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Effects of a Modified Diet on Iron Status, Menstrual Health, Injury Status, and Aerobic Performance in Adolescent Female Football Players: A 12-Week Controlled Intervention Study

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ABSTRACT: **Background**: Female football players face high iron insufficiency from poor nutrition, menstrual blood loss, and training stress, reducing performance. The objective was to assess iron status, menstrual health, injury status and aerobic performance of adolescent female football players following 12 weeks of intervention.

Materials and Methods: The study conducted on randomly selected 120 female football players (age: 16–18 years), parted into (i) Control group (CG; n = 40), (ii) Training with Normal Diet Group (TNDG; n = 40), and (iii) Training with Modified diet group (TMDG; n=40). The CG received no training, whereas the TNDG and TMDG followed training (2 hrs/d, 5d/week) for 12 weeks. The CG and TNDG consumed regular diet, whereas TMDG consumed modified diet enrich with folic acid, iron, vitamin B12. Socio- economic and injury status was done at baseline; menstrual health, nutritional status, VO2max, haemoglobin (Hb), haematocrit (Hct), and serum ferritin (SF) were assessed at 0 and 12 week.

Results: In TMDG, dietary iron, folic acid, vitamin B12, Hb, HCT, SF and VO2max, showed a significant ($p<0.05$) improvement than TNDG and CG after 12 weeks. The TNDG and TMDG showed more prevalent menstrual irregularity and heavy bleeding than CG. About 50% reduction in participation in sports was noted during menstruation. In injury, there is no significant difference between TNDG and TMDG. In TMDG dietary iron, folic acid, and vitamin B12 showed significant positive correlations ($p<0.05$) with Hb, Hct, SF, VO2max.

Conclusion: Modified diet rich in iron, folic acid, vitamin B12 and protein may enhance iron status and aerobic performance of female soccer players.

1. INTRODUCTION

Nearly 50% of sports participants are female athletes. To achieve optimal health and performance of athlete's optimal nutrition is important aspects. Not only in size women differ from men, but also separated themselves from each other's in terms of body composition and hormonal context [Holtzman and Ackerman, 2021]. Female athletic triad is linked with serious conditions such as menstrual issues, limited energy availability, and low bone mineral density. Fluctuations of oestrogen and progesterone effect metabolism and fluid retention. This can be suppressed with exogenous hormones. This manipulated ideal timing for performance [Carter, 2018].

Iron is essential to oxidative metabolism and thus is especially important for the endurance athlete to increase aerobic capacity [DellaValle, 2013]. Especially, the body's adaptation in response to different training loads haematological markers is important indicators [Walker et al., 2019]. For changes in haematological values and iron (Fe) stores, training intensity and volume could be the reason. Iron requirement are increased by approximately 70% for endurance athletes. Low iron stores are most prevalent nutrients deficiency especially among female athletes. Several ways such as sweating, haematuria, gastrointestinal bleeding, inflammation, and intravascular and extravascular hemolysis and increased injury risk etc., are the reason for iron loss [Manna, 2020]. Thus, ATP production reduces by iron deficiency and for this; the body make the ATP from glucose via anaerobic metabolism. Thereby, reduce endurance capacity. An exercise-induced plasma volume expansion is reason for the decline hematocrit (Hct), haemoglobin (Hb), and red blood cell (RBC) count. This takes place within few days of intensive training [Koehler et al., 2012]. For maximizing maximal and submaximal exercise capacity iron-containing diet is essential.

Despite the given importance of iron, limited research has focused on the combined effects of training and dietary modification on iron status and aerobic capacity among adolescent female football players. Therefore, the aim of the present study was to find out menstrual status, iron status, aerobic capacity, and injury profiles of female football players following 12 weeks nutritional intervention.

2. MATERIALS AND METHODS

2.1 Study Area and Study participants

The study was conducted on residents of West Midnapore district especially from Medinpur Sadar Subdivision during March, 2025 to June, 2025. The participants were from same socio economic group. For this study one hundred and twenty nine female football players (aged: 16-18 yrs.) playing district level football from the training camp at Paschim Medinipur district, West Bengal, India for the last two years were included randomly.

2.2 Sample Size Determination and Group Distribution

By G* power software with the version of 3.1.9.7, the sample size was determined. For this study minimum of 102 subjects were required where, inputting parameters were: effect size- 0.2, α error probability- 0.05, power- 0.95, number of groups- 3, number of measurements- 2 and output parameters were: critical F- 3.08. To avoid mid study dropout of each group, total sample size were increased to one hundred and twenty nine (25% additional) [Kang, 2021]. Among them due to illness and injury were excluded nine participants from the study. The remaining one hundred twenty participants were randomly parted into: (i) Control group (CG; n = 40), (ii) Training with Normal Diet Group (TNDG; n = 40), and (iii) Training with Modified diet group (TMDG; n=40). The participants of TNDG and TMDG engaged in competitive football training; whereas CG involved in sedentary activities.

2.3 Inclusion and Exclusion criteria

Subjects from the age of 16-18 years were considered for present study. Subjects free from history of recent disease and illness were covered in the study. By physicians medical examinations were done. On this the selection was done. Subjects from less than the age of 16 years and above 18 years were excluded from this study. Subjects have any medical issue or some serious health issues i.e. surgery, chronic illness etc. were excluded from the research study.

2.4 Ethical consideration:-

The present study involved dietary intervention without the use of pharmaceutical agents or controlled supplementation. Among all the participants and their parent/legal guardian, the study design, objectives and possible complications was interpreted and a written informed consent was taken. For this study the ethical approval was earned from the Institutional Ethics Committee (No: EC/2023-24/055; dated 03.12.2024). Declaration of Helsinki's ethical guidance with its 2013 revision, the study was done.

2.5 Experimental design

This study was designed as a 12-weeks randomly assigned experimental, parallel-group intervention study involving three groups. One hundred and twenty nine female football players (aged: 16-18 yrs.) were covered after medical examinations performed by the physicians. Due to illness and injury were excluded nine participants from the study; and the remaining one hundred twenty participants were segregated into: (i) Control group (CG; n = 40), (ii) Training with Normal Diet Group (TNDG; n = 40), and (iii) Training with Modified diet group (TMDG; n=40). All the participants of TNDG and TMDG followed the same training programme (2 hrs/d, 5d/week) for 12 weeks. The CG followed no training programme. The participants of CG and TNDG consumed regular normal diet, whereas participants of TMDG consumed modified diet (rich in iron, folic acid, vitamin B12 and protein). The evaluation of modified diet effects under similar training conditions which was the primary objective of the study. Therefore, the principal comparison was made between the TNDG and TMDG. Comparisons involving the control group were used as a reference to minimize the effects of training. Socio- economic and injury status was done at baseline; menstrual health, nutritional status, VO2max, haemoglobin (Hb), haematocrit (Hct), and serum ferritin (SF) were assessed at 0 and 12 week.

2.6 Training

The participants of TNDG and TMDG were taken general training program from qualified coaches, whereas the CG receives no training. Training includes strength and power training, endurance training, techniques and tactics. For 2 hours per day, 5 days per week for 12 weeks, the participants followed training programme [Bompa and Buzzichelli, 2018].

2.7. Dietary Intervention

The participants of TMDG consumed modified diet (rich in iron, folic acid, vitamin B12 and protein) following the recommended as per NIN and ICMR.

2.8 Development of tool

For the purpose of the data collection (for assessment socio economic, menstrual, injury and nutritional status) questionnaire tool were be used. The individuals were being questioned using a standard set of questions from the questionnaire.

2.9 Assessments Procedure

2.9.1 Assessment of Socio Economic Status (SES), Menstrual Status, Injury status and Nutritional Status

The SES were assessed by questionnaire method of modified Kupuswami scale, 2021 [Saleem and Jan, 2021]. The menstrual status of the participants was assessed by questionnaire method by employing 7-point Likert scale [Abed et al., 2015; Bargiota et al., 2016]. The Injury status of the participants was assessed by questionnaire method by employing 5-point Likert scale [Clarsen et al., 2013; Lohrer et al., 2016]. To collect the dietary information, A 24 hour recall method was used [Srilakshmi, 2020]. The macronutrients (carbohydrate, protein, fat) as well as micronutrients (vitamins and minerals) content of the diet was assessed by using Indian food composition, NIN and ICMR, 2017 [Longvah et al., 2017]. As per ICMR guidelines energy requirements of the football players were evaluated by using Harrish-Benedict formula [Srilakshmi, 2020].

2.9.2 Maximal aerobic capacity Measurement (VO_{2max})

Through Yo-Yo Intermittent Recovery Test 1 (YYIR1), maximal aerobic capacity (VO_{2max}) of the subject was assessed. To performed specific running test for 20 m distance, the subject was asked. For 20 m and 5 m for recovery, running track was created. When the subject was exhausted, the specific lap and shuttle was noted, the test was terminated and from that specific lap and shuttle and VO_{2max} was determined (Mirani and Patil, 2017).

2.9.3 Measurement of Biochemical Variables

From an antecubital vein of the participants, a five ml of 12 hours fasting blood was taken for determination of haemoglobin, haematocrit value and serum ferritin level by using standard procedure [Mukherjee, 2017].

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2.10 Statistical analysis

All the data of selected nutritional status, menstrual status, injury status, maximum aerobic capacity and haemoglobin level, haematocrit value, ferritin level shown to mean and standard deviation (SD). The participant's SES was compared by chi squire test. A Two-way ANOVA alongside multiple comparison test was employed to inquire both within and between-group differences. To find out within the differences in groups Paired t test was performed. The primary effects of time and group, and interaction effect were presented for each dependent variable with F-values, degrees of freedom, and partial eta squared (η^2p). To assess the relationship between variables Pearson's correlation analysis was used. At $p \leq 0.05$ level the statistical significance was set [Banerjee, 2018]. By using SPSS software all statistics were done (version 27.0; IBM Corp., Armonk, NY, USA).

3. RESULTS AND DISCUSSION

3.1. Results

Assessment of socio economic status of Control group (CG), Training with Normal Diet Group (TNDG), Training with Modified diet group (TMDG)

No significant difference was shown in SES among the participants of CG, Training with Normal Diet Group (TNDG), and Training with Modified Diet Group (TMDG). This is shown by chi-square analysis. Across the three socio-economic categories (lower, lower middle, and upper), the observed frequencies were compared. With 4 degrees of freedom, the chi-square value was 0.26. At the 0.05 level of p value with the critical value of 9.49 this was much lower. So, comparable across the three groups, the socio-economic status at baseline was not being a confounding factor in this study that followed.

Assessment of Menstrual status among the Control group (CG), Training with Normal Diet Group (TNDG), Training with Modified diet group (TMDG)

The group-wise comparison of Menstrual Attitude Questionnaire (MAQ) subscales revealed no significant changes in the Control Group (CG) after 12 weeks. The Training with Normal Diet Group (TNDG) showed significant ($p \leq 0.05$) changes in all five MAQ scales. That was mean in reductions of Debilitation, Bothersome, and Denial scores where the increment in Natural and Predictions score. On the side, with the reduction of Debilitation, Bothersome, and Denial from 34.10±4.75 to 22.07±7.05, 18.75±3.56 to 11.23±3.27, and 20.79±3.24 to 14.94±3.29, where with the increase of Natural and Predictable score from 14.02±3.04 to 20.31±2.44 and 13.24±2.21 to 17.35±1.74, the Training with Modified diet group (TMDG) showed most significant ($p \leq 0.001$) changes (Table 1).

Table 1: Assessment of Menstrual Attitude Questionnaire (MAQ) among the Control group (CG), Training with Normal Diet Group (TNDG), and Training with Modified diet group (TMDG)

	Control Group (CG; n=40)		Training with Normal Diet Group (TNDG; n=40)		Training with Modified diet group (TMDG, n=40)	
	0 week	12 weeks	0 week	12weeks	0 week	12 weeks
Debilitation (F1)	32.57 ±4.74	32.48^{NS} ±4.35	32.68 ±4.09	30.30* ±4.15	34.10 ±4.75	22.07# ±7.05
Bothersome (F2)	18.07 ±2.80	18.17^{NS} ±2.98	18.32 ±3.80	16.55* ±4.64	18.75 ±3.56	11.23# ±3.27
Natural (F3)	13.76 ±2.58	14.78^{NS} ±3.26	15.12 ±2.91	16.78* ±2.79	14.02 ±3.04	20.31# ±2.44
Predictable (F4)	12.86 ±2.44	12.81^{NS} ±2.47	13.00 ±2.02	14.12* ±1.91	13.24 ±2.21	17.35# ±1.74

Denial (F5)	20.13 ±3.20	19.85 NS ±3.43	20.51 ±4.34	18.55* ±3.34	20.79 ±3.24	14.94# ±3.29

Note: [Each data presented as mean ±SD, n=40, df=39; paired sample t-test was performed; The significant level was chosen at $p \leq 0.05$ (*) when comparing TNDG '0wk' with TNDG '12wks'; If TMDG '0 week' parameters significant with TMDG '12 weeks' parameters then-# $p \leq 0.001$ where critical t value is 2.02; non-significant-no sign.

Group-wise percentage distribution of menstrual characteristics showed noticeable variation. Menarche at 10–12 years was more common in the CG (62.5%), whereas 13–15 years predominated in TNDG and TMDG (62.5%). Regular cycles were reported by 55% of CG participants compared with 25% and 20% in TNDG and TMDG, respectively.

Table 2: Group-wise Percentage Distribution of Menstrual Characteristics of Control group (CG), Training with Normal Diet Group (TNDG), Training with Modified diet group (TMDG)

Menstrual History Questionnaire	Percentage of participants (%)		
	Control Group (CG; n=40)	Training with Normal Diet Group (TNDG; n=40)	Training with Modified diet group (TMDG, n=40)
Approximate age of first menstrual period (Menarche):			
10-12 yrs	62.5	37.5	37.5
13-15 yrs	37.5	62.5	62.5
16-18 yrs	0	0	0
Yet not started	0	0	0
How are your Periods?			
Regular	55	25	20
Irregular (Oligomenorrhea)	45	75	80
How is your periods flow?			
Normal	50	12.5	12.5
Mild	25	25	25
Moderate	17.5	50	45
Heavy	7.5	12.5	17.5
How long does period last?			
<5 days	25	62.5	62.5
5-7 days	50	25	25
>7 days	25	12.5	12.5
How often you had menstrual periods in the last year?			
Once every less than 21 days (Polymenorrhea)	7.5	0	0

Every 21-27 days	5	12.5	12.5
Every 28-35 days	50	50	50
Every 36-50 days	25	12.5	12.5
Every 3-4 months	25	0	0
Very irregular, sometimes monthly or sometimes skip	12.5	25	25
Intensity of your menstrual pain?			
Not at all	15	12.5	25
Mild	25	12.5	12.5
Moderate	50	50	25
Dysmenorrhea	10	25	37.5
Tolerable pain	5	12.5	20
Severe pain	5	12.5	17.5
During periods are you participated in game?			
Not at all	50	5	5
Participation with periods	12.5	25	25
Reduced participation	25	50	50
Full Participation	12.5	20	20
Have you ever experience that exercise or sports activity affected by periods?			
Not at all	37.5	25	25
slightly	50	62.5	62.5
Greatly	12.5	12.5	12.5
Have you experience any injuries the past year during periods?			
Not at all	87.5	62.5	62.5
Yes	12.5	37.5	37.5
Have you ever seen a medical practitioner about problems associated with your periods?			
No	50	75	75
Yes	50	25	25

Normal menstrual flow was more frequent in CG (50%), while moderate-to-heavy flow was more prevalent in the training groups. Menstrual duration of <5 days was reported by 62.5% of both training groups compared with 25% of CG. Most participants had cycles every 28–35 days (50% in all groups). Moderate menstrual pain was common in CG and TNDG (50%), whereas 25% of TMDG reported moderate pain. Notably, 50% of CG reported no participation in games during menstruation, compared with only 5% in both training groups. Most participants reported slight effects of menstruation on exercise (Table 2).

Assessment of Injury status among the Control group (CG), Training with Normal Diet Group (TNDG), Training with Modified diet group (TMDG)

Between the comparison of Training with Normal Diet Group (TNDG) and Training with Modified Diet Group (TMDG), demonstrate that no statistical significant differences was seen in any injury related domain. In those two groups i.e., TNDG and TMDG, Critical Injury Factor scores, Injury Affected Areas, Functional Impact Scale Energy Availability/Fatigue, Menstrual Function and Injury Link, and Bone Health/Stress Injury Indicators were showed into 5.20±1.93 and 4.53±2.13, 5.00±3.64 and 5.73±2.98, 20.20±6.87 and 19.06±5.83, 8.35±2.20 and 9.40±1.99, 3.60±1.53 and 3.46±1.64, 5.80±1.90 and 5.66±2.02. In this difference of those two groups, was not significant ($p>0.05$) with the equal variance. On the other hand, in Nutritional Risk Behaviours, there was also shown not significant difference ($p>0.05$) but with unequal variance ($p=0.755$). In both groups, no statistical significant ($p>0.05$) difference was seen in total injury score with equal variance ($p=1.00$). Overall, injury profiles were comparable between groups (Table 3).

Table 3: Assessment of Injury status among the Control group (CG), Training with Normal Diet Group (TNDG), and Training with Modified diet group (TMDG)

Injury Domains	Training with Normal Diet Group (TNDG, n =20)	Training with Modified Diet Group (TMDG, n = 15)	t test	Leven's test p value
Critical injury Factor domain	5.20 ±1.93	4.53 ±2.13	0.34 NS	0.872 EV
Injury affected Areas domain	5.00 ±3.64	5.73 ±2.98	0.53 NS	0.150 EV
Functional impact Scale domain	20.20 ±6.87	19.06 ±5.83	0.61 NS	0.393 EV
Energy availability / fatigue Domain	8.35 ±2.20	9.40 ±1.99	0.16 NS	0.750 EV
Menstrual Function and Injury Link Domain	3.60 ±1.53	3.46 ±1.64	0.81 NS	0.659 EV
Bone health / stress injury Indicators domain	5.80 ±1.90	5.66 ±2.02	0.84 NS	0.904 EV
Nutritional risk behaviours Domain	4.65 ±3.08	4.93 ±1.86	0.76 NS	0.031
Total Score	52.80 ±15.99	52.80 ±12.01	1.00 NS	0.088 EV

Note: [Each data presented as mean ±SD, Independent t-test was performed; the significant level was chosen at $p\leq 0.05$ (*) when comparing TNDG NS=non-significant-no sign, EV= Equal variance.]

Assessment of Nutritional Status of Control group (CG), Training with Normal Diet Group (TNDG), Training with Modified diet group (TMDG)

The assessment of nutritional status demonstrated significant differences among groups, across time, and in group × time interactions for most dietary parameters. A significant ($p<0.000$) group effect and group × time interaction ($p= 0.016$) with the F value of 14.002 and 4.222 were shown in carbohydrate intake. On the other side, a significant ($p\leq0.000$) group, time and interaction effect was shown in protein, energy, dietary iron, folic acid, vitamin B12, and vitamin C. In TMDG group, in macronutrients, Carbohydrate, protein, fat intake were increased from 353.07±38.03 to 381.32±46.04 g/day, 55.61±6.24 to 63.24±8.63 g/day, 58.73±8.14 to 66.87±6.93 g/day. That improvement was further increased overall energy intake i.e., 2163.35±162.12 to 2380.14±197.95 kcal/day. Besides that, in micronutrients, dietary iron, folic acid, vitamin B12, and vitamin C were increased from 17.07±2.16 to 29.14±9.95 mg/day, 199.25±34.99 to 298.80±67.23 µg/day, 2.21±0.55 to

3.28±0.85 μg/day, and 74.28±16.95 to 132.86±36.27 mg/day. In contrast, minimum or no significant (p>0.05) difference was shown in several nutrients in CG and TNDG (Table 4). These results indicate that much more improvement was shown in energy, macro and micronutrients which were for modified diet.

Table 4: Assessment of Nutritional status among the Control group (CG), Training with Normal Diet Group (TNDG), and Training with Modified diet group (TMDG)

Parameters	Control Group (CG; n=40)		Training with Normal Diet Group (TNDG; n=40)		Training with Modified Diet Group (TMDG, n=40)		Two-Way ANOVA (P Value) [Effect Size]		
	0 week	12 week	0 week	12week	0 week	12 week	Group	Time	Interaction
Carbohydrate (gram/day)	338.33 ±37.50	333.07^{NS} ±36.70	355.38$^{\$}$ ±36.91	357.62$^{\$}$ ±33.63	353.07 ±38.03	381.32$^{*\$\#}$ ±46.04	14.002 (0.000) [0.107]	2.874 (0.091) [0.012]	4.222 (0.016) [0.035]
Protein (gram/day)	55.94 ±7.79	53.55^{NS} ±6.87	58.04^{NS} ±7.42	59.10$^{\$}$ ±9.56	55.61 ±6.24	63.24$^{*\$\#}$ ±8.63	22.379 (0.000) [0.161]	22.210 (0.000) [0.087]	30.429 (0.000) [0.206]
Fat (gram/day)	59.22 ±5.50	60.14^{NS} ±6.11	60.68^{NS} ±6.19	61.84^{NS} ±6.59	58.73 ±8.14	66.87$^{*\$\#}$ ±6.93	4.429 (0.013) [0.036]	15.891 (0.000) [0.063]	7.645 (0.001) [0.061]
Energy (kilocalorie/day)	2110.10 ±158.74	2087.55^{NS} ±169.51	2199.93$^{\$}$ ±145.03	2223.54$^{\$}$ ±132.27	2163.35$^{\$}$ ±162.12	2380.14$^{*\$\#}$ ±197.95	26.527 (0.000) [0.185]	15.360 (0.000) [0.062]	15.567 (0.000) [0.117]
Dietary Iron (milligram/day)	17.35 ±2.17	17.58^{NS} ±2.24	18.18^{NS} ±1.91	18.64^{NS} ±1.63	17.07 ±2.16	29.14$^{*\$\#}$ ±9.95	36.419 (0.000) [0.237]	54.509 (0.000) [0.189]	45.738 (0.000) [0.281]
Folic acid (microgram/day)	213.69 ±39.70	207.72^{NS} ±33.08	203.82^{NS} ±31.56	216.27^{NS} ±32.77	199.25 ±34.99	298.80$^{*\$\#}$ ±67.23	22.801 (0.000) [0.163]	42.886 (0.000) [0.155]	36.356 (0.000) [0.237]
Vitamin B 12 (microgram/day)	2.48 ±0.62	2.19^{NS} ±0.57	2.57^{NS} ±0.65	2.60$^{\$}$ ±0.66	2.21$^{\#}$ ±0.55	3.28$^{*\$\#}$ ±0.85	7.767 (0.001) [0.062]	9.836 (0.002) [0.040]	23.074 (0.000) [0.165]
Vitamin C (mg/day)	83.67 ±23.98	83.39^{NS} ±20.05	92.20^{NS} ±19.37	94.44^{NS} ±19.65	74.28$^{\#}$ ±16.95	132.86$^{*\$\#}$ ±36.27	14.419 (0.000) [0.110]	43.855 (0.000) [0.158]	39.758 (0.000) [0.254]

Note. [Data presented as mean ± SD, n= 40 in each group (CG, TNDG, TMDG), Two-way ANOVA with replication followed by multiple comparison was performed. Paired sample t test was used to determine within group differences. In within group *p<0.05 when compared to 0-week with 12 week. On the other, in between group $p<0.05 when compared to CG with TNDG and TMDG, where #p<0.05 when compared to TNDG and TMDG. In the table calculated F values, partial eta squared (ηp2) and p value; SD = standard deviation, ANOVA= Analysis of Variance, CG = control group, TNDG = training with normal diet group, TMDG= training with modified diet group, NS= not significant]

Assessment of biochemical status and physiological variables among the Control group (CG), Training with Normal Diet Group (TNDG), Training with Modified diet group (TMDG)

The assessment of biochemical and physiological variables demonstrated significant differences among groups, across time, and in group × time interactions. With the F value of 33.442, 44.303, and 27.464, a significant ($p<0.000$) group, time and interaction effect showed in haemoglobin level respectively. On the other side, in HCT, a significant group ($F=9.457$, $p\leq0.000$), time ($F=7.127$, $p\leq0.008$) and interaction ($F=6.075$, $p\leq0.003$) effect whereas, in serum ferritin a significant group, time and interaction effect ($p\leq 0.000$) with the F value of 69.253, 82.249, and 74.957 was shown. In TMDG group, in haemoglobin, haematocrit, and serum ferritin level were increasing to 12.62±0.28 g/dL, 37.38±6.53 %, and 35.52±12.03 µg/L. Besides that, in VO2max, was increased from 36.99±0.44 to 40.72±2.00 ml/kg/min, with a significant ($p<0.000$) group, time and interaction effect; where F value were 104.048, 118.505, 96.889. In contrast, CG and TNDG showed minimal changes (Table 5). The modified diet combined with training produced substantial improvements in haematological status and aerobic capacity.

Table 5: Effect of biochemical status and physiological variables of Control group (CG), Training with Normal Diet Group (TNDG), Training with Modified diet group (TMDG)

	Control Group (CG; n=40)		Training with Normal Diet Group (TNDG; n=40)		Training with Modified diet group (TMDG, n=40)		F Values (P Value) [Effect Size]		
	0 week	12 week	0 week	12week	0 week	12 week	Group	Time	Interaction
Hb (gm/dl)	10.85 ± 0.83	10.93 NS ± 0.73	10.98 NS± 0.67	11.16 NS ± 0.55	10.98 NS± 0.79	12.62 *$#± 0.28	33.442 (0.000) [0.222]	44.303 (0.000) [0.159]	27.464 (0.000) [0.190]
HCT (%)	29.70 ± 6.13	29.76 NS ± 5.68	30.90 NS ± 6.05	31.19 NS ± 8.05	30.91 NS ± 6.82	37.38 *$#± 6.53	9.457 (0.000) [0.075]	7.127 (0.008) [0.030]	6.075 (0.003) [0.049]
SF (µg/L)	16.15 ± 2.74	16.06 NS ± 3.57	15.93 NS ± 3.32	16.64 NS ± 3.60	15.66 NS ± 3.83	35.52 *$#± 12.03	69.253 (0.000) [0.372]	82.249 (0.000) [0.260]	74.957 (0.000) [0.390]
VO_{2max} (ml/kg/min)	36.93 ± 0.62	36.99 NS ± 0.58	36.91 NS ± 0.30	37.10 NS ± 0.60	36.99 NS ± 0.44	40.72 *$#± 2.00	104.048 (0.000) [0.471]	118.505 (0.000) [0.336]	96.889 (0.000) [0.453]

Note. [Data presented as mean±Sd, n= 40 in each group (CG, TNDG, TMDG), Two-way ANOVA with replication followed by multiple comparison was performed. Paired sample t test was used to determine within group differences. In within group *p<0.05 when compared to 0-week with 12 week. On the other, in between group $p<0.05 when compared to control group with training group and iron rich diet group, where #p<0.05 when compared to training group and iron rich diet group. In the table calculated F values, partial eta squared (ηp2) and p value; SD = standard deviation, ANOVA= Analysis of Variance, CG = control group, TNDG = training with normal diet group, TMDG= training with modified diet group, NS= not significant, Hb=Haemoglobin, HCT= Haematocrit, SF= Serum Ferritin, VO_{2max}= Maximal aerobic capacity]

Correlational Effect between Variables

In the present study, debilitation (F1) and bothersome (F2) showed a negative correlation with dietary iron ($r = -0.563$, $p \leq 0.000$; $r = -0.590$, $p \leq 0.000$), folic acid ($r = -0.649$, $p \leq 0.000$; $r = -0.615$, $p \leq 0.000$), and haemoglobin ($r = -0.537$, $p \leq 0.001$; $r = -0.508$, $p \leq 0.001$). Here dietary iron showed positive correlation with dietary vitamin C ($r = +0.511$, $p \leq 0.001$); Hb showed positive correlation with dietary iron ($r = +0.536$, $p \leq 0.000$), folic acid ($r = +0.712$, $p \leq 0.000$) and vitamin B12 ($r = +0.522$, $p \leq 0.001$). On the other side, VO2max showed significant positive correlation with heamoglobin level ($r= +0.801$, $p<0.000$), haematocrit value ($r = +0.716$, $p \leq 0.000$) and ferritin level ($r = +0.712$, $p \leq 0.000$) of female football players.

3.2 Discussion

Sports nutrition is a system that not only covers nutrition, but also for the purpose of promoting optimal performance biochemical and physiological scientific knowledge is essential [Srilakshmi, 2019]. The physically active women and endurance athletes with iron deficiency is still going from past. This is due to notably high prevalence of latent iron deficiency seen in female athletes which in some cases is reported to be more than twice the level reported in their sedentary counterparts [Alaunyte et al., 2015]. Socio-economic status has a principle role to assess health status as well as nutritional status of family. It allows properly understanding the health services affordability, their purchasing capacity, amenities, and the health seeking behaviour. Though there was no significant difference in socio-economic status among the volunteers of CG, TNDG and TMDG.

For further research on women's sport, the effect of the menstrual cycle on physical performance is being key consideration [Carmichael et al., 2021]. Menstrual health status reported the age at menarche was predominantly within 13–15 years of TNDG and TMDG; however, a notable proportion of participants in the CG reported earlier menarche at the age of 10–12 years, which may reflect variations in nutritional and environmental factors and at an earlier age this consequence may be for those girls of upper socio-economic class have menarche than the girls of lower socio-economic class. As due to proper nutrition and better health they get a certain critical weight at an early age. On the other, menstrual irregularity in TNDG and TMDG was high than CG. By the influence of both heredity and socio-economic status mainly nutrition, this difference could be assigned [Öztürk and Güneri, 2021]. This suggests possible hormonal imbalance and energy deficiency, which are commonly observed in adolescent athletes, especially those from low socioeconomic backgrounds. Inadequate dietary intake, particularly of iron-rich foods, may contribute to menstrual dysfunction. High percentage of participants noted heavy bleeding in TNDG and TMDG than CG. This showed that a significant percentage of subjects had moderate to heavy bleeding. Heavy bleeding is the reason of iron shortage as well as decline of heamoglobin and ferritin level. This is especially for athletes, because iron deficiency may decrease oxygen delivery and reduce performance. Severe dysmenorrhea can negatively affect training participants and performance which may report reduced sports participation. With several studies in different parts these results are agreeing that, among the girls during menstruation dysmenorrhoeal was the most common medical problem [Chandra-Mouli and Patel, 2020]. According to most of the girl, their performance negatively affect by menstruation. That is why; recently during menstruation many girls and women not to join any physical activities [Neşe Şahin Ozdemir, 2013]. In MAQ subscales, the CG did not show any significant changes. That indicate that without intervention, menstrual attitudes remain largely unchanged over time; where, a significant improvements in menstrual attitudes, particularly in the TNDG and TMDG. At baseline, participants across all groups exhibited relatively higher scores in the debilitation and bothersome factors, indicating a tendency to perceive menstruation as physically limiting and distressing. After the intervention, the TNDG showed significant improvements across all MAQ subscales. In particular, decrease in debilitation and bothersome scores imply that frequent exercise may lessen menstrual discomfort. Exercise is well to improve blood circulation, lessen prostaglandin-mediated discomfort, and better the release of endorphin. These above criteria lead to a more favourable view of menstruation [Alfaro-Magallanes et al., 2022]. In TMDG, showed significant improvement in all menstrual subscales, which indicate more pronounced, changed in menstrual attitudes.

The marked reduction in debilitation and bothersome scores in this group suggests that improved nutritional status, particularly enhanced iron intake, may reduce fatigue and menstrual-related discomfort. Elevated haemoglobin and ferritin level is linked to improve oxygen transport and reduced weakness, lethargy type symptoms. These are frequently linked to negative impressions of menstruation [Nolte et al., 2025]. Moreover, in both TNDG and TMDG, there is increase in natural and predictable subscale scores. These indicate better understanding and acceptance of menstruation as a typical physiological phenomenon. This might be explained by both greater exposure to organized training situations, which can improve body awareness and confidence, and physiological adaptation. In TMDG, there is reduction in denial scores. This score indicate more aware of participant's menstrual health and less prone to ignore symptoms. This shift may facilitate better self-management and health-seeking behaviour [Kvalem et al., 2024].

In this study, between groups (TNDG and TMDG) no significant difference was shown in domain wise injury scores. According to similar findings from previous studies, especially in short-term interventions or small samples, no significant changes in composite injury or performance scores through nutritional or training interventions [Melin et al., 2014; Soligard et al., 2017]. Additionally, iron deficiency and inadequate dietary intake have an impact on female athletes' tiredness, recuperation, and susceptibility to injury, yet these effects may not always be evident in short-term quantitative score differences, which has been demonstrated [De Souza et al., 2014]. Furthermore, differences in variability of different injury domain did not substantially influence the overall findings of the study. Across the groups, the variance was generally similar, indicating stable group comparisons.

Female football players are thought to be more susceptible to iron insufficiency, which can develop to iron deficiency anaemia. Inadequate dietary iron intake, severe menstrual bleeding, perspiration, exercise-induced hemolysis, and foot striking hemolysis may all contribute to a negative iron balance. In female football players when excess blood loss is happened on that time training period is closed. In recent years, an epidemiological study of the iron deficiency was noted in athletes due to heavy training [Coates et al., 2017]. To prevent an energy deficit or excess energy balance as well as energy cost is most common factor. The reason of delayed puberty, short stature, and menstrual dysfunction, loss of muscle mass and increased fatigue, injury or illness, energy deficit is responsible [Hannon et al., 2020]. Nutrients like carbohydrate and protein must be needed because through gluconeogenesis protein help to maintain blood glucose when duration of exercise increases, which take place in liver. For the reduction of fatigue and the risk of disease and injury Improvement of Sports nutrition is accounted. This is for athletic performance and faster recovery. In baseline no significant difference was shown in macro and micronutrients, Hb level, HCT and ferritin level and VO2max among the volunteers of CG, TNDG and TMDG in this study. In TMDG, a significant improvement in macro and micro nutrients intake e.g. carbohydrate, protein, fat, dietary iron, folic acid, vitamin B12, vitamin C compared with the CG and TNDG as those were followed their regular diet which had dietary inadequacy. Maintaining iron status in female athletes requires eating a balanced diet high in iron and nutrients that aid in iron absorption. Practical strategies for raising iron levels in female athletes should involve meal composition modifications that improve iron bioavailability with the focus on dietary iron intake especially heme iron intake and dietary adjustments with healthy eating practices, where, in case of non-heme iron which is plant based that absorption is slow. That's why, vitamin C rich fruits and vegetable include in diet which enhance iron absorption [Alaunyte et al., 2015]. In TMDG, a significant improvement in macro and micro nutrients intake e.g. carbohydrate, protein, fat, dietary iron, folic acid, vitamin B12, vitamin C compared with the CG and TNDG as those were followed their regular diet which had dietary inadequacy. High dietary iron intake, folic acid and vitamin B12 intake contributed to improved iron stores, as indicated by ameliorate ferritin levels, which followed haemoglobin synthesis and red blood cell production. Adequate availability of all three nutrients ensures effective erythropoiesis, resulting in optimal haemoglobin concentration and haematocrit values [McKay et al., 2022]. Deficiency of iron leads to deplete ferritin and haemoglobin levels, whereas deficiencies of folic acid and vitamin B12 hamper red blood cell production. This indicates that adequate dietary iron, folic acid and vitamin B12 play a crucial role in maintaining optimal haematological status and aerobic capacity among adolescent female footballers. As maximal aerobic capacity is depend on haemoglobin level and haematocrit, so there have a significant increase in VO2max in TMDG group [Sim et al., 2019]. But, in CG no significant different was shown. Moreover, in TNDG, there is significant increase in VO2max which is due to training. But the level was very low due to not taking proper iron rich foods. On haemoglobin level athlete's oxidative potentiality is depended. For the sake of higher oxygen supply, increase VO2max is needed [Kenney et al., 2012]. Haemoglobin mass and/or concentration are related to VO2max because transportation of oxygen is primarily happened by haemoglobin. It declines aerobic capacity, raise hearty rate and elongates recovery time after exercise [Sim et al., 2019]. The results show that foods enrich with iron with proper balance diet to female football players for 12 weeks significantly increased Hb concentration, hematocrit value among the participants of TMDG which reflecting enhanced erythropoiesis and oxygen-carrying capacity [Alaunyte et al., 2015]. In CG and TNDG, there were no significant changes in haemoglobin and hematocrit values, which might be for the reason of different dietary nutrients deficiency like folic acid, vitamin B12, iron, and protein and also iron losses due to training-induced hemolysis, sweating, gastrointestinal bleeding, and inflammation. In addition, significantly increase in serum ferritin level was found only in TMDG, which indicated greater iron stores among the participants of TMDG [Pengelly et al., 2025].

In this study, in MAQ debilitation and bothersome scores measure how much menstruation is perceived to interfere with life and performance. As a negative correlation was noted between dietary iron, folic acid, Hb level, debilitation and bothersome, indicating after 12 weeks intervention adequate iron, folic acid, Hb level supports proper oxygen supply to the muscle, normal RBC production and more stable menstrual patterns, possibly leading to lower debilitation and bothersome scores. As a positive correlation was noted between dietary iron and dietary vitamin C, indicating by reducing ferric iron ($Fe3+$) to increase greater absorption of non-heme iron (found in plant-based foods) make it more soluble and absorbable form i.e., ferrous iron ($Fe2+$) in the stomach. This can significantly improve the bio-accessibility of iron [Domellöf and Sjöberg, 2024]. Greater absorption of dietary iron helps to synthesis haemoglobin; where improved dietary iron increased red blood cell and iron stores which indicate increased haematocrit value and ferritin level. So a positive correlation was noted between dietary irons and haemoglobin level, haematocrit value and ferritin level. On the other side, dietary folic acid and vitamin B12 intake showed a positive correlation with haemoglobin and haematocrit levels. This indicates DNA synthesis and red blood cell maturation. Haemoglobin showed the strongest positive relationship with VO_{2max} because it directly linked with oxygen transport to working muscles. Haematocrit also showed a positive correlation, indicating to improve red blood cell volume; where, serum ferritin exhibited an indirect association with VO_{2max}, suggesting that adequate iron stores are essential for maintaining optimal haemoglobin levels and aerobic efficiency. For optimal status of iron by enhancing endurance performance among adolescent female footballers, these findings are mainly responsible [Alaunyte et al., 2015; Kenney et al., 2012].

In this study a key strength is firstly the volunteers of TNDG and TMDG was from low socio economic backgrounds which showed the proper deficiency of diet. Also this study lies in the combined assessment of menstrual effect and nutritional intervention, providing a more comprehensive understanding of how menstrual effect was associated with health problems, which is the reason for the prime nutrients deficiency i.e. carbohydrate, protein, fat, dietary iron, folic acid, vitamin B12 those are essential for performance. So this 12 weeks intervention has showed the improvement of those nutrients which enhance iron status and performance of female football players.

4. CONCLUSION

With particular focus on improving iron bioavailability by increasing total dietary iron, especially haem-iron intake, should incorporate in female athletes diet. By enhancing iron absorption with increased Vitamin C, different iron rich foods can be given. On the side, absorption inhibitors like tea, coffee, and calcium containing foods should be avoided or decreased from meal. So, for the enhancement of performance, improvement of iron status should be accounted. Therefore, at regular basis monitoring iron status should be encouraged in female football players.

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Author Contributions

Pritee Singha: Investigation, Data collection and formal analysis,

Gurjeet Kaur Chawla: Experimental design, Supervision,

Indranil Manna: Experimental design, Writing – original draft, Supervision.

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Ethics Statement: The ethical approval was obtained from the Institutional Ethics Committee for this study (No: EC/2023-24/055; dated 03.12.2024).

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