for 10 minutes and then centrifuged at 10000 rpm for 15 minutes. Two different phases, i.e. organic and aqueous were obtained and collected each phase in different falcons. Both the phases were then dried in vacuum concentrator instrument. Dried organic phase and aqueous phase extract were then used for the estimation of fat soluble vitamins and water soluble vitamins, respectively.

2.3 Collection and processing of blood and urine samples

Fasting venous blood samples were collected during morning hours (0700-0800 h) in EDTA and heparin tubes. EDTA tubes were used for haematological analysis and heparin tubes were centrifuged for 15 min at 1000 x g at room temperature to fractionate blood into upper plasma and lower RBC layer. Plasma samples were stored in two different vials, one containing 10µl of 1 M dithiothretiol (DTT) used for estimation of water soluble vitamins and plain plasma vials for estimation of fat soluble vitamins kept on -80°C until analysis. Lower layer was aspirated and kept in fresh tubes which were further washed twice with 150mM KCl by centrifugation. Finally store RBC in cryovials for enzymatic analysis.

24 hours urine samples were collected in 6N HCl containing jerry cans. Total urine volume was recorded and 10 ml sample was stored at -80˚C for analysis.

2.4 Haematological profile analysis
Haematological profile of each participant was measured in both pre and posts 3 months follow-up EDTA blood samples using haematology analyser.

2.5 Vitamin Estimation
In plasma samples, fat soluble vitamins were estimated using protocol developed by Kandar *et al.*, 2012 [14] and water soluble vitamins were estimated using protocol developed by Giorgi *et al.*, 2011 [15]. Both the methods used hexane extraction procedure. Organic phase (hexane layer) used for analysis of fat soluble vitamins and aqueous phase used for the extraction of water soluble vitamins. Both the phases were injected in C18 column of RP-HPLC using autosampler. In urine samples, water soluble vitamins were estimated using the same protocol.

2.6 Biomarker Estimation
Erythrocyte contains transketolase enzyme for which vitamin B1 function as coenzyme. The activity of this enzyme increased by *in vitro* addition of the coenzyme i.e. vitamins. The degree of activation, i.e. activation coefficient of Erythrocyte Transketolase (ETK) by in vitro addition of Thiamine Pyrophosphate (TPP) has been suggested as an indirect measure of their respective vitamin content and to inversely relate to vitamin concentration [16].

2.7 Statistical analysis
Statistical analysis was performed using GraphPad Prism software version 5.0 for windows 8.0 (GraphPad Software, LaJolla, California, USA) with the level of statistical significance set at $p<0.05$. Paired t-test was used to compare physical and biochemical parameters in pre and post three months follow-up samples.

3. Results and Discussion
Body composition profile of all the participants at both pre and post 3-months follow-up is listed in Table 1. Body weight, Fat%, Fat free mass, total body water, and bone mass were maintained in the normal physiological range thereby, representing good health of participants selected for pre phase. No statistically significant change was observed after 3-months follow-up in comparison with pre phase. Hence, represents a well-balanced diet during these three months. Average BMI of participants was found to be 24.9 ± 0.3 as 29% participants were overweight considering 18-25 be the normal range of BMI for healthy persons.

Table 1: Body composition Profile

Variables	Pre (Mean ± SEM)	Post 3 Months Follow-up (Mean ± SEM)
Height (cm)	174.9 ± 0.5	174.9 ± 0.5
Weight (kg)	76.47 ± 1.10	76.05 ± 1.07
Fat%	22.41 ± 0.46	22.25 ± 0.46
Fat Mass (FM) (kg)	17.37 ± 0.57	17.25 ± 0.57
Fat Free Mass (FFM) (kg)	59.0 ± 0.61	59.29 ± 0.58
Muscle Mass (MM) (kg)	56.04 ± 0.57	56.22 ± 0.22
Total Body Water (TBW) (kg)	40.87 ± 0.53	41.3 ± 0.51
TBW %	53.54 ± 0.24	53.54 ± 0.3
Bone Mass (BM) (kg)	3.06 ± 0.03	3.07 ± 0.03
Basal Metabolic Rate (BMR) (kcal/d)	1670.71 ± 19.06	1676.73 ± 18.77
Body Mass Index (BMI)	24.92 ± 0.33	24.97 ± 0.32

Energy expenditure measured with the help of Actical® activity monitor watches is listed in Table 2. Basal metabolic rate (BMR) was found to be 1679 ± 38 kcal per day. Total energy expenditure was observed to be 3348.18 ± 184.78 kcal, physical activity level (PAL) value was calculated to be 2.0 ± 0.1 using TEE/BMR, indicating that participants were moderately active.

Table 2: Basal Metabolic Rate (BMR), Total Energy Expenditure (TEE), and Physical Activity Level (PAL) Value during 3 months (Moderately Active)

BMR (kcal/d)	1679.68 ± 38.88
TEE (kcal/d)	3348.18 ± 184.78
PAL value	2.0 ± 0.10

Knowing the fact that vitamins play an important role in erythropoiesis, we assessed the haematological profile of participants in both phases. Vitamin B12 and vitamin B9 are essential for the maturation of red blood cells [17]. Vitamin C deficiency causes lack of maturation failure in the process of erythropoiesis, which manifests clinically as reticulocytopenia, an abnormally low amount of reticulocytes. Erythroblasts require folate and vitamin B12 for proliferation during their differentiation. Deficiency of folate or vitamin B12 inhibits purine and thymidylate synthesis, impairs DNA synthesis, and causes erythroblast apoptosis, resulting in anaemia from ineffective erythropoiesis [17]. Haematology profile was measured using haematology analyser and is represented in Table 3. Concurrent with previous studies, we observed a significant change in various haematological parameters like RBC, MCV, HCT%, MCH, MCHC, RDW, PLCR% and PDW% in post phase in comparison with pre phase which may be due to moderate physical exercise [18].

Table 3: Haematology Profile

Variables	Pre (Mean ± SEM)	Post 3 Months Follow-up (Mean ± SEM)
WBC m/mm³	6.51 ± 0.21	6.68 ± 0.32
LYM %	22.52 ± 0.69	24.07 ± 0.89
MIX %	7.91 ± 0.27	8.53 ± 0.44
NEU %	69.57 ± 0.87	67.59 ± 1.22
RBC M/mm³	4.89 ± 0.06	4.56 ± 0.08***
MCV fl	95.65 ± 1.18	91.87 ± 1.19***
HCT %	46.47 ± 0.45	41.18 ± 0.46***
MCH Pg	27.31 ± 0.43	29.36 ± 0.42***
MCHC g/dl	28.52 ± 0.16	31.47 ± 0.56***
RDW	12.06 ± 0.16	11.10 ± 0.16***
HB (g/dl)	13.29 ± 0.17	13.20 ± 0.17
THR m/mm³	156.91 ± 5.52	154.38 ± 7.34
MPV fl	10.52 ± 1.57	9.31 ± 0.10
PLCR %	17.80 ± 0.83	22.18 ± 1.04***
PDW %	8.51 ± 0.43	8.80 ± 0.52*

Dietary intake of participants was calculated using plate sample analysis collected from unit mess and was found to be more than RDA as shown in Table 4. Also, mean energy intake was calculated using raw food items issued in unit mess and was found to be 3500 Kcal which was more than their total energy expenditure i.e. 3348 kcal. Hence, it was assessed that dietary intake was adequate as it was more than expenditure.

Table 4: Dietary intake of each vitamin (mean ± SD) and RDA values

Vitamin	RDA	Dietary Vitamin Intake
Vitamin A (ug/d)	600	752 ± 209
Vitamin E (mg/d)	1000	1105 ± 196
Vitamin B1 (mg/d)	1.4	1.5 ± 0.2
Vitamin B2 (mg/d)	1.6	2.0 ± 0.2
Vitamin B3 (mg/d)	18	25.2 ± 6.8
Vitamin B5 (mg/d)	5	6.2 ± 1.0
Vitamin B6 (mg/d)	2.0	2.31 ± 0.4
Vitamin C (mg/d)	40	149 ± 93
Vitamin B9 (ug/d)	200	273 ± 99
Vitamin B12 (ug/d)	1	1.5 ± 0.3

Table 5 represents 24-h urinary excretion of water soluble vitamins level. Recovery rate shown in table 5 represents the percentage of 3 days mean dietary vitamin intake excreted out through urine and was calculated using (24-h urinary excretion/ 3 days mean intake) X 100. Urinary vitamin excretion increases with increase in levels of physical exercise [19]. But our study shows the contradictory results, although the participants were performing moderate physical exercise even then 24-h urinary excretion of vitamins falls under normal physiological range [20]. This is because dietary vitamin intake of participants is sufficient enough to maintain their excretion level even with physical exercise.

Table 5: Quantitative representation of 24-h urinary excretion of vitamins, 3-days mean vitamin intake and recovery rate calculated percentage of intake excreted out through urine.

Vitamins	24-h Urinary excretion of Vitamin (mean ± SD) [a]	3 Days Mean Vitamin Intake (mean ± SD) [b]	Recovery Rate % [r]
Vitamin B1 (mg/d)	0.272 ± 0.131	1.5 ± 0.2	18.1
Vitamin B2 (mg/d)	0.246 ± 0.215	2.0 ± 0.2	12.3
Vitamin B3 equivalent (mg/d)	11.314 ± 3.701	25.2 ± 6.8	44.9
Vitamin B5 (mg/d)	4.395 ± 1.465	6.2 ± 1.0	70.9
Vitamin B6 (mg/d)	1.59 ± 0.53	2.31 ± 0.4	68.6
Vitamin C (mg/d)	47.382 ± 4.135	149 ± 93	31.8
Vitamin B9 (µg/d)	12.285 ± 4.071	273 ± 99	4.5
Vitamin B12 (µg/d)	0.021 ± 0.011	1.5 ± 0.3	1.4

[a] represents urinary excretion for each vitamin corresponds to thiamine for B1, riboflavin for B2, sum of nicotinamide for B3 equivalents, pantothenic acid for B5, 4-Pyridoxic acid for B6, ascorbic acid for C, Folate for B9, cobalamin for B12.
[b] represents mean dietary intake was calculated using daily dietary intake for each individual using plate sample analysis.
[r] represents recovery rate was derived from 24-h urinary excretion/ 3 days mean intake.

Physical exercise increases the need for vitamins due to increased need for antioxidant vitamins like A, E [21]. Conversely, if physically active men had poor dietary choices, then they show a low plasma vitamin level [22]. Although the participants of our study were consuming proper diet, we assessed that whether moderate physical activity affects plasma vitamin level or not. Fig 1 represents fat soluble vitamin concentration in plasma samples of pre and post phases compared statistically using paired t-test with scattered dot plot. Fig 1(A) shows plasma vitamin A level which is found to be significantly ($p = 0.0388$) changed in post (94.87 ± 3.33 µg/dl) in comparison with pre (95.53 ± 3.37 µg/dl) plasma samples. The minimum (49.82µg/dl in pre and 51.21µg/dl in post) and maximum (190.0µg/dl in pre and 192.1µg/dl in post) plasma vitamin A concentration falls under the normal physiological range (50-200 µg/dl). Fig 1(B) shows plasma vitamin E level which is found to be significantly ($p<0.001$) changed in post (10.86 ± 0.34 mg/L) in comparison with pre (11.16 ± 0.35 mg/L). The minimum (5.00 mg/L in pre and 5.04 mg/L in post) and maximum (21.92 mg/L in pre and 21.13 mg/L in post) plasma vitamin E concentration falls under normal physiological range (5.5-17mg/L). Furthermore, higher levels of physical activity are generally associated with better serum vitamin D status, presumably because more outdoor physical activity can increase sun exposure and vitamin D production in the skin [23]. But, it has been reported that vitamin D status is closely linked with obesity. Fig 1(C) shows plasma vitamin D level which is found to be significantly ($p<0.001$) changed in post (3.37 ± 0.20 µg/dl) in comparison with pre (3.63 ± 0.22 µg/dl). Although, average plasma vitamin D3 level of participants falls under normal physiological range (2-8 µg/dl) but it was observed that 17% of participants showed severe deficiency and 20 % of participants showed mild to moderate deficiency. This may be due to the fact that around 40% of the participants were overweight with BMI of >25 as it was reported previously that poor vitamin D status is is strongly linked with obesity [24].

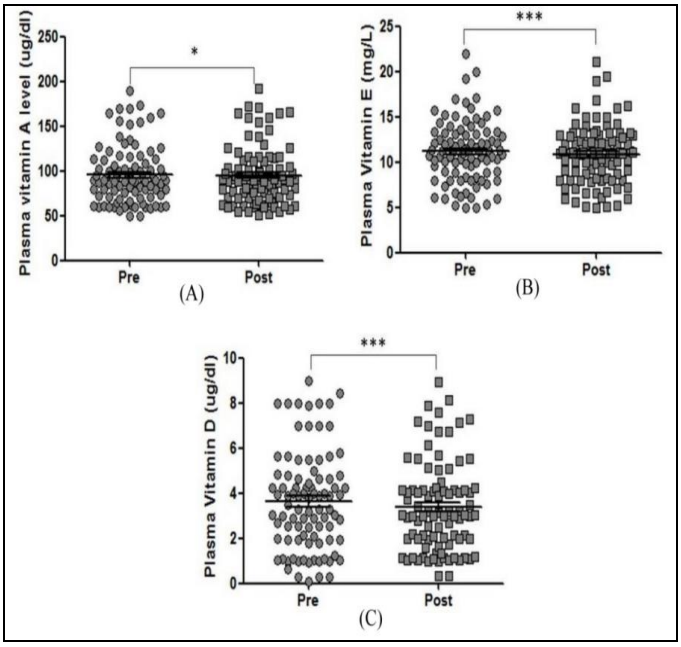

Fig 1: Fat soluble vitamin concentration in plasma samples of both pre and post phases: compared statistically using paired t-test with scatter dot plot (Line represents mean value and error bars represents Standard error mean)

Exercise triggers metabolic pathways that depend on thiamine, riboflavin, and vitamin B-6, the requirements for these vitamins may be increased in athletes and active individuals [25]. Conversely, if persons increase their physical activity and restrict their energy intake, the need for these vitamins may increase further [26]. Although the participants of our study have dietary vitamin intake more than RDA, but to check whether the diet is adequate enough to meet the required vitamin demand in respect with energy expenditure, we assessed concentration of water soluble vitamin in plasma samples of pre and post phases which is depicted in Fig 2, compared statistically using paired t-test with scattered dot plot. Fig 2(A) represents vitamin B1 concentration in plasma samples which is found to be significantly ($p = 0.0224$) changed in post (4.91 ± 0.17 µg/dl) with respect to pre (5.04 ± 0.17 µg/dl) phase. Concurrent with the previously reported literature [27], approximately 7% participants showed marginal vitamin B1 deficiency in both phases. Result of the status of vitamin B1 was further confirmed by analysis of erythrocyte transketolase enzyme activation coefficient. Mean value of activation coefficient of enzyme erythrocyte transketolase was 1.14 ± 0.52 (mean ± SD) which was considered to be in normal range i.e. < 2.0 for normal healthy vitamin B1 status. But, concurrent with the findings of plasma vitamin B1 level 8 out of 100 participants shows their activation coefficient to be more than 2.0. Hence, we can interpret here that 7-8% participants shows deficiency for vitamin B1. We found a significant ($p<0.0001$ and Pearson r = -0.4608) negative correlation between concentration of vitamin B1 and activation coefficient of enzyme erythrocyte transketolase [27]. Participants with low vitamin B1 concentration in plasma have more enzymatic activation coefficient as represented in Fig 3. Fig 2(B) represents vitamin B2 concentration in plasma samples which is found to be significantly ($p = 0.0004$) changed in post (7.64 ± 0.55 µg/L) with respect to pre (8.24 ± 0.63 µg/L). The minimum (1.34µg/L in pre and 1.12µg/L in post) and maximum (23.92µg/L in pre and 21.16µg/L in post) plasma vitamin B2 concentration falls under the normal physiological range (1-19 µg/L). Fig 2(C) represents vitamin B3 concentration in plasma samples which is found to be significantly changed in post (3.88 ± 0.21 µg/ml) in comparison with pre (4.14 ± 0.22 µg/ml). The minimum (0.57 µg/ml in pre and 0.11µg/ml in post) and maximum (9.05µg/ml in pre and 10.02µg/ml in post) plasma vitamin B3 concentration falls under the normal physiological range (0.5-8.45 µg/ml). Fig 2(D) represents vitamin B5 concentration in plasma samples which is found to be almost similar in both pre (90.91 ± 3.44 µg/L) and post (90.61 ± 3.46 µg/L) phases and falls under the normal physiological range (37-147 µg/L). Contradictory with the previous literature [28], Fig 2(E) represents vitamin B6 concentration in plasma samples which is found to be almost similar in both pre (23.72 ± 1.24 µg/L) and post (24.44 ± 1.31) phases and falls under the normal physiological range (5-50 µg/L). Fig 2(F) represents vitamin B9 concentration in plasma samples which is found to be changed significantly ($p<0.0001$) in post (16.00 ± 0.57 ng/ml) in comparison with pre (12.73 ± 0.88 ng/ml) phase. Fig 2(G) represents vitamin B12 concentration in plasma samples which is found to be significantly changed in post (262.8 ± 7.47 ng/L) in comparison with pre (245.4 ± 8.04 ng/L) phase. Fig 2(H) represents vitamin C concentration in plasma samples which is found to be significantly ($p<0.0001$) changed in post (7.49 ± 0.12 mg/L) in comparison with pre (6.88 ± 0.17mg/L) phase.

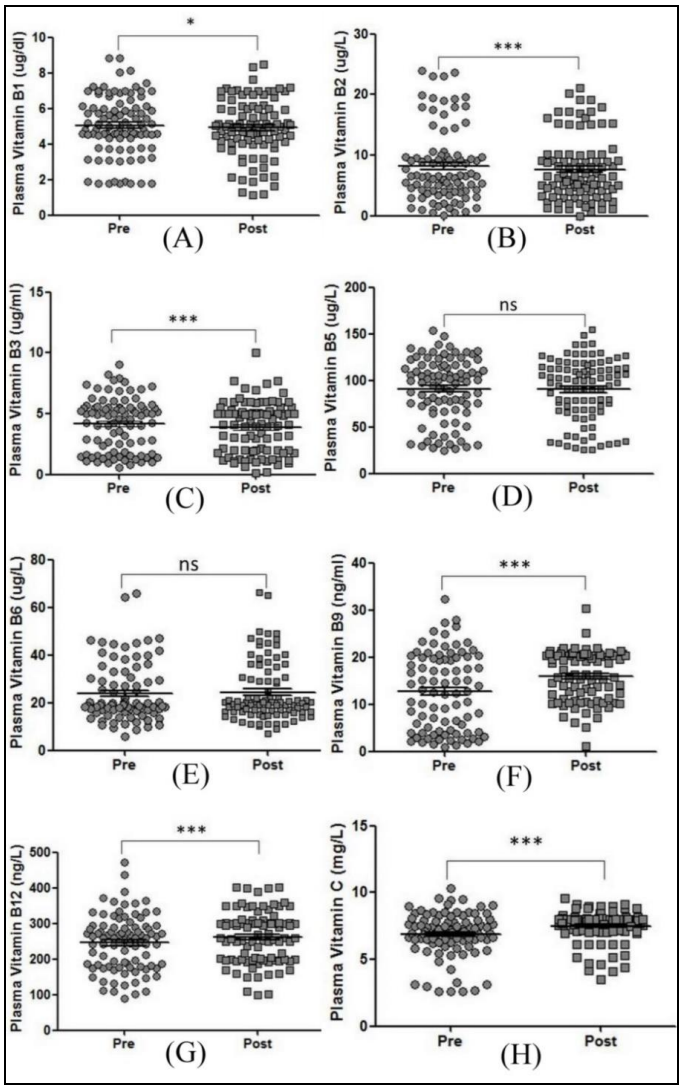

Fig 2: Water soluble vitamin concentration in plasma samples of both pre and post phases: compared statistically using paired t-test with scatter dot plot (Line represents mean value and error bars represents Standard error mean)

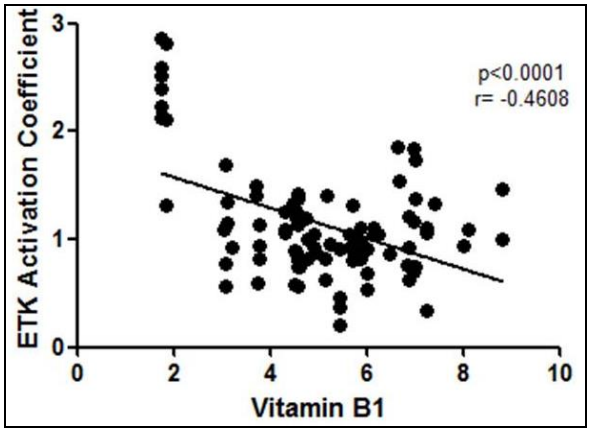

Fig 3: Inverse correlation of plasma vitamin B1 concentration and ETK activation coefficient.

4. Conclusion

The result of the study shows that moderate physical activity with PAL value of 2.0 affects vitamin concentration in plasma. Dietary intake was more than the RDA. Urinary loss of vitamins was in normal physiological range. Hence, we can conclude that during moderate physical exercise, dietary intake should be adequate so as to maintain plasma vitamin levels in normal physiological range and prevents urinary loss.

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