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Research Article / Araştırma Makalesi

Effect of eight-week controlled dietary modification trial on nutritional biomarkers in young Indian athletes

Genç Hintli sporcularda sekiz haftalık kontrollü diyet değişikliği denemesinin beslenme biyobelirteçleri üzerine etkisi

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ABSTRACT

Objective: Present study was intended to examine the effects of dietary modification on nutritional biomarkers in young Indian male athletes.

Methods: Eighty-eight footballers and hockey players were randomly segregated into ad-libitum group (ALG) and nutrition counselling group (NCG). Eight weeks of dietary modification trial was implemented on NCG, resulting in 9.5, 2.6, and 2.1 g/kgbw/day of carbohydrate, protein, and fat respectively. Rate of changes in daily nutrient intake and nutrition biomarkers were captured.

Results: NCG revealed significant increases in total serum protein (5.6%, p<0.001), haemoglobin (3.9%, p<0.001) and vitamin E (2.9%, p<0.001). They also exhibited rises in serum vitamin C (6%, p=0.004), folate (4.5%, p=0.004), ferritin (4.8%, p=0.003), calcium (4.2%, p=0.009), serum vitamins B12 (3.9%, p=0.012) and D (12.3%, p<0.001). Haemoglobin levels were positively correlated with daily protein (p<0.01), iron (p<0.05), vitamins B12 and vitamin C (p<0.05 both) intakes. Serum calcium positively correlated with daily calcium (p<0.01), phosphorus and protein (p<0.05 both) intakes. Serum zinc was positively correlated with daily protein, calcium, vitamin B9 and vitamin D intake, while serum ferritin and vitamin B12 were positively correlated with protein and iron intake. Post-intervention, NCG reported optimal blood levels of calcium, zinc, protein, Hb, ferritin, vitamins B12, C and E, whereas the folate and vitamin D values were suboptimal. On the other hand, the ALG revealed marginal levels of zinc and total protein as well as noticeably low levels of calcium, folate, and vitamin D.

Conclusion: Enhanced blood levels of nutritional biomarkers noted after eight-weeks of controlled dietary modification was supported by positive correlations observed with the daily nutrient intakes.

Keywords: *Controlled dietary modification, nutritional biomarkers, nutrient intake, nutritional status, physiological profile*

ÖZ

Amaç: Bu çalışmanın amacı, genç Hintli erkek sporcularda diyet değişikliğinin beslenme biyobelirteçleri üzerindeki etkilerini incelemekti.

Yöntem: Seksen sekiz futbolcu ve hokey oyuncusu rastgele olarak isteğe bağlı gruba (ALG) ve beslenme danışmanlığı grubuna (NCG) ayrıldı. NCG üzerinde sekiz haftalık diyet değişikliği denemesi uygulandı ve vücut ağırlığının her günü için sırasıyla 9.5 g, 2.6 g ve 2.1 g karbonhidrat, protein ve yağ hedeflendi. Günlük besin alımındaki ve beslenme biyo-belirteçlerindeki değişim oranları saptandı.

Bulgular: NCG grubu toplam serum proteininde (%5.6, p<0.001), hemoglobinde (%3.9, p<0.001) ve E vitamininde (%2.9, p<0.001) anlamlı artışlar gösterdi. Ayrıca C vitamini (%6.0, p=0.004), folat (%4.5, p=0.004), ferritin (%4.8, p=0.003), kalsiyum (%4.2, p=0.009), serum B12 (%3.9, p=0.012) ve D vitaminlerinde (%12.3, p<0.001) de artışlar sergiledi. Hemoglobin düzeyi; günlük protein (p<0.01), demir (p<0.05), B12 ve C vitaminleri (p<0.05) alımıyla pozitif yönde ilişkiliydi. Serum kalsiyumu, günlük kalsiyum (p<0.01), fosfor ve protein (her ikisi p<0.05) alımıyla pozitif yönde ilişkiliydi. Serum çinkosu günlük protein, kalsiyum, B9 ve D vitaminleri alımıyla pozitif korelasyon sergilerken, serum ferritin ve B12 vitamini protein ve demir alımıyla pozitif korelasyon gösterdi. Girişim sonrasında NCG grubunda kanda optimal kalsiyum, çinko, protein, Hb, ferritin, B12, C ve E vitaminleri düzeyleri gözlenirken, folat ve D vitamini değerleri optimalin altında kaldı. Öte yandan, ALG grubunda marjinal düzeylerde çinko ve toplam proteinin yanı sıra gözle görülür derecede düşük kalsiyum, folat ve D vitamini düzeyleri ortaya çıktı.

Sonuç: Sekiz haftalık kontrollü diyet değişikliği sonrası kaydedilen beslenme biyobelirteçlerinin kan seviyelerindeki artış, günlük besin alımıyla gözlenen pozitif korelasyonlarla desteklendi.

Anahtar Sözcükler: *Kontrollü diyet değişikliği, beslenme biyobelirteçleri, besin alımı, beslenme durumu, fizyolojik profil*

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INTRODUCTION

Training for athletic excellence requires adequate calories and macronutrients to enhance performance, and support growth and development. Vitamins and minerals are essential for energy metabolism, and deficiencies can have a significant impact on exercise capacity (1).

Iron is an essential micronutrient for athletic performance, as it is involved in the production of red blood cells, oxygen transport and electron transport during oxidative phosphorylation. A fall in ferritin, a form of iron store in the body, is a sign of iron deficiency. Low blood haemoglobin concentration (anaemia) may result from an inadequate iron intake in the diet, and may also be due to the deficiency of vitamins B12 or folate (2). Athletes require special attention with regard to calcium needs and its adequate intake. Factors such as weight-bearing exercise, electrolytes lost via sweat, and magnesium homeostasis are important for optimizing adequate dietary calcium intake. Additionally, magnesium homeostasis is important for the regulation of energy and calcium metabolism, acting as a cofactor and activator for a number of enzymes (3). Zinc plays an important role in tissue repair and physical activity, while vitamin D3 is essential for muscle growth and improved fitness (4). Vitamin C is essential for the functioning of biochemical pathways, particularly exercise metabolism, as it counteracts oxidative stress (5). Similarly, vitamin E is an important antioxidant and membrane stabilizer for improving performance, minimizing muscle damage, and maximizing recovery from exercise (6).

Nutrition is essential for athletic excellence, but little research has been done to examine the impact of structured dietary modification protocols on Indian athletes in terms of biochemical parameters. Hence, the present study intended to examine the changes in blood levels of the nutritional biomarkers brought about by intervening in a controlled dietary modification programme in accordance to the standard nutritional guidelines among young Indian male athletes. It was hypothesized that nutritional status and blood levels of the nutritional biomarkers would improve after engaging athletes in the eight weeks of recommended dietary modification trial.

Study Participants

The present investigation was carried out on 88 state level young male athletes (Football: 42; Hockey: 46) who had at least three years of formal training history. They were randomly segregated into an ad-libitum group (ALG; n=43, age: 16.4±1.4 years) and a nutrition counselling group (NCG; n=45, age: 16.1±1.9 years). The test protocol was implemented during their pre-competitive phase, subjects were clinically examined by physicians specialized in sports medicine following standard procedure (7). The study was conducted in accordance with the Declaration of Helsinki after obtaining ethical clearance from the Institutional Human Ethical Committee, Department of Physiology, University of Calcutta (Ref. No. IHEC/AB/P83/2019).

Athletes followed a customary training protocol designed by qualified coaches and scientific experts for eight weeks, with three hours of training per day, six days a week. Training consisted of cardio-respiratory workouts, sprint intervals, and game-specific skill training. NCG received controlled dietary modification for eight weeks, while ALG were instructed to follow ad-libitum intake. All assessments were repeated for post-intervention testing.

Nutritional Intervention

Eight weeks of a dietary modification trial led by an experienced sports nutritionist was implemented on NCG. Controlled and individualized dietary changes were made in accordance with the standardized nutrition and hydration guidelines set by collaborative efforts of the Indian Life Science Institute, National Institute of Nutrition, and Sports Authority of India. The daily diet plan was designed to fetch 70 kcal/kgbw/day of total energy, with 550 g of cereals, 40 g of pulses, 150 g of leafy greens, 200 g other vegetables, 150 g of roots and tubers, 750 ml of milk and dairy products, 75 g of fats and butter, 250 g of meat, 100 g of egg and 150 m of fruits. Individual dietary modification was catered to all the athletes in NCG, by implementing minor adjustments in the number of servings of various food groups and calories contributed through macronutrient distribution. In each subsequent review, compliance with dietary recommendations was confirmed, and nutritional modifications were made in accordance with the goals of their distinctive sports training and the individual needs of the athletes. Considering the young population of the participants, diversity in food choices and individual preferences were also considered to increase adherence.

Training Regimen

Under the supervision of a scientific expert, certified coaches administered the systematic training programme. With the exception of the training, practically all of the games in the present research included a similar training regimen, which was carried out for an average of 4 to 5 hours every day except Sunday, totaling about 30 hours each week. The physical training program included flexibility exercises, and strength and endurance training in accordance with the needs of respective sports.

Nutritional Assessment

A 24-hour dietary recall questionnaire was used to assess dietary habits such as meal pattern, frequency, intake of food groups, and portion sizes. Cooked foods were converted to raw amounts and nutrients were calculated using the Dietsoft software (NIN, ICMR, IFCT 2017). Nutrient intake, including calories, carbohydrate, protein, fat, dietary fibre, calcium, phosphorus, iron, zinc, vitamins B9, B12, C, D, E and B was calculated and compared with nutrition and hydration guidelines for athletes before and after dietary modification (8).

Anthropometric and Body Composition Assessments

Physical characteristics including body height and weight was measured using anthropometric rod and digital weighing machine respectively, and body mass index (BMI) was calculated by using standard equation. Fat mass (FM), fat free mass (FFM), muscle mass (MM), body cell mass (BCM) was assessed using Bioelectrical Impedance Analyzer (BIA) (Maltron Bioscan 920-2, Made in UK) following standard testing manual of Maltron International.

Biochemical Assessment

Venous blood sample was collected from the antecubital vein between 7 and 8 am in the pre-prandial state. Each sample was transferred into aliquots which were stored in a cooler box until centrifuged at 2500 rpm for 15 min at 4°C. The samples were then transferred into two different crayovials and preserved at -20°C for biochemical analyses. Serum calcium and zinc concentrations were determined using atomic absorption spectrophotometry (9,10). Serum ferritin and haemoglobin were measured using colorimetric assay kit (11). Total serum protein determination was done on the serum samples using standard Lowry method (12). ELISA kit was used for measuring folic acid (Cell Biolabs, Inc), vitamin B12 and vitamin 25(OH) D (Calbiotech Inc.) (13,14). Analysis for plasma vitamins E and C were done through spectroscopic determination following protocols standardized by Baker and Frank, and Roe and Kuether (15,16).

Statistical Analyses

Statistical Package for Social Sciences (SPSS) version 25.0 (SPSS Inc., Chicago, Il, USA) was used for statistical analysis of the data. Differences between paired data for all variables obtained before and after the intervention were calculated using paired t-test and percentage change analysis. Pearson's product-moment correlation coefficient was used to assess the association between different variables. The differences between the cases and controls were also analysed using sample T test. Level of significance was set at $p \leq 0.05$.

RESULTS

Table 1 depicts the change rates in anthropometric parameters and daily macronutrient intake in NCG and ALG from pre- to post-intervention. Although insignificant changes in body weight and BMI were noted in both groups, fat content decreased significantly by 16.7% in the NCG ($p=0.004$), whereas ALG revealed a 12.8% increase in fat ratio ($p=0.020$). Significant increases of 12.1% in FFM ($p \leq 0.001$) and 17.8% in MM ($p \leq 0.01$) occurred among NCG, whereas ALG depicted decreases of 0.7% ($p>0.05$) and 5.4% ($p \leq 0.05$), respectively. Also, the BCM/FFM ratio was found to be significantly increased by 5.7% in the NCG ($p \leq 0.05$), whereas the same decreased by 1.8% ($p \leq 0.01$) in the ALG. Substantial upsurge in the g/kgbw/day intake of carbohydrate (31.3%, $p<0.001$), protein (15.8%, $p<0.001$) and fat (15.4%, $p<0.001$) were noted among the NCG post-intervention. The ALG displayed wan insignificant change in carbohydrate intake ($p=0.083$), however protein intake declined to 6.3% ($p=0.022$) and a steep 8.3% increase in fat intake ($p=0.002$) was observed. Intake of total dietary fibre significantly improved to 50.9% among the NCG ($p<0.001$), while ALG disclosed an increase of 8.9% ($p=0.048$).

Concerning the differences noted in the anthropometric variables and macronutrient intake among the NCG and ALG after the intervention period; significant differences were noted in FFM ($p<0.001$), fat ratio ($p=0.003$), BCM/FFM ($p=0.001$) and MM among the NCG and ALG:

the former revealed higher FFM (53.2±4.7 kg) and MM (16.2±3.3 kg) values along with higher BCM/FFM ratio (0.56±0.05), whereas ALG had higher fat ratio (16.2±5.7%). The distribution of macronutrient intake post-intervention depicts that NCG were eating significantly higher proportions of carbohydrate (56.8±2.5%; $p<0.001$) and protein (18.7±1.1%; $p<0.001$) in their daily diet, whereas the proportion of fat in the daily diet was significantly higher among the ALG (28.3±2.8%; $p<0.001$). Further, NCG was found to have significantly higher dietary fibre intake (36.3±10.8 g; $p<0.001$) after the intervention period.

Table 2 illustrates the changes in micronutrient intake from pre-to post-dietary modification among the ALG and NCG. The daily calcium ($p<0.001$) and phosphorus intake ($p<0.001$) significantly increased to 21.2% and 21.8% respectively among the NCG, whereas ALG displayed no changes for the same. A rise of 9.0% in iron ($p=0.01$) and 5.7% in magnesium ($p<0.001$)

Table 1. Comparison of anthropometric variables and macronutrient intake in the pre- and post-intervention phases, and post-intervention differences among NCG and ALG

Parameters	Groups	Pre-intervention	Post-intervention	Level of significance	p Value	% Change
Weight	NCG	57.7±7.5	58.3±7.2	-1.539	0.131 (ns)	1.0
(kg)	ALG	61.4±9.8	61.5±9.4	-0.100	0.921 (ns)	0.1
BMI	NCG	20.2±1.9	20.3±2.0	-0.812	0.421 (ns)	0.9
(kg/m^2)	ALG	20.8±2.0	20.8±2.0	-0.071	0.944 (ns)	0.1
FFM	NCG	47.5±4.5	53.2±4.7	-8.627	0.000***	12.1
(kg)	ALG	48.7±5.0	49.1±4.9**c**	-0.487	0.634 (ns)	0.7
Fat ratio	NCG	15.3±6.8	12.7±4.9	3.025	0.004*	-16.7
(%)	ALG	14.3±5.8	16.2±5.7**b**	-2.413	0.020*	12.8
BCM/FFM	NCG	0.53±0.05	0.56±0.05	-2.606*	0.023	5.7
	ALG	0.54±0.04	0.53±0.03**b**	3.975**	0.002	-1.8
MM	NCG	13.8±3.8	16.2±3.3	-3.515	0.004**	17.8
(kg)	ALG	13.8±4.5	13.1±3.9**c**	2.850	0.014*	-5.4
Energy	NCG	2655±284	3333±406	-13.194	<0.001***	25.5
(kcal)	ALG	2479±318	2532±319	-1.210	0.233 (ns)	2.2
Carbohydrate	NCG	364±45	473±61	-13.615	<0.001***	29.8
(g)	ALG	332±50	331±49	0.130	0.897 (ns)	-0.3
Carbohydrate	NCG	6.42±1.14	8.44±1.20	-11.503	<0.001***	31.3
(g/kgbw/day)	ALG	5.53±1.18	5.49±1.04**c**	0.251	0.803 (ns)	-0.7
Carbohydrateintake (% energy)	NCG	54.9±2.6	56.8±2.5	-4.801	<0.001***	3.5
	ALG	53.5±2.5	52.2±4.1**c**	1.687	0.099 (ns)	-2.4
Protein	NCG	96.7±10.4	151.0±13.8	-31.262	<0.001***	56.1
(g)	ALG	97.6±11.7	92.8±13.7	1.865	0.069 (ns)	-4.9
Protein	NCG	1.90±0.31	2.20±0.30	-7.528	<0.001***	15.8
(g/kgbw/day)	ALG	1.60±0.33	1.50±0.28**c**	2.371	0.022*	-6.3
Protein intake	NCG	15.0±1.0	18.7±1.1	-21.597	<0.001***	24.7
(% energy)	ALG	16.2±1.0	15.1±1.4**c**	4.868	<0.001***	-6.8
Fat	NCG	73.9±11.1	79.0±13.6	-2.914	0.006*	6.9
(g)	ALG	72.6±11.0	79.7±15.2	-4.351	<0.001***	9.8
Fat	NCG	1.30±0.27	1.50±0.25	-4.664	<0.001***	15.4
(g/kgbw/day)	ALG	1.20±0.23	1.30±0.25**c**	-3.975	<0.001***	8.3
Fat intake	NCG	25.0±2.3	21.3±2.2	11.677	<0.001***	-14.8
(% energy)	ALG	26.5±2.8	28.3±2.8**c**	-3.418	0.001***	6.8
Dietary fibre intake (g)	NCG	24.0±9.0	36.3±10.8	-10.853	<0.001***	50.9
	ALG	23.8±7.8	25.9±7.6**c**	-2.034	0.048*	8.9

Values are mean ± SD; pre-post differences *: p<0.05, **: p<0.01, ***: p<0.001, ns: not significant; post-intervention inter-group **b**: p<0.01, **c**: p<0.001; NCG: nutrition counselling group, ALG= ad-libitum group

Intake were also noted among the NCG, whereas ALG revealed insignificant decreases in mineral intake. Further, NCG reported significant increases in vitamins B: 6.6% in vitamin B2 (p=0.028), 5.5% in B3 (p=0.013), 8.7% in B5 (p=0.011), 4.5% in B6 (p=0.001), 7.2% in B9 (p<0.001), and 20.2% in vitamin B12 (p=0.013). Contradictorily, insignificant decreases were recorded among ALG along with significant reduction of 4.8% in vitamin B2 (p=0.044) and 4.1% in B3 intake (p=0.049), respectively. Also, the NCG exhibited increased intakes of 14.3% in vitamin A (p=0.005), 17.0% in vitamin C (p<0.001), 7.6% in vitamin E (p<0.001) and 6.8% in vitamin D (p=0.009); whereas ALG had a significantly decreased intake of 4.7% in vitamin A (p=0.034).

Comparing biomarkers' status (Table 3), NCG displayed a significant increase of 5.6% in total serum protein (p<0.001), 3.9% in haemoglobin (p<0.001) and 2.9% in vitamin E (p<0.001). They also exhibited increases of 6.0% in vitamin C (p=0.004), 4.5% in folate (p=0.004), 4.8% in ferritin (p=0.003) and 4.2% in calcium (p=0.009). Serum vitamins B12 (p=0.012) and D (p<0.001) also improved to 3.9% and 12.3%, respectively, post-intervention. However, the increase in zinc was insignificant in this group. ALG exhibited no substantial changes in blood biomarker levels except for significant decreases in vitamins C (p=0.004) and E (p=0.010) after the intervention.

Changes were noted in nutritional biomarkers of NCG and ALG after the intervention period. NCG revealed significantly higher values for serum calcium (p<0.001), Hb (p=0.009), folate (p<0.014), and vitamin D (p=0.052). Whereas, the differences in serum zinc, total serum protein, ferritin, vitamin B12, and vitamin C were found to be insignificant (p>0.05).

Table 2. Comparison of pre- and post-intervention micronutrient intakes among NCG and ALG

Parameters	Groups	Pre-intervention	Post-intervention	Level of significance	p Value	% Change
Calcium	NCG	1130±181	1368±148	-7.796	<0.001***	21.2
(mg)	ALG	1166±179	1159±100	0.259	0.797 (ns)	-0.6
Phosphorus (mg)	NCG	2048±297	2495±309	-11.988	<0.001***	21.8
	ALG	1925±339	1902±290	0.545	0.589 (ns)	-1.2
Iron	NCG	15.2±3.1	16.6±3.6	-2.681	0.010**	9.0
(mg)	ALG	15.0±4.3	14.9±3.9	0.256	0.799 (ns)	-0.9
Zinc	NCG	6.15±1.75	6.69±2.16	-1.622	0.112 (ns)	5.5
(mg)	ALG	6.20±1.86	5.98±1.82	0.762	0.450 (ns)	-3.5
Magnesium	NCG	405±75	428±80	-4.613	<0.001***	5.7
(mg)	ALG	398±86	403±75	-0.406	0.687 (ns)	1.2
Vitamin A	NCG	546±164	624±184	-2.990	0.005*	14.3
(µg)	ALG	584±121	556±107	2.191	0.034*	-4.7
Vitamin B1	NCG	1.68±0.33	1.76±0.42	-1.837	0.073 (ns)	4.7
(mg)	ALG	1.52±0.40	1.45±0.36	1.566	0.125 (ns)	-4.6
Vitamin B2	NCG	1.51±0.20	1.61±0.32	-2.273	0.028*	6.6
(mg)	ALG	1.46±0.30	1.39±0.19	2.079	0.044*	-4.8
Vitamin B3	NCG	12.5±1.6	13.2±1.89	-2.600	0.013*	5.5
(mg)	ALG	11.3±1.7	10.9±1.6	2.026	0.049*	-4.1
Vitamin B5	NCG	1.03±0.18	1.12±0.23	-2.647	0.011*	8.7
(mg)	ALG	1.02±0.31	1.00±0.29	0.352	0.727 (ns)	-1.9
Vitamin B6	NCG	0.66±0.16	0.69±0.15	-3.668	0.001***	4.5
(mg)	ALG	0.67±0.24	0.61±0.18	1.952	0.058 (ns)	-8.9
Vitamin B9	NCG	208±31	224±28	-4.141	<0.001***	7.2
(µg)	ALG	212±46	206±44	1.270	0.211 (ns)	-3.1
Vitamin B12	NCG	0.94±0.38	1.13±0.42	-2.577	0.013*	20.2
(µg)	ALG	1.02±0.54	0.96±0.44	0.873	0.388 (ns)	-5.8
Vitamin C	NCG	66.7±11.8	78.1±14.7	-4.546	<0.001***	17.0
(mg)	ALG	47.0±10.1	49.0±11.0	-1.097	0.279 (ns)	4.3
Vitamin E	NCG	7.06±1.05	7.60±1.02	-4.628	<0.001***	7.6
(mg)	ALG	7.09±0.84	7.12±0.86	1.750	0.086 (ns)	0.4
Vitamin D	NCG	4.83±1.31	5.16±1.21	-2.744	0.009*	6.8
(µg)	ALG	4.54±0.70	4.41±0.82	1.895	0.065 (ns)	-2.8

Values are mean±SD, *: p<0.05, **: p<0.01, ***: p<0.001, ns: not significant, NCG= nutrition counselling group, ALG: ad-libitum group

Table 3. Comparison of nutritional biomarkers recorded pre- and post-intervention, and post-intervention differences among NCG and ALG

Parameters	Reference range	Groups	Pre-intervention	Post-intervention	Level of significance	p Value	% Change
Calcium (mg/dl)	9.2-11.0	NCG	8.85±1.04	9.22±1.01	-2.728	0.009*	4.2
		ALG	8.43±0.86	8.36±0.84**c**	1.788	0.081 (ns)	-0.8
Zinc (µg/dl)	70-150	NCG	78.2±16.6	80.4±15.5	-1.285	0.206 (ns)	2.7
		ALG	77.0±8.6	76.3±8.7	-2.352	0.023*	-0.9
Total serum protein (g/dl)	6.0-8.0	NCG	6.01±0.75	6.35±0.70	-4.107	<0.001***	5.6
		ALG	6.17±0.53	6.13±0.56	0.801	0.428 (ns)	-0.6
Haemoglobin (g/dl)	13.5-15.0	NCG	14.7±0.9	15.3±0.9	-4.426	<0.001***	3.9
		ALG	14.7±0.8	14.8±0.9**b**	-0.416	0.680 (ns)	0.2
Ferritin (ng/ml)	50-300	NCG	60.9±9.9	63.8±10.6	-3.186	0.003*	4.8
		ALG	63.0±9.3	62.1±8.9	-2.211	0.032 (ns)	-1.4
Vitamin B12 (pg/ml)	400-700	NCG	516±71	536±57	2.617	0.012**	3.9
		ALG	520±78	515±72	-1.177	0.245 (ns)	-1.1
Folate (ng/ml)	6-20	NCG	4.85±0.70	5.07±0.67	-3.075	0.004*	4.5
		ALG	4.77±0.70	4.70±0.72**b**	-2.521	0.015**	-1.5
Vitamin C (µmol/L)	23-114	NCG	56.0±6.3	59.3±7.6	-3.066	0.004*	6.0
		ALG	55.9±7.5	57.5±7.2	-3.093	0.004*	2.9
Vitamin E (µmol/L)	11.9-30	NCG	26.8±3.1	27.6±3.3	-7.691	<0.001***	2.9
		ALG	27.1±2.9	26.4±2.8	2.705	0.010**	-2.5
Vitamin D (ng/ml)	>40.0	NCG	21.2±5.9	23.8±5.7	5.112	<0.001***	12.3
		ALG	22.5±6.7	21.1±6.9**a**	1.647	0.107 (ns)	-6.1

Values are mean±SD, *: p<0.05, **: p<0.01, ***: p<0.001, ns: not significant; post-intervention inter-group **a**: p<0.05, **b**: p<0.01, **c**: p<0.001; NCG: nutrition counselling group, ALG: ad-libitum group

In terms of interrelations;, haemoglobin levels were found to have positive correlation with serum zinc and total protein (p<0.01 both) levels, serum ferritin (p<0.05), and vitamins B12, B9 and C (p<0.01 each) plasma levels. Also, daily protein (p<0.01) and iron (p<0.05), and vitamin B12 and vitamin C (p<0.05 both) intakes were found to positively correlate with haemoglobin levels. Serum calcium displayed positive correlation with total serum protein (p<0.01), and vitamin D (p<0.05) levels. It has also been observed that a significant positive correlation of serum calcium with daily intakes of calcium (p<0.01), phosphorus and protein (p<0.05 both) existed. Serum zinc was also found to be po–

sitively and significantly correlating with total serum protein, vitamins B12 and D ($p<0.05$ each) levels, along with daily dietary intake of protein ($p<0.01$), calcium and vitamin B9 ($p<0.05$ both). Total serum protein positively correlated with serum ferritin ($p<0.01$) and vitamin D ($p<0.05$) along with daily dietary intakes of protein ($p<0.01$), fat, calcium, and vitamins A, B1, B6 and B12 ($p<0.05$ each). Serum ferritin exhibited significant positive correlations with daily iron ($p<0.01$) and protein intake ($p<0.05$). Vitamin B12 levels positively correlated with daily protein and iron intakes ($p<0.05$ both). Vitamin D positively correlated with dietary vitamin D intake ($p<0.01$). Serum folate also was positively correlated with daily dietary intakes of vitamin B9 and vitamin C ($p<0.01$ both). Plasma vitamin C positively correlated with vitamin C ($p<0.01$) intake. Plasma vitamin E positively correlated with daily fat ($p<0.05$) and vitamin E intake ($p<0.01$).

DISCUSSION

Depending on the volume of exercise regimen, carbohydrate and protein intake are enhanced to meet the energy requirements (17). After eight weeks of dietary modification in the present study, the daily intake recommendations were met by 88% (8.4±1.2 g/kgbw/day, $p<0.001$) for carbohydrates, 85% for proteins (2.2± 0.3 g/kgbw/day, $p<0.001$), and 63% for fats (1.5± 0.25 g/kgbw/day, $p<0.001$), with regards to the standardized guidelines set for team game athletes, whereas before the intervention NCG were only meeting 67%, 73% and 54% of the recommendations, respectively. The NCGs displayed significant increases in macronutrient intake to meet the total calorie need and create an equilibrium in healthy fat and quality protein intakes with healthy carbohydrate choices. Additionally, the proportion of carbohydrate: protein: fat intake changed from 54%:15%:25% to 57%:19%:21%, which resulted in a 16.7% decrease in fat mass post-intervention. Combining training regimen with dietary modification can significantly modify body composition (17,18).

On the contrary, ALG revealed a 6.3% decline in protein intake ($p=0.022$) along with a 8.3% increase in their daily fat intake ($p<0.001$). Athletes who are prone to micronutrient deficits are those who restrict overall consumption, implement extreme measures for weight loss, and reject one or more food groups from their diet. This was noticed among the ALG, as there were insignificant changes in daily calorie consumption during the curriculum, suggesting that athletes remain deficient in macronutrients and micronutrients (19).

Changes in body weight attained by both the NCG and ALG were significant. FFM and FM are the two most crucial components for a player's optimal health and strength (17). The body composition profile of the NCG was significantly positive, with substantial decreases in fat ratio and gains in fat-free mass across all athletic disciplines. This resulted in better FM:FFM ratios. The reduction in FM despite higher energy intake is likely due to a change in the ratios of calorie contribution by macronutrients combined with a strategically designed exercise routine. The comparative results after the intervention period depicted NCG to have a more favourable macronutrient distribution than that of ALG, which further might have resulted in significant favorable changes in the BCM/FFM ratio and a lower fat percentage in the former.

Furthermore, energy intake rose, but shortfalls in intake (200-300 kcal/day) may have contributed to NCG fat mass decreases. Overall weight increases were supported by significant increases in FFM, BCM, total body protein and MM, which resulted in proportional increases in their BCM/FFM ratios and glycogen stores. This is important for improving physical fitness profiles of athletes (2). The present study revealed a significant improvement in fat ratio and active cell mass in the studied population following a dietary intervention programme. However, the ALG recorded significant decreases in BCM/FFM, protein and MM, as well as significantly raised FM across all sports disciplines. This suggests that extra body fat accumulation is contributing to increased weight. Performance parameters have not been studied, but the improvement in fat ratio and active cell mass is consistent with previous research (20).

Blood biomarkers for athletic excellence include proteins, metabolites, minerals, electrolytes, and other molecules. Deficits such as energy, protein, vitamin D, and calcium can negatively affect overall health and body function (21). Research has revealed that NCG with increased energy and protein consumption can lead to increased calcium and vitamin D intakes, which can lead to significant increases in total serum protein, calcium, vitamins D and B12. Studies have also shown an inverse interrelationship between vitamin D levels and body fat ratio (22). It is speculated that for individuals with excess body fat, provitamin D3 remains trapped deep within their rigid subcutaneous fat which subsequently impairs the conversion of vitamin D3 to 25(OH) D (20). Athletes with lower body fat mass had higher vitamin D concentrations, which are essential for calcium uptake and incorporation into bone (23). Increased intake of calcium, phosphorus, and vitamin D among NCG may have resulted in increased calcium concentration in blood (24).

Dietary modification through iron-rich foods is recommended to ensure optimal iron intake and maintain iron status

(3). Increased protein intake, such as organ meat, poultry, and legumes, has been linked to improved iron status in the NCG. Serum ferritin levels have also improved, suggesting healthy iron stores. Vitamin C intake also facilitates iron absorption, correlated with plasma status and prevalence of deficiency (25). The poor iron and vitamin C intake among the ALG may be the reason for no significant changes in their haemoglobin and ferritin concentrations. Implementation of controlled dietary modification recommended to NCG resulted in significant increases in the intake of protein and iron, vitamins B9, B12 and C, which synergistically might have resulted in improved total serum protein, haemoglobin, and vitamin B12 levels. This correlation is in line with previously done studies depicting a direct proportional relationship of haemoglobin level with daily intake of dietary protein, iron, vitamin B12 and vitamin C (26,27).

Nutritional biomarkers such as serum zinc, protein and ferritin have a positive correlation with haemoglobin levels (28). Vitamins B12, folate and C have been linked to the occurrence of different types of anaemia (29,30). Serum calcium levels are associated with total serum protein, as observed in this study (31). The positive bridging between calcium and vitamin D is essential for the body to maintain sufficient levels of vitamin D to regulate calcium homeostasis (4). This study found associations between serum zinc, vitamin D, total serum protein and vitamin B12, which are essential for enzymes involved in DNA synthesis, mitosis, cell division and protein synthesis (27,29).

Protein, calcium, folate and vitamin D rich foods are a good source of zinc, and total serum protein and ferritin both have a direct proportional relationship with dietary protein intake. Total serum protein levels also provided a parallel link with serum ferritin and vitamin D (31). Depleted iron stores can affect ferritin levels, and dietary iron intake has been linked to the same (27). Vitamin B12 levels are positively correlated with serum zinc, protein, and iron intake due to its presence in animal food sources (29). Folate and vitamin C levels in blood are correlated with dietary vitamin C intake, but vitamin E has a significant positive correlation with daily fat intake more than dietary vitamin E (32). Improved nutritional profile and adequate dietary intake by athletes are essential for balancing nutritional biomarkers and improving performance.

This study revealed that controlled dietary modification among NCG resulted in significant increases in macro- and micronutrient intake as observed in previous researches, which may have resulted in better blood levels of total serum protein, haemoglobin, serum ferritin, plasma zinc, serum calcium and vitamin B12 (33). Furthermore, comparative results between the NCG and ALG post-intervention disclosed significant differences only in the blood levels of calcium, Hb, and vitamin D. After the completion of the intervention period, NCG was found to have optimal blood levels of calcium, zinc, total protein, Hb, ferritin, and vitamins B12, C and E, whereas the values captured for folate and vitamin D were found suboptimal, leaving space for improvement. On the other side, ALG depicted borderline values for serum zinc and total protein levels along with significantly deficient blood levels of calcium, folate and vitamin D, which collectively signifies their daily diet to be lacking in protein sources, supported by the poor contribution of protein to total daily calorie intake.

Further, the body composition profile was markedly positive among the NCG post-intervention, with significant reductions in fat ratio and gains in fat-free mass. Similarly, among the ALG, significant declines in BCM/FFM and MM, along with raised FM, suggest increases in weight contributed by extra body fat accumulation. Our study contributes to the body of evidence that athletes intervened with controlled dietary modification ameliorate body composition profile and blood biomarkers that plays a pivotal role in athletic performance (34)

The main limitation of this study was that the meals were served from a centralized kitchen although the dietary recommendation was individualized at the athlete level. Compliance was checked through verbal communication, but close monitoring of individuals' daily dietary intake onground was a challenge and was not exclusively controlled.

CONCLUSION

This study revealed enhanced blood levels of nutritional biomarkers after eight-weeks of controlled dietary modification.

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Ethics Committee Approval / Etik Komite Onayı

The approval for this study was obtained from University of Calcutta, Institutional Human Ethical Committee Deperatment of Physiologoy, Kolkata, India (Decision no: IHEC/AB/P83/2019, Date: 27/02/2019).

Conflict of Interest / Çıkar Çatışması

The authors declared no conflicts of interest with respect to authorship and/or publication of the article.

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Author Contributions / Yazar Katkıları

Concept: MD, SKD; Design: MD, AB; Supervision: AB, SKD, GD; Materials: MD, SKD, GD; Data Collection and/or Processing, MD; Analysis and Interpretation: MD; Literature Review: MD, GD; Writing Manuscript: MD, AB; Critical Reviews: AB

REFERENCES

1. Witard OC, Garthe I, Phillips SM. Dietary protein for training adaptation and body composition manipulation in track and field athletes. *Int J Sport Nutr Exerc Metab.* 2019;29(2):165-74.
2. Xu W, Barrientos T, Andrews NC. Iron and copper in mitochondrial diseases. *Cell Metab.* 2013;17(3):319-28.
3. Clarkson P, Haymes E. Exercise and mineral status of athletes: calcium, magnesium, phosphorus and iron. *Med Sci Sports Exerc.* 1995;27(6):831-43.
4. Larson-Meyer D. The importance of vitamin D for athletes. *Gatorade Sports Sci Exch.* 2015;28:1-8.
5. Hughes RE. Recommended daily amounts and biochemical roles. The vitamin C, carnitine, fatigue relationship. In: Counsell JN, Hornig DH editors. *Vitamin C (Ascorbic Acid).* London: Applied Science Publishers. 1981; p. 75-86.
6. Asha Devi S, Prathima S, Subramanyam MVV. Dietary vitamin E and physical exercise: I. Altered endurance capacity and plasma lipid profile in ageing rats. *Exp Gerontol.* 2003;38(3):285-90.
7. Debnath M, Roy M, Chatterjee S, Dey S K. Body composition profile of elite Indian male and female archers: a comparative study. *Int J Health Phys Edu Comp Sci Sport.* 2016;23(1):19-25.
8. Panandiker DHP, Satyanarayana K, Ramana YV, Sinha R. *Nutrition and Hydration Guidelines for Excellence in Sports Performance*. International Life Sciences Institute India. 2007.
9. Butrimovitz GP, Purdy WC. The determination of zinc in blood plasma by atomic absorption spectrometry. *Anal Chim Acta.*1977;94(1):63-73.
10. Thin CG, Thomson, PA. Estimation of calcium and magnesium in serum and urine by atomic absorption spectrophotometry. *JClin Pathol.* 1967;20(3):280-2.
11. Karamizrak SO, Işlegen C, Varol SR, Taskiran Y, Yaman C, Mutaf I, et al. Evaluation of iron metabolism indices and their relation with physical work capacity in athletes. *Br J Sports Med.* 1996;30(1):15-9.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Bio lChem.*1951;193(1):265-75.
13. Scott JM, Molloy AM. The discovery of vitamin B_{12}. *Ann Nutr Metab.* 2012;61(3):239-45.
14. Benetti E. Mastrocola R, Chjiazza F, Nigro D, D'Antona G, Bordano V, et al. Effects of vitamin D on insulin resistance and myosteatosis in diet-induced obese mice. *PLoS One.* 2018;13(1):e0189707.
15. Baker AF, Frank G. Estimation of vitamin E in tissues. In: Stahl, editor. *Dunnschicht Chromotographic ein laboratorium Handbuch*. Berlin: Springer-Verlag; 1951. p. 41-52.
16. Roe JH, Kuether CA. The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J Biol Chem.* 1943;147(2):399-407.
17. Kerksick CM, Kulovitz M.. Requirements of energy, carbohydrates, proteins and fats for athletes. In: *Nutrition and Enhanced Sports Performance*. Elsevier; 2013. p. 443-59.
18. Fleming JA, Catháin CÓ, Harper LD, Naughton RJ. Dietary intake and daily distribution of carbohydrate, protein and fat in youth tennis players over a 7-day training and competition period. *J Sports Sci Med.* 2021;20(3):413-20.
19. Jordan SL, Albracht-Schulte K, Robert-McComb JJ. Micronutrient deficiency in athletes and inefficiency of supplementation: Is low energy availability a culprit? *PharmaNutrition* [Internet]. 2020;14:100229.
20. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine joint position statement. Nutrition and athletic performance. *Med Sci Sports Exerc*. 2016;48(3):543-68.
21. Harvey LJ, Armah CN, Dainty JR, Foxall RJ, DJohn Lewis D, Langford NJ. Impact of menstrual blood loss and diet on iron deficiency among women in the UK. *Br J Nutr.* 2005;94(4):557-64.
22. Viegas Raimundo F, Moreira Faulhaber GA, Kalinka Menegatti P, da Silva Marques L, Weber Furlanetto T. Effect of high- versus low-fat meal on serum 25-hydroxyvitamin D levels after a single oral dose of vitamin D: a single-blind, parallel, randomized trial. *Int J Endocrinol.* 2011;809069.
23. American College of Sports Medicine, American Dietetic Association, Dietitians of Canada. Joint position statement: Nutrition and athletic performance. *Med Sci Sports Exerc*. 2000;32(12):2130-45.
24. Groff JL, Stepnick Gropper SA. *Advanced Nutrition and Human Metabolism.* Belmont, CA: Wadsworth Thompson Learning*;* 2000.
25. Hoppe M, Brün B, Larsson MP, Moraeus L, Hulthén L. Heme iron-based dietary intervention for improvement of iron status in young women. *Nutrition*. 2013;29(1):89-95.
26. Gera T, Sachdev HS, Boy E. Effect of iron-fortified foods on hematologic and biological outcomes: systematic review of randomized controlled trials. *Am J Clin Nutr*. 2012;96(2):309-24.
27. Habte K, Adish A, Zerfu D, Kebele A, Moges T, Tesfaye B, et al. Iron, folate, and vitamin B12 status of Ethiopian professional runners. *Nutr Metab (Lond)*. 2015;12:62.
28. El Hendy HA, Yousef MI, Abo El-Naga NI. Effect of dietary zinc deficiency on haematological and biochemical parameters and concentrations of zinc, copper, and iron in growing rats. *Toxicology.* 2001;167(2):163-70.
29. Krzywanski J, Mikulski T, Pokrywka A, Mlynczak M, Krysztofiak H, Fraczek B, et al. Vitamin B12 status and optimal range for haemoglobin formation in elite athletes. *Nutrients.* 2020;12(4):1038.
30. Finkelstein FO, Juergensen P, Wang S, Santacroce S, Levine M, Kotanko P, et al. Haemoglobin and plasma vitamin C levels in patients on peritoneal dialysis. *Perit Dial Int.* 2011;31(1):74-9.
31. Payne RB, Little AJ, Williams RB, Milner JR. Interpretation of serum calcium in patients with abnormal serum proteins. *Br Med J.* 1973;4(5893):643-6.
32. Monsen ER. Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium, and carotenoids. *J Am Diet Assoc*. 2000;100(6):637-40.
33. Baranauskas M, Stukas R, Tubelis L, Žagminas K, Šurkienė G, Švedas E, et al. Nutritional habits among high-performance endurance athletes. *Medicina (Kaunas).* 2015;51(6):351-62.
34. Guest NS, Horne J, Vanderhout SM, El-Sohemy A. Nutrigenomics: personalized nutrition for athletic performance. *Front Nutr.*2019;6:8.