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Energy Expenditure and Nutritional Status of Sailors During One Month of Extensive Physical Training

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ABSTRACT

The present study was conducted to determine nutritional requirements during extensive physical training by sailors of Indian Navy. A total of 37 sailors who were undergoing physical trainers course at training establishment of Indian Navy participated in this study. Energy expenditure, energy intakes, nutrient status and body composition changes during one month of training were recorded. Mean energy expenditure was found to be 4035 kcal/day ± 733 kcal/day and an average intake of 4478 kcal/day ± 340 kcal/day with sufficient amount of micro and macronutrients. The level of vitamin and minerals in blood and their excretion were in the normal range. Body composition was also maintained with a marginal decrease in body fat content. Increase in grip strength of passive hand was observed (Basal: 41.5 kg ± 8.8 kg, after 1 month of training: 46.5 kg ± 6.1 kg). Results indicate adequate nutritional support from the diet and positive effects of the training on health.

Keywords: Body composition; Energy expenditure; Navy; Nutritional status; Nutrients

1. INTRODUCTION

Physical training of Indian Navy encompasses all physical and recreational training activities required for optimal performance during the peace and war. Energy expenditures during training tend to be significantly high depending upon training requirements, intensity and environmental conditions[1-5]. Protein requirements for soldiers are also more in comparison with general population[6]. To meet the functional needs like promoting skeletal-muscle protein accretion and physical strength, dietary intake of 1.6 g protein/kg body weight per day is recommended for persons with intense physical activity and long-term consumption of 2.0 g/kg is considered safe for healthy adults[7]. The energy expenditure by Military personnel reported being in the range of 3109 kcal - 7131 kcal. The US Army Ranger Students during ranger training spent about 5200 kcal, which under cold and hilly conditions may go as high as 7131 kcal[8]. In case of Indian soldiers during Basic Military Training the physical activity level of 2.66 has been recorded using DLW technique. Energy expenditure has been found to be 61.2 kcal/kg body weight/day[9]. In our earlier study energy expenditure of Sailors at the ship was found in a range of 2449 kcal/day - 4907 kcal/day with a mean of 3313 kcal/day ± 578 kcal/day, while in case of submariners it was 3168 kcal/day ± 282 (2606-3907) kcal/day[10]. The energy expenditure of the marine commandos MARCOs and divers ranged 3200 kcal/day - 4700 kcal/day (mean value 4055 kcal/day ± 465 kcal/day)[3].

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To obtain maximum benefit of training adequate dietary support is very important. The high energy expenditure increases requirement of thiamin and riboflavin, the vitamins which participate in energy metabolism and antioxidant nutrients like Vitamin C, Vitamin E, β-carotene, Zinc and selenium to protect from exercise induces oxidative stress[11-16].

Bio-availability of different micronutrients especially of zinc, iron and calcium is low from Indian diets due to presence of high amount of phytates and oxalates.

Keeping all these aspects into consideration the present study was conducted to evaluate energy and nutrient requirements of PT trainers of Indian Navy during their advanced training.

2. MATERIALS AND METHODS

A total of 37 trainees, who were undergoing training in three different courses at INPT School, Goa participated in the study. The climate during the study period was warm and humid with a temperature range of 25)C - 30 ˚C.

Subjects were explained about study procedure that was approved by Ethics Committee of the institute and written consent for participation in the study was obtained. All investigations were carried out initially and repeated after one month with monitoring of food intake from galley/ mess. All trainees were consuming food at the common mess and were on authorised ration. The food intake was *ad libitum* and any food item provided extra to mess or consumed individually were also recorded.

Energy expenditure was measured using accelerometer based actical devices (Respironics, Mini Mitter Company, Inc,

Bend, OR97701 USA). Monitoring devices were worn on the wrist by a group of 20 subject for a period of 7 day. Devices are waterproof therefore participants wore these during all type of activities including swimming in the pool and open sea etc. Minute by minute data for energy expenditure was recorded. This system is a good tool to give a reliable measure of energy expenditure in case of subjects following a set pattern of activities and was validated against Doubly Labeled Water (DLW) technique[9,18].

The food and nutrient intake were evaluated by inventory method. Monitoring of galley from where study volunteers were taking food was made instead of making any separate kitchen arrangement. Total strength of persons taking food was recorded daily to get a mean intake. All raw food items have weighed and the wastage in the raw stage was recorded. The standard tables were used to compute nutritional composition of diet[19]. Use of Tables was restricted only to determine the proximate composition of ration consumed.

For actual intake of micronutrients, duplicate food samples were collected from different participants at different times (breakfast, lunch, and dinner) so that entire range of menu was represented. The samples were weighed, homogenised in a mixer grinder and aliquots were stored in frozen condition with thymol (0.1 % w/w, 1ml of 10 % (w/v) thymol dissolved in isopropanol) as a preservative for analysis of nutrients. Participants were asked to keep records of twenty-four-hour dietary intakes for a period of 7 day to have an estimate of extra intake if any.

Calorie content of food was analysed using computer controlled bomb calorimeter (Toshniwal Brothers Pvt. Ltd. India). Protein content was analysed by the micro Kjeldahl method using automated Nitrogen Analyser (Pelican Equipments, Chennai, India). Fat content was measured using solvent extraction technique. Vitamin contents (vitamin A, vitamin E, thiamin, riboflavin, pyridoxine, niacin) were analysed using standard techniques[20]. Minerals and trace elements were analysed using Atomic Absorption Spectrophotometer[20]. Measurement of body weight, stature, and circumferences (waist and hip) using standard measuring devices were made [Seca Human weighing scale, Hamburg, Germany (least count 0.1 kg) used for body weight, Martin's Anthropometer for height]. The waist to hip ratio and body mass index (BMI) was calculated. Body composition viz. body fat, body water, lean body mass were measured by bioelectrical impedance analysis (BIA) using Body Composition Analyser (Tanita, Model No BC-420MA, USA)

Hematological variables i.e. hemoglobin, leucocytes counts, red blood cell, platelet counts, hematocrit, RBC indices [mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)] were determined using Sysmex Automatic Hematology Analyser, UK.

Venous blood samples were drawn in the morning (0700 h - 0800 h) after an overnight fast. Samples were collected in heparinised tubes, centrifuged at 1000 g for 10 min to isolate plasma. An aliquot of 400 µl of each plasma sample was mix with DTT (Dithiothreitol) after separation for estimation of water-soluble vitamins. Erythrocytes were washed three times with 150 mM KCl and 10 per cent lysates (v/v) were prepared with distilled water for estimation of enzyme markers of vitamin status. Samples were brought in frozen condition to a laboratory where they were stored at -70 ˚C until assayed. Twenty-four hours' urine samples from volunteers were collected in PVC cans containing a known volume of 6N HCl as a preservative. Aliquots of urine were stored after measuring total volume and used for various estimations. Total plasma protein was estimated using a modified method of Lowry et al[21]. Plasma albumin was estimated using colorimetric method after separation[20]. Globulin levels were obtained by subtracting albumin level from total proteins, A/G ratio was also calculated. Plasma vitamin A, β-carotene, vitamin D, vitamin E were estimated after extraction using High-Pressure Liquid Chromatography (UPLC, Thermo Fisher, USA) with Diode array detector. Ascorbic acid (vitamin C) in 24-hour urine was estimated using α-α dipyridyl[22]. Erythrocyte transketolase (EC 2.2.1.1) activity and its activation by thiamin pyrophosphate (TPP) were assayed spectrophotometrically for vitamin B_1 status[20]. Erythrocyte glutathione reductase (GR) (EC 1.6.4.2) activity and its activation by Flavin Adenin Dinucleotide (FAD) was measured for riboflavin (vitamin B_2) status[23]. Erythrocyte aspartate aminotransferase (AST) (EC 2.6.1.1) activation by pyridoxal 5' phosphate (PLP) was used as the measure of pyridoxine (vitamin B_6) status using colorimetric method[20]. Urinary methylmalonic acid for vitamin B_{12} status was measured by the method described by Giorgio[24]. Urinary thiamin estimated using thiochrome method[20] and urinary riboflavin by a method described by Najjar and Holt[25]. Urinary nitrogen estimation was done by the micro Kjeldahl method, creatinine in plasma and urine samples were estimated using alkaline picrate method[26]. Lipid profile: Total cholesterol, HDL cholesterol, and triglycerides in plasma were estimated using commercially available kits. LDL was calculated using Friedwalds formula i.e. LDL Cholesterol = Total Cholesterol – (HDL Cholesterol + Triglcerides/5). One-fifth of triglycerides were taken as very low-density lipoproteins (VLDL) content. Hydroperoxide levels were measured using FOX-1 assay[27] and total antioxidant levels using ABTS method[28]. Plasma levels of Cu, Zn and Mg were estimated using Atomic Absorption Spectrometer[20]. Clinical examination for any nutritional deficiency symptom was carried out by Principal Medical Officer of the unit.

Passive handgrip test for muscular strength was carried out using Grip Strength Dynamometer (TKK5001, Grip A, Takaki Scientific Instruments Co. Ltd., Japan, least count 0.5 kg). The technique was explained to the participants before conducting the measurement and encouraged to press dynamometer by their nonworking hand (i.e. left hand for right-hand users) with full grip strength and readings were recorded.

Statistical analysis was made using software Graph Pad PRISM Version 5.02. Comparison of data obtained initially and after one month was made using Student 't' test and p-value <0.05 was considered as a significant change.

3. RESULTS AND DISCUSSION

The study subjects represented to different states of the country. The average age (± SD) of the study participants

was 25.2 Years ± 4.8 Years (Range: 19-34 Y). As dietary preferences are concerned 15 participants were vegetarian and 23 participant were non-vegetarians. Occasional intake of alcoholic beverages and smoking was reported by 13 and 3 participant respectively. However, they were asked to refrain from alcohol intake during the study period.

Average energy expenditure of the study group was taken as the criterion for arriving at energy requirements of trainees. Analysis of time spent in different grades of activities was made on the basis of metabolic equivalents (METS) using Actical devices as shown in Table 1.

Table 1. Time spent in different activity grades and energy expenditure of trainees

Time (min) spent in activities				Total energy expenditure kcal/day
Sedentary	Light	Moderate	Vigorous	
484 ± 124 (33.6 %)	526 ± 80.8 (36.5 %)	345 ± 86 (24.0 %)	85 ± 16 (5.9 %)	4035 ± 733 (3242-5761)

Values are Mean ± SD (n=20). Light/moderate cut- point 0.31 kcal/min/kg or 3.0 METs, Moderate/vigorous cut-point 0.83 kcal/min/kg or 6.0 METs.

The mean total energy expenditure (TEE) of the study group was found to be 4035 kcal ± 733 kcal (3242 kcal - 5761 kcal). TEE, when expressed in terms of per kg body weight, comes to be 64 kcal/kg ± 6 kcal/kg. Using factorial method i.e. calculated using BMR multiplied with 2.3 (PAL used for calculating RDA for heavy activity group by ICMR), the energy requirement comes to be in the range of 2889 kcal/kg - 4315 kcal.

In our earlier study energy expenditure of Sailors at the ship was found in a range of 2449 kcal/day - 4907 kcal/day with a mean of 3313 kcal/day ± 578 kcal/day, while in case of submariners it was 3168 ± 282 (2606-3907) kcal/day[3,10]. The energy expenditure of the MARCOs and divers ranged 3200 kcal/day - 4700 kcal/day (mean value 4055 kcal/day ± 465 kcal/day). The energy expenditure of trainees is almost similar to MARCOs.

The common component among training schools leading to very high energy expenditure is that instructors keep students physically active for extended hours. Purpose of the long physically active training are i) to give students as much training as possible in a short period of time and ii) to impose a physical and psychological stress upon these soldiers to prepare them for the stress of combat and missions including survival training.

Energy and nutrient intake computed from raw ration as well as analysis of cooked food samples is as given in Table 2. The mean intake (± SD) of energy from food consumed from establishment galley and extra items was computed to be 4478 kcal ± 340 kcal. Energy contribution from carbohydrate fat and protein was 58.8 per cent, 27.9 per cent and 13.3 per cent of total calories respectively. This ratio is well balanced in terms of macronutrients. Intake of micronutrients (vitamins and minerals) was well within limits of safe intake. Extra items taken by trainees outside mess were found to be juices, banana shake, groundnuts, roasted Bengal grams, almonds, chocolates, dry fruits, Indian sweets and electrolytes.

Table 2. Nutrient intake by trainees

Nutrients	Intake	RDA 2010
Energy (kcal)	4478 ± 340*	3490
Protein (g)	149 ± 54	60
Fat (g)	139 ± 40	40 [visible fat]
CHO (g)	658 ± 54	-
Vitamin A (µg)	1062 ± 120	600
Vitamin C (mg)	150 ± 25	40
Thiamin (mg)	3.3 ± 0.3	1.7
Riboflavin (mg)	2.3 ± 0.3	2.1
Niacin (mg)	28.7 ± 5.4	21
Iron (mg)	34.7 ± 7.6	17
Calcium (mg)	1356 ± 318	600
Sodium (mg)	8180 ± 180	1100-3300
Potassium (mg)	3679 ± 250	1875-5625
Zinc (mg)	16.3 ± 1.4	12
Copper (mg)	3.3 ± 0.5	2.2
Crude Fiber (g)	33.9 ± 10.5	-

*Calories contributed from carbohydrate 58.8 per cent, fat 27.9 per cent and protein 13.3 per cent of total intake.

No clinical deficiency symptoms were noted in study participants. Changes in body weights and body composition during the study are as given in Table 3. There was a small but statistically significant change in body composition like a decrease in body weight, body fat and the increase in muscle mass indicating positive effects of training.

Table 3. Body weight and body composition changes of trainees

Variable	Initial	After 1 Month
Height (cm)	170.2 ± 4.8	170.8 ± 4.8
Body weight (kg)	64.5 ± 8.3	63.9 ± 8.0
Body fat (%)	17.9 ± 4.4	16.5 ± 4.3
Body fat (kg)	11.82 ± 4.12	10.82 ± 3.82
LBM (kg)	52.69 ± 4.84	53.16 ± 5.02
Muscle mass (kg)	49.95 ± 4.60	50.40 ± 4.80
Body water (L)	35.48 ± 3.72	35.97 ± 3.75
Body water (%)	64.83 ± 1.65	64.12 ± 1.94
WHR	0.880 ± 0.036	0.875 ± 0.040
BMR (kcal)	1502 ± 142	1514 ± 146
BMI (kg/m^2)	22.3 ± 2.4	21.9 ± 2.3

Values are Mean ±SD (n=37; P< 0.05 except for height in comparison with initial. LBM- Lean Body Mass, WHR- Waist to Hip Ratio, BMR- Basal Metabolic rate, BMI – Body Mass Index

BMI was maintained or decreased marginally in study participants. BMI (kg/m^2) of 31 participant were well within the suggested healthy limits for Indians i.e. 17.5 - 23[29-31]. Hemoglobin, blood cell counts and RBC indices are as given in Table 4. All values were within normal range. The marginal but statistically significant decrease in Hb and RBC indices indicates more requirement of iron due to increased physical activity, even though observed intakes were more than RDA. This also reflects that iron absorption from diet may not be adequate due to presence of phylates and oxylates[32].

Table 4. Hematological variables of trainees

Variable	Initial	After 1 month	Normal Range
WBC (m/mm^3)	6.7 ± 1.3	6.5 ± 1.6 ^{ns}	3.0-12.0
RBC (M/mm^3)	5.4 ± 0.9	5.1 ± 0.3*	4.0 – 5.9
MCV (fl)	85.0 ± 5.2	86.4 ± 5.3*	83.0 – 98.0
Hct (%)	45.7 ± 5.4	43.4 ± 2.0*	35.0 – 54.0
MCH (pg)	27.8 ± 2.1	27.6 ± 2.2 ^{ns}	25.0 – 33.0
MCHC (pg)	32.7 ± 1.0	30.3 ± 3.2*	28.0 – 40.0
Hemoglobin (g/dL)	14.9 ± 1.8	13.8 ± 0.8*	12.0 –18.0
Thrombocytes (m/mm^3)	297 ± 48	295 ± 48 ^{ns}	150 – 450

Values are Mean ± SD (n=37). *p<0.05, ns = not significant in comparison with initial values.

The total plasma protein and albumin levels were within normal range (normal range for total proteins 6.4 g/dL - 8.3 g/dL). The plasma levels of creatinine were normal and 24 hour excretion in urine was also in the normal range (Tables 5 and 8).

Table5. Plasma proteins, creatinine, lipid profile and hydroperoxide levels of trainees

Variable	Initial	after 1 month
Total Protein (g/dL)	7.22 ± 0.51	8.03 ± 0.54*
Albumin (g/dL)	2.6 ± 0.58	3.26 ± 0.60*
Globulin (g/dL)	4.62 ± 0.27	4.77 ± 0.36*
A/G Ratio	0.57 ± 0.14	0.69 ± 0.16*
Creatinine (µmol/L)	135 ± 11	141 ± 22 ^{ns}
Uric Acid (mg/dL)	6.8 ± 2.1	6.4 ± 1.7 ^{ns}
Cholesterol (mg/dL)	117.8 ± 32.3	134.9 ± 27.7*
HDL (mg/dL)	57.6 ± 10.3	58.4 ± 11.8 ^{ns}
LDL (mg/dL)	51.6 ± 28.7	66.4 ± 24.4*
Triglycerides (mg/dL)	43.3 ± 25.5	50.2 ± 25.5 ^{ns}
Total Antioxidants (mM)	0.74 ± 0.22	0.54 ± 0.20*
FOX-1 (µ mol/ml)	40.2 ± 11.4	45.8 ± 16.1*

Values are mean ± SD (n=37). *p< 0.05 in comparison with initial.

Levels of urinary nitrogen studied initially and after training indicated a positive nitrogen balance. Computation of nitrogen balance [Nitrogen intake (g/24 h.) – {urinary Nitrogen (g/24h) + 2 g /24 h}] is important and oldest method for the study of the adequacy of protein nutrition. It is excellent but cumbersome biochemical measurement that truly reflects both the somatic and visceral protein pools[33]. This indicates adequacy of existing ration scale in terms of protein content.

Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured in plasma samples. The values were well within the desirable range of each variable. Increase in total cholesterol and LDL-cholesterol was observed and this may be the effect of exercise-induced mobilisation of lipids. Values need to be normalised against total lipids. Similar observations were made during our earlier study conducted on Ghatak Trainees at Belgaum[34].

Both lipid and water-soluble vitamins were measured in plasma samples. Values were in the optimal range indicating optimal intake.

The β– Carotene and carotenoids have got antioxidant property and also protect from degenerative diseases and a certain type of cancers[35-38]. The vitamin A in the form of retinal is essential for vision, growth, and differentiation of epithelial tissue and is required for bone growth, immunity, reproduction and embryonic development. Vitamin A is obtained from the diet in two major forms. Preformed vitamin A as retinol is obtained from animal sources such as liver, butter, cheese, margarine, dried milk, cream, fortified milk, kidneys and some seafood.

Provitamin A is provided as β– carotene, α– carotene and other carotenoids. In current RDA[17] the efficiency ratio of conversion of β– carotene to retinol is taken as 0.16. Important sources of provitamin A are pumpkin, squash, carrots, tomatoes, peaches, mangoes and most of the green leafy vegetables.

Vitamin D deficiency is prevalent in general population. However, participants of present study have optimal levels of 25-OH Cholecaciferol and this may be because of good amount of sun exposure during different outdoor games and activities. Vitamin E acts as antioxidant vitamin. Acceptable levels are considered as ≥ 7.0 µg/ml (or ≥ 7.0 mg/L). Vegetable oils, nuts, seeds and most grains are good sources of vitamin E. All tocol and tocotrinol derivatives, which exhibit activity of α-tocopherol, are given generic name vitamin E.

Table 6. Plasma Levels of vitamins and minerals

Variable	Initial	After 1 month	Normal range
Vitamin A (µg/dL RE)	64.9 ± 14.4	60.5 ± 11.4	15-65
β-Carotene (µg/dL)	26.1 ± 1.7	30.1 ± 2.2	15-300
Vitamin E (mg/L)	11.2 ± 3.1	7.82± 4.0	> 7.0
Vitamin D_3 (ng/mL)	32.0 ± 3.8	37.8 ± 4.0 *	> 30
Vitamin C (mg/dL)	0.8 ± 0.12	1.04 ± 0.22*	0.4-2.0
Thiamin ((µg/dL)	5.74 ± 1.55	6.64± 1.55	2.5-7.5
Riboflavin (µg/L)	11.5 ± 3.4	10.0 ± 5.4	1-19
Vitamin B_{12} (pg/ml)	340 ± 91	420 ± 52 *	210-911
Cu (mg/L)	0.61 ± 0.16	0.56 ± 0.12	0.6-1.4
Zn (mg/L)	0.76 ± 0.13	0.70 ± 0.11	0.66-1.1
Mg (mg/L)	46.18 ± 12.6	47.78 ± 7.5	18-30

Values are mean ± SD (n=37). *p< 0.05 in comparison with initial. RE-Retinol equivalent

The levels of vitamin C were estimated both in plasma and 24 h urine samples. Plasma levels of vitamin C were found to be in normal range (0.4 mg/dL - 1.5 mg/dL). Excretion of vitamin C in 24 h urine was also more than reported normal range i.e. 6 mg/day - 18 mg/day as intakes were much higher than RDA (Table 8) and excesses is not stored in the body.

Vitamin C has a primary role in formation of collagen[39], helps in wound healing, formation and maintenance of cartilage, teeth, gums, bones, muscles and skin[40]. Vitamin C participates in hydroxylation of lysine to hydroxylysine and hydroxylation of proline to hydroxyproline[41]. Vitamin C participates in the

synthesis of tyrosine, carnitine, adrenal hormones, vasoactive amines, microsomal drug metabolism, leukocyte function and wound healing. Ascorbic acid acts as major antioxidant and a free radical scavenger thus protects cells from oxidative damage[39]. Ascorbic acid is absorbed very efficiently from diet[42].

Thiamin (vitamin B_1) status was assessed using erythrocyte transketolase activity as shown in Table 7 as a functional test[23,43,44].

Table 7. Erythrocyte Enzyme markers of vitamin status

Variable	Initial	After 1 Month of Training
Transketolase TPP Activation Factor	1.22 ± 0.77 (8)	1.44 ± 0.57 (13)
Glutathione reductase FAD Activation Factor	1.02 ± 0.07 (2)	1.06 ± 0.10 (4)
Aspartate Aminotransferase PLP Activation Factor	2.05 ± 1.18 (17)	2.46 ± 2.1 (14)

Values are Mean ± SD. Values in parenthesis indicates participants with lower levels than the acceptable limit i.e. 1.15 for TK, 1.50 for GR & 1.70 for AST indicative of lower levels of thiamine, riboflavin and Vitamin B_6 respectively.

Table 8. Urinary excretion of vitamins and metabolites

Parameters	Initial	After 1 month
Thiamine (µg/day)	305 ± 214	246 ± 90
Riboflavin (µg/day)	664 ± 310	720 ± 392
N- Methyl Nicotinamide (mg/g creatinne)	21.4 ± 12.3	20.8 ± 12.2
Methyl Malonic Acid (nmol/day)	1.78 ± 1.50	1.05 ± 0.70
Vitamin C (mg/day)	44 ± 23	39 ± 32
Urinary Nitrogen (g/day)	13.1 ± 7.40	11.1 ± 8.2
Creatinine (g/day)	1.24 ± 0.42	1.21 ± 0.45

Values are Mean ± SD.

The relative enhancement of erythrocyte transketolase activity by *in vitro* saturation with TPP is considered to be a sensitive and specific measure for the detection and evaluation of thiamin status. This measurement is referred as TPP effect (in present) or as the erythrocyte thiamin transketolase activity coefficient. Activation factor using TPP were found more than 1.15 in 8 participant initially and the number increased to 13 after 1 month of training, which indicates risk of deficiency.

We have also measured urinary thiamin excretion using thiochrome method. Acceptable levels of thiamin excretion are ≥ 66 µg/g creatinine[23]. The excretion levels were found to be normal. Good source of thiamin are pork, ham, beef, liver, green pea, whole grain wheat, cornmeal and brown rice. In Western countries, 25 per cent thiamin intake is provided by thiamin fortified foods. Riboflavin (vitamin B_2) status was assayed using erythrocyte glutathione reductase activity and stimulation of activity by in vitro addition of flavin adenine dinucleotide (FAD) an shown in Table 7. The acceptable range of activation is 1.20 or 20 per cent more than normal activity.

However, Prasad[45], *et al.* on the basis of supplementation studies and on well to do children have suggested that the cut off values of activation factor of glutathione reductase for prediction of normal riboflavin status should be 1.5 or even higher. Taking this into account the values obtained are showing optimal riboflavin status. Only 2 participant initially and 4 after training showed a low status of riboflavin. The urinary excretion was also found to be normal (normal range 0.5 mg/day to 0.8 mg/day). The ration scale contains sufficient amount of riboflavin as general requirements are 0.6 mg/1000 kcal. The vitamin is heat stable and has limited water solubility; the very little amount is lost during the cooking and processing of food. Suboptimal dietary intakes of riboflavin occur in many third world countries including Thailand, Gambia, India, Nigeria, and China[46]. Deterioration of riboflavin status can occur due to extensive physical activity as flavin enzymes are involved in cellular respiration and energy production. Several non-dietary factors may also contribute to high prevalence of subclinical (biochemical) riboflavin deficiency[47].

Pyridoxine (vitamin B_6) status was determined using an estimation of erythrocyte Aspartate aminotransferase and its activation by pyridoxal 5' phosphate in vitro. The activation coefficient <1.70 is considered acceptable[42]. Out of the 37 participant initially 17 and after 1 month of training 14 participant showed lower levels of pyridoxine.

Niacin status was evaluated by determining N' - methyl nicotinamide in urine and was found normal. Vitamin B^{12} status was determined by measuring plasma levels as well as excretion of methylmalonic acid in 24-hour urine.

Methyl malonyl CoA mutase has B^{12} as a coenzyme and in B^{12} deficiency methyl malonyl coenzyme A accumulates and is excreted as methylmalonic acid (MMA) or its salt. In a normal person the excretion of MMA is the 0-35 µmol/24 h and with the colorimetric method that we have used here, the normal range is 0 µmol/24 h – 93 µmol/24 h[26]. Values were within a normal range indicating optimal levels of vitamin B^{12} (Table7). In hematological profile, the Mean corpuscular volume (MCV) was also normal.

There is a possibility of increased requirement of antioxidants to ameliorate oxidative stress during increased physical activity. There are several reports indicating increased oxidative stress and decreased antioxidant levels during exercise[11,15,48-49]. The hydroperoxide levels were measured using FOX-1 assay and levels were found in a normal range[27]. The total antioxidant activity was also well within reported normal range (0.5 mmol/L - 2.0 mmol/L). However, a decrease in total antioxidant levels along with a rise in hydroperoxides is indicative of an increased requirement for antioxidants in the diet. Blood pressure (systolic and diastolic), resting heart rates were in normal healthy range (Table 9) indicating proper functioning of the cardiovascular system and there was no change after 1 month of training.

Handgrip strength was increased during one month in comparison to initial (initial: 41.5 kg ± 8.8 kg, after 1 month 46.5 kg ± 6.1 kg, $p<0.05$). Measurement of the hand grip is considered as an easy functional measure of nutritional status[50]. Being PT Trainers of Navy the study participants were very much aware of their nutritional, requirements. This may also be one of the key factors behind the observed good nutritional status in spite of very high energy expenditure.

Table 9. Resting blood pressure, heart rate & BMR

Variable	Mean ± SD
Blood Pressure (mmHg)	
Systolic	116 ± 12
Diastolic	72 ± 9
Heart Rate (Beats/min)	75 ± 8
BMR (kcal/day)	1615 ± 111

Values are Mean ±SD

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