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Original Article

Vitamin C and E supplementation and high intensity interval training induced changes in lipid profile and haematological variables of young males

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ABSTRACT

High intensity interval training (HIIT) causes oxidative stress and haematological alteration. Present study was aimed to evaluate the effect of 8 weeks' supplementation of vitamin C and E on HIIT induced changes in lipid profile parameters and haematological variables. Hundred six male adolescent players were randomly assigned into five age-matched groups, i.e., Control (no exercise + placebo), HIIT (placebo), HIIT + vitamin-C (1 000 mg/day), HIIT + vitamin-E 400 IU/day) and combined HIIT + vitamin C and E. Morning and evening sessions (90 min) of HIIT included 4 phases (15 min each) with 3 sets (4 min each). Each 4 min HIIT set consisted of 2 min intense sprint workout (90%–95% of heart rate maximum [HR_{max}]) followed by 1 min active recovery (60%–70% HR_{max}) followed by 1 min of complete rest (1:1 work-rest ratio). Lipid profile parameters, haematological variables, endurance capacity and vertical jump were evaluated by standard protocols. Significant decrease in body weight, fat%, total cholesterol, triglyceride, Total Cholesterol/High Density Lipoprotein-Cholesterol and significant increase in High Density Lipoprotein-Cholesterol, maximal oxygen consumption, vertical jump were observed for all four intervention groups. White blood cell count, red blood cell count, haemoglobin percentage and haematocrit values were significantly decreased while platelet count and platelet-to-leukocyte ratio (PLR) ratio were increased significantly only for HIIT group. Blood level of tocopherol and ascorbic acid was significantly increased (values were within the normal range) in all the respective vitamin supplemented groups. Supplementation of vitamin C and E secures health protection with suppressed haemolysis and improved inflammatory blood variables with enhanced explosive leg strength and lipid profile parameters without any concomitant change in endurance capacity.

Introduction

High-intensity interval training (HIIT) is a time-efficient strategy and an efficient alternative to traditional endurance training among athletes to develop both the aerobic and anaerobic systems within a short period.[1] But strenuous exercises like eccentric intervals/high-intensity training inflict metabolic and mechanical stress due to the need for excessive energy in a very short time. This higher need for energy increase oxygen consumption leading to the generation of mitochondrial reactive oxygen species (ROS).[2,3] Studies depict that high-intensity exercises elicit detrimental effects on skeletal muscle[2,4] and increase circulatory proinflammatory cytokines (interleukin-6 [IL-6] and tumour necrosis factor-alpha [TNF-α]) in proportion to ROS generation.[5,6]

High-intensity/eccentric exhaustive training induces oxidative stress and alters the haematological profile by facilitating haemolysis along with a decrease in ferritin, haemoglobin (Hb) content, and haematocrit value (HCT). However, the erythrocyte-related changes occur simultaneously with decreased leukocyte count, increased platelet count, and platelet-to-leukocyte ratio (PLR) due to the effect of HIIT.[7] Examination of the literature revealed that antioxidant vitamins (e.g., vitamin A, vitamin C and vitamin E) are effective in preventing exercise-induced inflammation-like responses and adverse haemorrhagic changes.[8,9]

Vitamin C and vitamin E are the most prevalent vitamin supplements

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List of abbreviations

HIIT	high-intensity interval training
ROS	Reactive oxygen species
IL-6	interleukin 6
TNF-α	tumour necrosis factor alpha
HR_{max}	heart rate maximum
BMI	Body mass index
BF %	body fat percentage
TC	total cholesterol
TG	triglyceride
HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
VLDL-C	very high-density lipoprotein cholesterol
TC/HDL-C	total cholesterol/high-density lipoprotein cholesterol ratio
WBC	white blood cell
RBC	red blood cell
Hb	haemoglobin
HCT	haematocrit
PLR	platelet-to-leukocyte ratio
$\dot{V}O_{2max}$	maximal oxygen consumption
VJ	vertical jump
LES	leg explosive strength
LCFA	long-chain fatty acid
$FABP_{pm}$	fatty acid-binding protein
CS	citrate synthase
β-HAD	beta hydroxyacyl coenzyme A dehydrogenase
AMPK	AMP-activated protein kinase
MAPK	p38 mitogen-activated protein kinase
MI	myocardial infarction
PGC-1α	peroxisome proliferator-activated receptor-gamma coactivator one-alpha

which are used by approximately 20% of the sports population either individually or in combined form. Vitamin C and E ingestion are still widely recommended as beneficial supplements among sportspersons to improve their performance irrespective of any specific guidelines or doses of these supplements.[2,3] A couple of studies predicted the beneficial effects of vitamin C and E supplementation on haematological variables.[8,10–12] None of the previous studies were designed to examine the effect of vitamin C and E supplementation on high-intensity training-induced alterations in haematological variables. Hindering the effect of antioxidant vitamins over cellular adaptations to endurance training has also been reported.[13] Therefore, controversies exist regarding the effectiveness of antioxidant vitamin supplementation on training-induced adaptive responses. Moreover, the most effective mode of vitamin C or E supplementation method (combined or alone) has not yet been explored.

The present study aimed to investigate the effects of antioxidant vitamin (C and E) supplementation on HIIT-induced changes in lipid profile and haematological variables concerning some selected physical fitness variables in young team game players. Further, the study was also focused to hypothesise the most effective mode of antioxidant vitamin (vitamin C and E) supplementation, i.e., either individually or in combined doses.

Material and methods

Selection of subjects

Hundred six young Indian male team game players (football [$n = 49$] and field hockey [$n = 57$]) were recruited for the present study. Participants were randomized to one negative control group (no HIIT, no vitamin), one positive control group (HIIT with no vitamin), and three experimental groups (vitamin supplemented groups). The negative control group ($n = 20$; mean age = [15.3 ± 1.64] years [yrs]) participated in control and was supplemented with a placebo. The placebo control group ($n = 20$; mean age = [15.6 ± 1.53] years [yrs]) participated in HIIT and was supplemented with a placebo. The remaining three experimental groups took part in the same HIIT protocol and were supplemented with vitamin C alone ($n = 22$; mean age = [16.0 ± 1.97] yrs), vitamin E alone ($n = 21$; mean age = [15.5 ± 1.54] yrs) and combined vitamin C + E ($n = 23$; mean age = [15.8 ± 1.81] yrs), using a fixed randomization scheme generated by the Moses-Oakford algorithm[14] with a block size of 8 and an allocation ratio of 1:1:1:1. So the groups were classified as follows.

i) Control: No HIIT + Placebo (No vitamin)
ii) HIIT: HIIT intervention + Placebo (No vitamin)
iii) Vitamin C: HIIT intervention + vitamin C
iv) Vitamin E: HIIT intervention + vitamin E
v) Vitamin C + E: HIIT intervention + vitamin C + vitamin E

All participants had a minimum of 4 years of professional training experience and were only recruited after the clinical examination.[15]

Ethical approval

Written informed consent was obtained from each player and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki. Ethical clearance (Ref No. IHEC/AB/P82/2019) was obtained from the Institutional Human Ethical Committee (IHEC), Department of Physiology, University of Calcutta.

Vitamin intervention

Participants were supplemented with active vitamin C tablets (1 000 mg ascorbate/tablet/day) and corresponding placebo tablets (dicalcium phosphate, 380 mg/tablet/day), both purchased from Consolidated Midland Co., India, and active vitamin E capsules (400 IU d-α-tocopheryl acetate/capsule/day) and corresponding placebo capsules (soybean oil), both donated by Henkel Co. India. Both the negative control and positive control group were treated as placebo-controlled one with HIIT intervention and one without HIIT intervention and the study endures the placebo-controlled double-blinded study intervention. Vitamins dose was prescribed by government-authorized physicians. Participants were instructed not to change their diet or take other vitamin supplements for the next two months. Supplementation adherence was assessed by the average pill counts at each follow-up visit, changes in serum levels of ascorbic acid and α-tocopherol from baseline and self-reports. The supplementation consumption logs were recorded by the research team member to ensure participants' compliance with supplement treatment.

Detailed training program

Three hours of HIIT (sprint intervals) practice was completed three times a week on alternate days (i.e., Monday, Wednesday, and Friday) and continued for eight weeks. The formulation and implementation of the HIIT protocol were accomplished by qualified coaches under the guidance of scientific experts. Three hours of HIIT was divided into two sessions (both morning and evening sessions of 90 min each). Each 90 min training phase started with a warmup session and ended with a cool-down session (each session consisted of 15 min of slow running at an intensity of 50% of heart rate maximum [HR_{max}]). During HIIT (total of

60 min/morning or evening session), subjects were asked to perform three all-out HIIT sets. Each small HIIT set consisted of a 2 min intense sprint workout (at 90%–95% of heart rate max [HR_{max}]) followed by 1 min active recovery (60%–70% HR_{max}) which was followed by 1 min of complete rest. Thus, the whole training workload had a work-rest ratio of 1:1. Finally, each 2 min intense sprint workout set was intervened with a brief stride of repetition maximum (rep.) running in increasing manner throughout the 8 weeks i.e., 5 rep. in 1st – 2nd week, 6 rep. in 3rd – 4th week, 7 rep. in 5th – 6th week and 8 rep. in 7th - 8th week.

On the other hand, the control group players continued systematic low-volume physical activity/recreational exercise which includes lower intensity (training load at around 60% HR_{max}) physical activity (i.e., stretching, jogging, low-intensity running, etc) for 3 days/week (on alternate basis) till 8 weeks interval to match the HIIT training duration (Table 1).

Measurement of anthropometric parameters

General physical characteristics, i.e., body height (metre - m) and body weight (kilogram - kg) were measured by using the Seca Alpha stadiometer (model – 213, Seca Deutschland, Germany) and Seca Alpha weighing scale (model – 770, Seca Deutschland, Germany) respectively. Body mass index (BMI) was calculated by using the standard formula.[16] Body fat percentage (BF%) was calculated by the standard formula of Brozek, after measuring the biceps, triceps, subscapular, and suprailiac skinfold thicknesses by using Harpenden skinfold calliper (with constant tension).[17]

Assessment of biochemical parameters

Process of blood collection and plasma sample preparation

Blood samples were collected from the antecubital vein into centrifuge tubes for serum preparation (without anticoagulant) between 6:00 a.m.–8:00 a.m. in the pre-prandial state (after 8–10 h of overnight fasting) to avoid possible differences due to diurnal variation. Each blood supernatant was centrifuged at 3 000 rpm for 15 min to ensure complete serum separation. The samples were transferred into cryo-vials and stored and preserved at −20 °C for later biochemical analyses.[18] All laboratory tests were performed at room temperature varying from 23 °C to 25 °C with a relative humidity of 50%–60%.

Assay for lipid profile parameters

Serum lipid profile include total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and TC/HDL-C ratio. The TC and the HDL-C fraction were estimated by the method of Manna et al.[19] Whereas, TC was measured via chod-pod method, TG via GPO-PAP method, where TC, TG, HDL were measured at 505 nm on a spectrophotometer. VLDL-C and LDL-C were indirectly measured by using the Friedewald equations. All values of TC, TG, HDL-C, LDL-C, and VLDL-C were expressed in milligram (mg/dl). Quality control measures were taken during the lipid profile assessment where samples were reconstituted with 10 ml of double distilled water and kept in 500 μL aliquots at −20 °C to be thawed only once before use. The stability of the samples is 3–4 h at room temperature, 7 days at 2 °C −8 °C, and 4–5 days at −20 °C. Both the pre- and post-test conditions for blood lipid analysis were kept similar and were conducted at the same laboratory, but pre- and post-testing were not completed at the same time.

Determination of complete blood count

The complete blood count included white blood cell (WBC) count, red blood cell (RBC) count, platelet count, haemoglobin percentage (Hb%), haematocrit (HCT), and platelet-to-leukocyte (PLR) ratio was analysed using a Beckman Coulter ACT 5 DIFF CP analyser (Bera, California, US).[20]

Assessment of vitamin C and vitamin E

Serum ascorbic acid levels were measured based on the reduction of Fe(III) to Fe(II) by ascorbic acid, followed by chromogenic chelation of Fe(II) with ferrozine. Intra-assay *CV* (coefficient of variation) of this assay was 3.2%. Serum α-tocopherol levels were measured by isocratic high-performance liquid chromatography. Intra-assay *CV* of this assay was 3.3%.[14]

Determination of physiological variables

Maximal oxygen consumption ($\dot{V}O_{2max}$) was measured using an incremental exercise protocol on a bicycle ergometer (Ergoline, VIA Sprint 150P, Germany). Athletes were asked to start pedalling without any load for 1st min. An initial 25-Watt (W) workload was applied for 2 min and the work rate progressively increased by 25 W every 2 min until exhaustion.[15] During the incremental test, breath by breath metabolic gas analyser (MetaMax 3B, CORTEX Biophysik GmbH, Leipzig, Germany) was used to determine $\dot{V}O_{2max}$ depending on the following criteria: i) plateau in $\dot{V}O_2$ (2 $ml \cdot kg^{-1} \cdot min^{-1}$) despite the increased work rate, ii) respiratory exchange ratio (RER) $\geq$ 1.1, iii) > 90% of age-predicted HR_{max} ± 5%, iv) voluntary exhaustion.[15,21] A polar heart rate monitor (Polar RS800CX, Polar Electro OY, Kempele, Finland) was used to measure HR and HR_{max} throughout the exercise protocol.[15] Vertical jump (VJ) test was used to measure leg explosive strength (LES). Subjects were asked to jump as high as possible from a standing position just beside a wall and the maximum jump distance was measured to the nearest 0.1 cm and two attempts were given to each athlete.[22]

Dietary recall and nutritional intake calculations

Participants' 24-h diet recall information was recorded by means of a self-administered, and semi-structured questionnaire. Cooked food items were converted to raw amounts, and the nutrients were calculated accordingly. Three consecutive dietary recalls were repeated to reduce

Table 1
Details of training programme including type, sessions, intensity of exercise.

Daily training	HIIT Sessions	Training details			Training type	Training intensity
3 h daily training	Morning session (1.5 h)	Warmup (15 min)				
		HIIT (60 min)	1st and 2nd week	3 sets × 5 rep.	2 min intense sprit workout	90%–95% HRmax
			3rd and 4th week	3 sets × 6 rep.		
			5th and 6th week	3 sets × 7 rep.		
			7th and 8th week	3 sets × 8 rep.		
		Cooldown (15 min)				
	Evening session (1.5 h)	Warmup (15 min)				
		HIIT (60 min)	1st and 2nd week	3 sets × 5 rep.	2 min intense sprit workout	90%–95% HRmax
			3rd and 4th week	3 sets × 6 rep.		
			5th and 6th week	3 sets × 7 rep.		
			7th and 8th week	3 sets × 8 rep.		
		Cooldown (15 min)				

HIIT = high-intensity interval training, HRmax = heart rate maximum, min = minute, h = hour, rep. = repetition.

the imprecision in nutrient intakes. Nutrients were calculated using Diet soft software (version 1.1.6). The nutrients in the software are based on the values published in the "Nutritive value of Indian Foods" by ICMR, 2017.[23] Questionnaire constituting of 20 and 12 questions was structured using a previous study and those referenced by Debnath et al.[24] Nutritional intake calculations were only done once before starting the study and these values were considered during supplementation.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 18.0 (SPSS Inc., Chicago, Il, USA) was used to analyse the data. Data were expressed as mean ± standard deviation (*SD*). The Shapiro-Wilk test was done to check the normality distribution. Two-tailed paired sample *t*-test was used to determine the difference between the means of pre- and post-training data set against every variable of each group. Two-way/mixed ANOVA was introduced to check the main effect of group and moment among all the groups at a time. Pearson's product-moment correlation, simple regression analysis, and analysis of % change were also performed for better interpretation of the data. A 95% confidence interval was set as the level of significance (see Table 2).

Results

The effect of vitamin C and E supplementation on HIIT-induced changes in general anthropometric parameters are presented in Table 3. A significant ($p < 0.001$) decrease in BF% was observed in all four intervened groups following training but an increase in BF% in the control group. Bodyweight and BMI were significantly decreased following intervention in three vitamin groups and the HIIT group except for weight in the HIIT group. Two-way ANOVA has depicted a significant difference in treatment only ($p < 0.01$) effect for BF% and time × treatment effect ($p < 0.001$) for height, body height, BMI, and BF%. Whereas, a significant time-only effect was observed in the case of variables i.e., height, weight, BMI, and BF%.

The effects of vitamin C and E supplementation on HIIT-induced changes in lipid profile variables are presented in Table 4. Significant ($p < 0.001$) decreases in TC, TG, and TC/HDL-C ratio were observed in all four groups (HIIT and three vitamins supplemented) following training in comparison to their corresponding pre-training values (except TG under the vitamin C + E group at $p < 0.01$). A significant ($p < 0.001$) increase was observed in HDL-C in all four groups (HIIT and three vitamins supplemented) following training (except HDL-C under the vitamin C group at $p < 0.01$). A significant ($p < 0.05$) decrease in VLDL-C was observed in HIIT and vitamin E post-training groups. On the other hand, LDL-C was the only lipid profile variable that was not altered post-training. Two-way ANOVA depicted significant differences in both time × treatment effect ($p < 0.001$) and time-only effect ($p < 0.001$) for TC, TG, HDL-C, and TC/HDL-C. Whereas, a significant time-only effect ($p < 0.01$) and treatment-only effect ($p < 0.05$) was observed in the case of HDL-C and TC/HDL-C ratio respectively.

Table 2
Comparison of nutritional intake among different intervention groups.

Parameters	Control ($n = 20$)	HIIT ($n = 20$)	Vitamin C ($n = 22$)	Vitamin E ($n = 21$)	Vitamin C + E ($n = 23$)
Energy (kCal)	2 654.71 ± 210.47	2 638.62 ± 269.92	2 522.26 ± 172.54	2 518.06 ± 195.54	2 585.19 ± 200.15
Carbohydrate (gm)	359.72 ± 40.34	360.98 ± 39.84	339.64 ± 36.48	337.29 ± 31.95	351.19 ± 37.19
Protein (gm)	108.95 ± 6.69	108.02 ± 8.27	105.57 ± 6.14	105.38 ± 7.88	106.83 ± 7.20
Fat (gm)	74.57 ± 6.19	72.85 ± 11.10	71.07 ± 3.71	71.46 ± 5.33	71.75 ± 5.23
Vitamin C (mg)	42.93 ± 13.23	43.80 ± 12.68	39.01 ± 12.04	40.83 ± 10.21	40.19 ± 11.39
Vitamin E (mg)	0.41 ± 0.17	0.44 ± 0.25	0.38 ± 0.17	0.40 ± 0.13	0.39 ± 0.18

Values are mean ± *SD*, HIIT = high-intensity interval training.

The effect of vitamin C and E supplementation on HIIT-induced changes in selected haematological parameters is presented in Table 5. A significant decrease was observed in WBC count, RBC count, Hb%, and HCT only in the HIIT group when compared post-training to the pre-training values. Whereas, platelet count and PLR depicted significant ($p < 0.05$ and $p < 0.01$ respectively) increase only in the HIIT group post-training part when compared to pre-training data. All other remaining vitamin-supplemented groups did not show any statistically significant variation among the haematological parameters. The two-way ANOVA result depicted no statistically significant difference when compared among haematological variables of all studied groups.

The effect of vitamin C and E supplementation on HIIT-induced alterations in vitamin C, vitamin E, $\dot{V}O_{2max}$, and VJ are presented in Table 6. Significant ($p < 0.001$) increases in both vitamin C and vitamin E were observed in all three-vitamin supplemented at post-training with a significant decrease in HIIT post-training group in comparison to pre-training values. On the other hand, significant ($p < 0.001$) increases in both $\dot{V}O_{2max}$ and VJ were observed in all four intervened groups (HIIT and three vitamins-supplemented groups) following training. The two-way ANOVA result depicted a significant ($p < 0.001$) difference in time × treatment effect and time-only effect for vitamin C, vitamin E, $\dot{V}O_{2max}$, and VJ. Whereas, significant treatment effect only was observed for vitamin C, vitamin E, and VJ variables but not except for $\dot{V}O_{2max}$.

Table 7 depicts the Pearson's product moment correlation coefficient of some selected lipids, haematological parameters, and physical fitness variables. Among all physical fitness variables, $\dot{V}O_{2max}$ and VJ showed a significant negative correlation with platelet count ($p < 0.05$) and PLR ($p < 0.01$) respectively.

The scatterplot-based relationship between VJ vs PLR and $\dot{V}O_{2max}$ vs platelet is presented in Fig. 1. Both the scatter plots of VJ vs PLR (R^2 linear values were 0.006, 0.278, 0.296, 0.014) and $\dot{V}O_{2max}$ vs platelet (R^2 linear values were 0.181, 0.002, 0.295, 2.243) are observed against HIIT, vitamin C, vitamin E, and combined vitamin C-E group respectively.

Discussion

The present study aimed to depict the effect of vitamin C and E supplementation on HIIT-induced changes in lipid profile parameters and haematological variables concerning some selected physical fitness variables of young team-game players. Supplementation of the antioxidant vitamins over the HIIT resulted in a significant increase in HDL-C, vitamin C, vitamin E, $\dot{V}O_{2max}$, VJ, and a decrease in body weight, BMI, BF%, TC, TG, TC/HDL-C. Though not statistically significant, WBC, RBC, platelet, PLR were somewhat decreased but not significantly while Hb% and HCT were somewhat but not significantly increased after the antioxidant supplementation.

In the present study, HIIT intervention improved the body composition profile by significantly reducing BF% (7.8%, $p < 0.001$) and BMI (0.7%, $p < 0.05$). These findings are in agreement with an earlier report.[25] High-intensity training generally hypothesized a reduction in body weight and the present study depicted a similar decrease in both body weight and BMI and that might be due to the excess intense training-induced lipolysis.[25,26] Skeletal muscle fat oxidation is a highly regulated process limited by several long-chain fatty acid (LCFA) membrane transporters among which fatty acid-binding protein ($FABP_{pm}$) and fatty acid translocase/CD36 are most important.[27] HIIT was suggested to increase the lipolysis rate/fat oxidation by increasing certain hormonal profiles such as catecholamine release which controls the β-adrenergic receptors in adipose tissue and increased $FABP_{pm}$ content in skeletal muscle.[26] On the other hand, the present study reported vitamin C- and E−induced improvements in body weight (0.7%, $p < 0.01$) and fat% (6.6%, $p < 0.001$) which might be due to the increased skeletal muscle fat oxidation resulted from several adaptations, including an increase in

Table 3
Comparison of anthropometric parameters between pre- and post-training phases of different intervened groups.

Parameters	Control ($n = 20$)	HIIT ($n = 20$)	Vitamin C ($n = 22$)	Vitamin E ($n = 21$)	Vitamin C + E ($n = 23$)	Two-way ANOVA	p value
Body height (cm)							
Pre-training	168.20 ± 5.22	169.65 ± 4.17	167.78 ± 6.15	170.09 ± 5.51	165.70 ± 6.07	Time × Treatment	< 0.001***
Post-training	168.24 ± 5.21	169.80 ± 4.09	167.97 ± 6.08	170.30 ± 5.43	166.00 ± 5.93	Treatment	0.078 NS
t value	−3.559**	−5.081***	−6.731***	−4.920***	−7.545***	Time effect	< 0.001***
p value	0.002	< 0.001	< 0.001	< 0.001	< 0.001		
% change	0.02 (↑)	0.1	0.1	0.1	0.2		
Body weight (kg)							
Pre-training	59.19 ± 5.37	59.80 ± 4.74	58.01 ± 6.28	59.98 ± 8.09	55.67 ± 7.54	Time × Treatment	<0.001***
Post-training	60.08 ± 4.73	59.49 ± 4.44	57.59 ± 6.21	59.31 ± 7.70	55.28 ± 7.14	Treatment	0.129 NS
t value	−3.192**	2.084 NS	3.527**	3.341**	3.175**	Time effect	0.027*
p value	0.005	0.051	0.002	0.003	0.004		
% change	1.5 (↑)	0.5	0.7	1.1	0.7		
BMI ($kg{\cdot}m^{-2}$)							
Pre-training	20.92 ± 1.59	20.77 ± 1.40	20.56 ± 1.28	20.66 ± 1.93	20.23 ± 2.06	Time × Treatment	< 0.001***
Post-training	21.23 ± 1.46	20.63 ± 1.30	20.36 ± 1.23	20.38 ± 1.80	20.02 ± 1.94	Treatment	0.423 NS
t value	−3.164**	2.748*	4.109**	3.802**	4.719***	Time effect	0.001**
p value	0.005	0.013	0.001	0.001	< 0.001		
% change	1.5 (↑)	0.7	1.0	1.3	1.0		
BF%							
Pre-training	11.90 ± 2.92	12.08 ± 3.04	15.05 ± 5.30	14.18 ± 4.94	17.17 ± 6.54	Time × Treatment	< 0.001***
Post-training	12.24 ± 2.85	11.14 ± 3.09	14.23 ± 4.79	13.02 ± 4.44	16.03 ± 5.90	Treatment	0.004**
t value	−8.643***	14.505***	5.443***	7.055***	7.577***	Time effect	< 0.001***
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		
% change	2.9	7.8	5.4	8.2	6.6		

Values are mean ± *SD*. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS = not significant, HIIT = high-intensity interval training, BMI = Body mass index, BF % = body fat percentage.

Table 4
Comparison of lipid profile parameters between pre- and post-training phases of different intervened groups.

Parameters	Control ($n = 20$)	HIIT ($n = 20$)	Vitamin C ($n = 22$)	Vitamin E ($n = 21$)	Vitamin C + E ($n = 23$)	Two-way ANOVA	p value
Total cholesterol (mg/dl)							
Pre-training	149.95 ± 30.71	147.60 ± 22.70	157.23 ± 20.83	154.48 ± 22.88	163.61 ± 26.30	Time × Treatment	< 0.001***
Post-training	159.70 ± 26.78	136.50 ± 18.73	151.09 ± 20.07	149.43 ± 21.06	152.52 ± 23.24	Treatment	0.234 NS
t value	−5.185***	4.888***	8.503***	5.538***	7.387***	Time effect	< 0.001***
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		
% change	0.3↑	7.5↓	3.9↓	3.3↓	6.8↓		
Triglyceride (mg/dl)							
Pre-training	63.65 ± 9.30	61.15 ± 12.23	63.00 ± 11.07	65.52 ± 10.49	65.04 ± 13.81	Time × Treatment	< 0.001***
Post-training	65.25 ± 9.87	55.85 ± 10.34	59.91 ± 10.68	61.76 ± 10.30	58.22 ± 14.84	Treatment	0.495 NS
t value	−3.018**	7.491***	8.316***	17.322***	3.414**	Time effect	< 0.001***
p value	0.007	< 0.001	< 0.001	< 0.001	0.002		
% change	0.3↑	8.7↓	4.9↓	5.7↓	10.5↓		
HDL-C (mg/dl)							
Pre-training	43.05 ± 8.49	44.20 ± 7.42	43.14 ± 7.67	44.19 ± 8.22	45.91 ± 8.38	Time × Treatment	< 0.001***
Post-training	43.00 ± 7.73	47.70 ± 7.49	45.45 ± 6.97	47.38 ± 6.71	50.30 ± 5.52	Treatment	0.218 NS
t value	0.076 NS	−20.571***	−3.051**	−7.440***	−5.409***	Time effect	< 0.001***
p value	0.940	< 0.001	0.006	< 0.001	< 0.001		
% change	0.2	7.9↑	5.3↑	7.2↑	9.6↑		
LDL-C (mg/dl)							
Pre-training	87.30 ± 21.14	86.20 ± 18.01	84.41 ± 19.75	88.05 ± 16.13	87.57 ± 18.99	Time × Treatment	0.185 NS
Post-training	88.95 ± 20.46	84.60 ± 17.09	90.50 ± 16.04	81.95 ± 24.94	94.17 ± 28.42	Treatment	0.828 NS
t value	−2.196*	0.406 NS	−1.385 NS	1.185 NS	−1.303 NS	Time effect	0.542 NS
p value	0.041	0.689	0.181	0.250	0.206		
% change	0.7↑	1.8↓	7.2↑	6.9↓	7.5↑		
VLDL-C (mg/dl)							
Pre-training	16.45 ± 5.48	16.80 ± 2.93	17.32 ± 5.09	16.95 ± 3.38	15.53 ± 6.85	Time × Treatment	0.123 NS
Post-training	17.10 ± 4.72	15.78 ± 3.25	15.55 ± 5.66	14.38 ± 6.32	12.57 ± 6.14	Treatment	0.309 NS
t value	−2.436*	2.765*	1.453NS	2.538*	1.987NS	Time effect	0.001**
p value	0.025	0.012	0.161	0.020	0.059		
% change	1.2↑	6.1↓	10.2↓	15.2↓	19.0↓		
TC/HDL-C							
Pre-training	3.53 ± 0.66	3.37 ± 0.45	3.72 ± 0.68	3.55 ± 0.52	3.66 ± 0.84	Time × Treatment	< 0.001***
Post-training	3.76 ± 0.53	2.89 ± 0.36	3.37 ± 0.50	3.17 ± 0.35	3.06 ± 0.51	Treatment	0.031*
t value	−2.526*	9.147***	4.937***	7.616***	6.463***	Time effect	< 0.001***
p value	0.021	< 0.001	< 0.001	< 0.001	< 0.001		
% change	2.9↑	14.2↓	9.4↓	10.7↓	16.4↓		

Values are mean ± *SD*, *$p < 0.05$, ***$p < 0.001$, NS = not significant, HIIT = high-intensity interval training, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, VLDL-C = very high-density lipoprotein cholesterol, TC/HDL-C = total cholesterol/high-density lipoprotein cholesterol ratio.

mitochondrial regulatory steps, rate of lipolysis, and fatty acid transportation.[27–29]

In the present study, high-intensity training significantly ($p < 0.001$) reduced serum TC, TG, TC/HDL-C, and increased HDL-C. These changes

Table 5
Comparison of selected haematological parameters between pre- and post-training phases of different intervened groups.

Parameters	Control ($n = 20$)	HIIT ($n = 20$)	Vitamin C ($n = 22$)	Vitamin E ($n = 21$)	Vitamin C + E ($n = 23$)	Two-way ANOVA	p value
WBC ($*10^3/\mu l$)							
Pre-training	6.84 ± 1.20	6.63 ± 1.45	6.81 ± 1.64	6.73 ± 1.87	6.98 ± 1.95	Time × Treatment	0.999 ^{NS}
Post-training	6.71 ± 1.67	6.41 ± 1.27	6.62 ± 1.46	6.57 ± 1.49	6.85 ± 1.49	Treatment	0.923 ^{NS}
t value	0.326 ^{NS}	3.953**	0.460 ^{NS}	0.542 ^{NS}	0.625 ^{NS}	Time effect	0.225 ^{NS}
p value	0.748	0.001	0.650	0.594	0.538		
% change	1.9↓	3.3↓	2.8↓	2.4↓	1.9↓		
RBC ($*10^6/\mu l$)							
Pre-training	5.42 ± 0.49	5.22 ± 0.34	5.38 ± 0.45	5.21 ± 0.43	5.29 ± 0.48	Time × Treatment	0.630 ^{NS}
Post-training	5.35 ± 0.50	5.10 ± 0.29	5.29 ± 0.29	5.15 ± 0.24	5.33 ± 0.41	Treatment	0.213 ^{NS}
t value	1.000 ^{NS}	5.282***	0.831 ^{NS}	0.762 ^{NS}	−0.617 ^{NS}	Time effect	0.079 ^{NS}
p value	0.330	< 0.001	0.416	0.455	0.543		
% change	1.3↓	2.3↓	1.7↓	1.1↓	0.8↑		
Platelet ($*10^3/\mu l$)							
Pre-training	247.40 ± 58.93	235.60 ± 51.99	242.95 ± 41.62	251.19 ± 58.25	255.74 ± 58.98	Time × Treatment	0.321 ^{NS}
Post-training	244.60 ± 57.32	248.40 ± 50.15	239.00 ± 37.00	246.24 ± 50.93	247.35 ± 48.32	Treatment	0.947 ^{NS}
t value	0.836 ^{NS}	−2.412*	1.586 ^{NS}	1.496 ^{NS}	0.607 ^{NS}	Time effect	0.663 ^{NS}
p value	0.413	0.026	0.128	0.150	0.550		
% change	1.1↓	5.4↑	1.6↓	2.0↓	3.3		
Haemoglobin % (gm/dl)							
Pre-training	14.76 ± 0.93	14.66 ± 0.68	14.95 ± 0.90	14.46 ± 0.91	14.85 ± 1.02	Time × Treatment	0.371 ^{NS}
Post-training	14.59 ± 0.72	14.38 ± 0.59	14.90 ± 0.73	14.53 ± 0.74	14.93 ± 0.87	Treatment	0.194 ^{NS}
t value	1.398 ^{NS}	7.604***	0.223 ^{NS}	−1.078 ^{NS}	−0.415 ^{NS}	Time effect	0.284 ^{NS}
p value	0.178	< 0.001	0.826	0.294	0.682		
% change	1.2↓	1.9↓	0.3↓	0.5↑	0.5↑		
HCT							
Pre-training	43.86 ± 2.53	43.45 ± 3.07	44.29 ± 2.43	43.30 ± 2.22	43.71 ± 3.00	Time × Treatment	0.565 ^{NS}
Post-training	43.37 ± 2.86	42.74 ± 2.82	44.20 ± 2.13	43.57 ± 1.94	44.27 ± 2.34	Treatment	0.440 ^{NS}
t value	0.573 ^{NS}	4.188***	0.127 ^{NS}	−0.403 ^{NS}	−1.156 ^{NS}	Time effect	0.742 ^{NS}
p value	0.574	< 0.001	0.900	0.691	0.260		
% change	1.1↓	1.6↓	0.2↓	0.6	1.3↑		
PLR							
Pre-training	36.83 ± 9.22	36.14 ± 7.22	37.00 ± 8.32	38.94 ± 10.36	38.32 ± 10.67	Time × Treatment	0.646 ^{NS}
Post-training	37.71 ± 9.79	39.08 ± 6.04	37.35 ± 8.45	38.56 ± 8.83	37.70 ± 11.05	Treatment	0.972 ^{NS}
t value	−0.478 ^{NS}	−3.736**	−0.161 ^{NS}	0.220 ^{NS}	0.334 ^{NS}	Time effect	0.429 ^{NS}
p value	0.638	0.001	0.874	0.828	0.742		
% change	2.4↑	8.1↑	0.9↑	1.0↓	1.6↓		

Values are mean ± *SD*, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, NS = not significant, HIIT = high-intensity interval training, WBC = white blood cell, RBC = red blood cell, HCT = haematocrit, PLR = platelet-to-leukocyte ratio.

Table 6
Comparison of plasma vitamin C and E, maximal oxygen consumption ($\dot{V}O_{2max}$) and vertical jump (VJ) between pre- and post-training phases of different intervened groups.

Parameters	Control ($n = 20$)	HIIT ($n = 20$)	Vitamin C ($n = 22$)	Vitamin E ($n = 21$)	Vitamin C + E ($n = 23$)	Two-way ANOVA	p value
Vitamin C (μmol/L)							
Pre-training	56.15 ± 7.96	58.29 ± 9.38	57.98 ± 10.13	56.29 ± 7.11	59.71 ± 7.45	Time × Treatment	< 0.001***
Post-training	56.03 ± 7.97	58.05 ± 9.18	69.48 ± 9.91	57.36 ± 7.04	69.05 ± 6.91	Treatment	0.001**
t value	2.541*	3.751**	−42.853***	−22.066***	−44.784***	Time effect	< 0.001***
p value	0.020	0.001	< 0.001	< 0.001	< 0.001		
% change	0.2 (↓)	0.4↓	19.8↑	1.9↑	15.6↑		
Vitamin E (μmol/L)							
Pre-training	26.48 ± 2.54	26.32 ± 2.87	26.93 ± 3.30	27.22 ± 2.81	27.17 ± 3.12	Time × Treatment	< 0.001***
Post-training	26.37 ± 2.52	26.13 ± 2.76	27.38 ± 3.24	31.07 ± 2.47	31.96 ± 2.82	Treatment	< 0.001***
t value	1.952 ^{NS}	7.266***	−16.431***	−26.236***	−31.358***	Time effect	< 0.001***
p value	0.066	< 0.001	< 0.001	< 0.001	< 0.001		
% change	0.4 (↓)	0.7↓	1.7↑	14.1↑	17.6↑		
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)							
Pre-training	51.43 ± 4.05	49.70 ± 2.68	50.12 ± 3.04	49.08 ± 3.01	50.96 ± 3.43	Time × Treatment	< 0.001***
Post-training	51.87 ± 3.66	56.22 ± 2.88	56.39 ± 2.89	55.05 ± 3.43	57.51 ± 3.17	Treatment	0.074 ^{NS}
t value	−2.368*	−20.934***	−29.704***	−21.894***	−36.420***	Time effect	< 0.001***
p value	0.029	< 0.001	< 0.001	< 0.001	< 0.001		
% change	0.9 (↑)	13.1↑	12.5↑	12.2↑	12.8↑		
Vertical jump (cm)							
Pre-training	45.90 ± 3.84	45.10 ± 3.65	46.91 ± 5.83	45.95 ± 5.35	47.57 ± 5.66	Time × Treatment	< 0.001***
Post-training	45.38 ± 3.34	49.05 ± 3.32	51.05 ± 5.06	49.67 ± 4.13	52.17 ± 4.81	Treatment	0.029*
t value	2.146*	−12.033***	−6.930***	−6.618***	−18.478***	Time effect	< 0.001***
p value	0.045	< 0.001	< 0.001	< 0.001	< 0.001		
% change	1.1 (↓)	8.7↑	8.8↑	8.1↑	9.7↑		

Values are (mean ± *SD*), $**p < 0.01$, $***p < 0.001$, NS = Not significant, HIIT = high-intensity interval training, $\dot{V}O_{2max}$ = maximal oxygen consumption.

Table 7
Pearson's product moment correlation coefficient of some selected lipid profile and haematological parameters with physical fitness variables.

Variables	$\dot{V}O_{2max}$	VJ
Cholesterol	0.081	0.062
HDL	0.010	0.020
HDL/Chol ratio	0.079	0.053
WBC	−0.128	0.198
RBC	−0.098	0.096
Platelet	−0.250*	−0.187
Hb	0.058	0.048
HCT	0.008	0.112
PLR	−0.058	−0.329[a]

[a] $p < 0.01$, *$p < 0.05$, VO_{2max} = maximal oxygen consumption, VJ = vertical jump, HDL = high-density lipoprotein, HDL/Chol ratio = high-density lipoprotein to cholesterol ratio, WBC = white blood cell, RBC = red blood cell, Hb = haemoglobin, HCT = haematocrit, PLR = platelet-to-leukocyte ratio.

might be due to the training intensity-dependent improvement in fat oxidation and lipolysis.[7,25,26,] The longer continuous interval and/or higher work: rest ratio is suggested to be more effective in raising HDL-C and lowering TG in response to intermittent training.[25] A similar increase in HDL-C and decrease in the atherogenic index (TC/HDL-C) were previously reported after 8 weeks (1:1 = work: rest) of interval training.[25] Such changes are attributed to the activation of AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK) for increasing glucose and fatty acid oxidation.[7] Vitamin C and E supplementation are found to improve TC (10.5%) and HDL-C (14.6%) levels. This kind of improvement in lipid profile might result from the vitamin C- and E−induced increase in skeletal muscle fat oxidation which is likely due to the upregulation of CS (citrate synthase) and β-HAD (beta hydroxyacyl coenzyme A dehydrogenase) enzyme, gross increase in mitochondrial volume and mass.[28] These changes alter several regulatory steps to include adipose tissue lipolysis of TG to fatty acids,[29] transport of fatty acid into the cell, intramuscular lipolysis of TG to fatty acids, and ultimately fatty acid transport into the mitochondria.[27] On the other hand, PLR is another important haematological index of cell-to-cell interactions that may facilitate atherothrombosis and immune-inflammatory reactions. Ultimately leading to the pathogenesis of inflammatory diseases, tissue damage, and cardiovascular diseases.[30,31] In the present study, high-intensity training without any supplementation facilitated an 8% increase in PLR count post-training but that decreased by 1.6% in the combined vitamin C-E group. The vitamin-induced reduction in PLR interaction is likely due to the improved immune suppression (reduced plasma levels of IL-6 mRNA, IL-1ra, and cortisol) with decreased oxidative stress/burst.[2,3,32,33] However, Enas et al.[34] predicted lower HDL-C and higher TC/HDL-C values with the occurrence and recurrence of myocardial infarction (MI) and stroke with severe coronary heart disease. Ballantyne et al.[35] reported that each 1 mg/dl increase in HDL-C can cause a 2% reduction in risk for coronary heart disease in men.

Present findings observe a significant ($p < 0.001$) reduction in RBC count, Hb%, and HCT value in the HIIT group. This finding might be due to the haemolysis along with a similar significant reduction in WBC and an increase in platelet count.[7,12] The strenuous exercise-induced haemolysis might have occurred due to oxidative stress[8] and/or physical trauma in circulation.[36] The damaged erythrocyte membrane can lead to haemolysis due to the reduced cellular deforming capability and increased membrane rigidity.[12,37] The present study also revealed improvement in RBC, WBC, platelet, Hb%, and HCT in terms of preventing the high-intensity exercise-induced alterations after vitamin C and E supplementation. This finding is in agreement with an earlier report by Chou et al.[12] who reported that vitamin C and E supplementation effectively suppressed the exercise-provoked red-blood-cell haemolysis while stabilizing Hb% and HCT value. However, in terms of differential count (DC), neutrophils and lymphocyte were significantly increased, and monocyte and eosinophil were significantly decreased in the combined exercising vitamin E group in comparison to exercising alone or vitamin alone groups.[11]

Clarkson and Thompson[38] reported lower ascorbic acid levels (20%) after HIIT, and these values remained lower 24–48 h after exercise. Increased myeloperoxidase was also found and reflected polymorphonuclear neutrophil activation with decreased ascorbic acid concentration. This finding might be due to the use of vitamin C for quenching free radicals.[38] So, these circumstances may provide insight into the need for antioxidant vitamin supplementation, especially during high-intensity exercise. In the present study, the basal intake calculation among all five groups shows that vitamin C and vitamin E intake ranges from 39 to 44 mg and 0.38–0.44 mg respectively. Further, supplementation for vitamin C and vitamin E of 1 000 mg and 400 mg was easily given to optimize the availability of these antioxidant vitamins in the blood.[8,10,12] In the present study, serum levels of ascorbic acid (19.8%) and α-tocopherol (14.1%) were significantly increased ($p < 0.001$) for the vitamin C and vitamin E groups, respectively. The combined vitamin C-E supplemented group was found to have higher levels of both ascorbic acid (15.6%) and α-tocopherol (17.6%) at $p < 0.001$. The finding of a significant increase in plasma levels of vitamin C and E in the vitamin-supplemented groups agrees with the work of Huang et al.[14] These supplemented increased vitamin levels were postulated as the

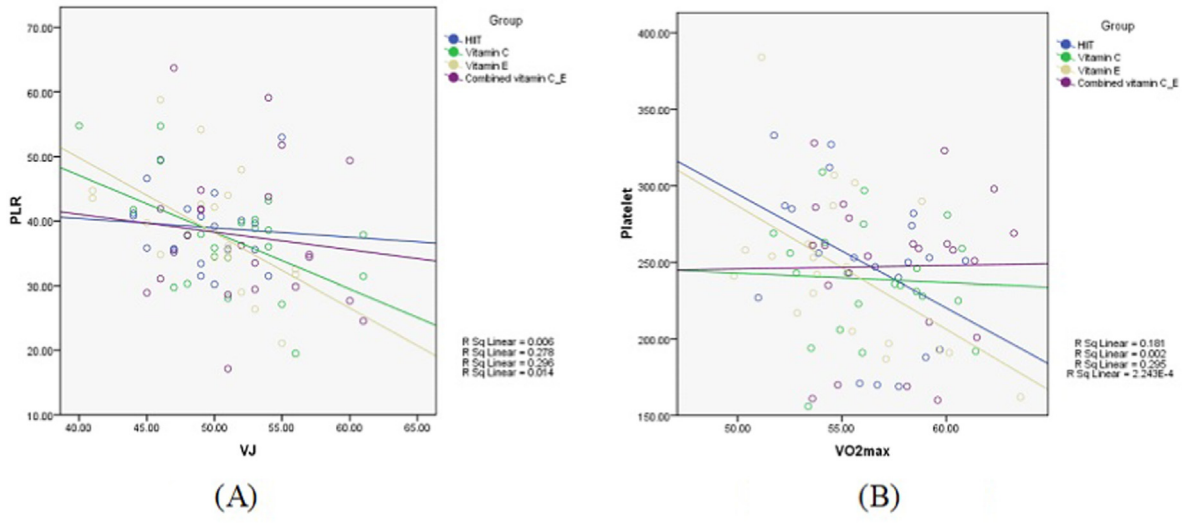

Fig. 1. The scatterplot-based relationship between VJ vs PLR and $\dot{V}O_{2max}$ vs platelet.

main cause for the protective measure against oxidative stress and muscle damage in the post-supplemented phase.

The present study depicted 13.1%, 12.5%, 12.2%, and 12.8% improvement in $\dot{V}O_{2max}$ after HIIT for vitamin C, vitamin E, and combined vitamin C-E groups, respectively while no such difference in $\dot{V}O_{2max}$ was found for the HIIT and combined vitamin C-E group. This finding is in agreement with earlier reports.[2,13] So, the antioxidant vitamin supplementation induced improved lipid profile and haematological variables, but any added effect was not found. No further significant change was identified against the $\dot{V}O_{2max}$ increment when compared among the HIIT group $\dot{V}O_{2max}$ increment with vitamin supplemented group $\dot{V}O_{2max}$ change. The only significant change in $\dot{V}O_{2max}$ was found when comparing the control group $\dot{V}O_{2max}$ with both HIIT and vitamin-supplemented group data. The present intervention group alteration might be due to the reduced or abolished exercise-associated skeletal muscle mitochondrial biogenesis, activation of peroxisome proliferator-activated receptor-gamma coactivator one-alpha (PGC-1α) signalling in skeletal muscle and PPARγ and LXRα target-gene upregulation in monocytes.[39–41] Paulsen et al.[13] reported a reduction in endurance training-induced increase of COX4 which suggests a blunted mitochondrial biogenesis. The inhibited redox-sensitive signalling comes with blunted induction of the PGC-1α gene which limits further improvement in $\dot{V}O_{2max}$.[13,42] Also reported is that vitamin C and E supplementation did not alter training adaptations, as assessed by the changes in CS and β-HAD activity.[32]

Physical fitness variables such as $\dot{V}O_{2max}$ and VJ were negative correlation with platelet count (correlation efficient $= -0.250$, $p < 0.05$) and PLR (correlation coefficient $= -0.329$, $p < 0.01$), respectively. This finding infers that platelet count and PLR were the most promising variables among all the haematological parameters for predicting athlete endurance capacity and leg explosive strength. However, both the scatter-plots for VJ vs PLR and $\dot{V}O_{2max}$ vs platelet depict the comparative correlation fit lines among which the combined vitamin C-E group showed the lesser extent of significant negative (R^2 linear $= 0.014$ and 2.243 respectively) between the variables. This result may confer that combined antioxidant vitamin (C and E) supplementation was able to reduce the training-induced stress on haematological variables compared with any other way of interventions used in the present study to further ameliorate the training adaptations optimally.

Present vitamin C and E supplementation was found to attenuate HIIT-induced haemolysis and secures some protection against haematological alterations along with improved fat oxidation and lipid profile. However, the antioxidant vitamin supplementation was able to significantly improve explosive leg strength but doesn't provide further improvement in $\dot{V}O_{2max}$ over HIIT-induced changes. One of the limitations of this study is probably the small sample size, which might cause some insignificance of some variables of supplementation groups. The study was unable to predict the gender variation as the study design did not include women participants. The study only focused on one particular HIIT sprint training with no variations in training intensities, and thus, the study does not suggest an intensity-wise HIIT effect. The present study training program does not cater to the energy expenditure calculation and thus counted as another limitation. Therefore, subsequent studies should use larger sample sizes and various training intensities combined with both men and women be conducted to evaluate the effect of vitamin (C and E) supplementation.

Conclusion

The present study evaluated antioxidant (vitamin C and E) supplementation and was able to provide findings for physiological protection towards exercise-induced haemolysis with increased RBC count and haemoglobin content, improved WBC and platelet alterations, and improved inflammatory profile. Further, the combined vitamin supplementation was reported to provide better fat oxidation and improved lipid profiling with increased HDL-C and decreased atherogenic index (TC/HDL-C) values which are associated with heart disease. Additionally, the combined vitamin-induced haematological improved explosive leg strength. However, the present study doesn't find any added improvement in $\dot{V}O_{2max}$ after vitamin supplementation. Antioxidant vitamin supplementation is only recommended if a vitamin deficit or serious oxidative burst or muscle damage or severe haemolysis takes place due to execrative/acute intense training protocol. The present study findings may be useful for future research. Finally, the present findings may also be helpful when preparing a suitable exercise training protocol for aerobic metabolism-based players, especially in the pre-competitive phase.

Submission statement

All authors have read and agree with manuscript content. The manuscript is only communicated to the Journal, the manuscript will not be submitted elsewhere for review and publication.

Ethical approval

Written informed consent was obtained from each player and study protocol conformed to the ethical guidelines of the Declaration of Helsinki. Ethical clearance (Ref No. IHEC/AB/P82/2019) was obtained from the Institutional Human Ethical Committee (IHEC), Department of Physiology, University of Calcutta.

Conflict of interest

All authors have agreed to publish the present article and declare no conflict of interest.

Authors' contributions

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4. Funding acquisition: Dr. Gouriprosad Datta and Dr.Swapan Kumar Dey.
5. Methodology: Surojit Sarkar and Dr.Swapan Kumar Dey.
6. Resources:Dr.Swapan Kumar Dey and Dr.GouriprosadDatta.
7. Supervision:Dr. Amit Bandyopadhyay, Dr. Swapan Kumar Dey and Dr. GouriprosadDatta.
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11. Confirmation of write up: All authors.

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