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Investigating the Relationship Between a Low Carbohydrate Diet Score and Inflammatory and Oxidative Stress Biomarkers in Female Students

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ABSTRACT

Objective: A healthy diet is a major component of lifestyle management for maintaining optimum health. Dietary carbohydrates may induce inflammation and oxidative stress. We aimed to assess the relationship between adherence to a low-carbohydrate diet pattern with oxidative stress status and a panel of blood biomarkers of inflammation in healthy young women.

Methods: In this study, 171 healthy young women participated. We estimated the low-carbohydrate diet scores using a food frequency questionnaire. The total antioxidant capacity and free radical scavenging activity and malondialdehyde of serum and urine were quantified using the ferric reducing/antioxidant power, α, α-diphenyl-β-picrylhydrazyl, and formation of thiobarbituric acid reactive substances methods. Several routine hematological parameters were measured including white blood cells, neutrophil counts, and mean platelet volume. Neutrophil:lymphocyte ratio and platelet:lymphocyte ratio were derived from these measures.

Results: A higher low-carbohydrate diet score indicates a lower intake of carbohydrates and fiber and a higher intake of protein and fat. A greater adherence to a low-carbohydrate diet pattern was related to a lower level of hematological inflammatory indices including neutrophil count and neutrophil:lymphocyte ratio. Multivariable-adjusted odds ratios (95% CIs) demonstrated that a higher adherence to low-carbohydrate diet (third vs. first tertile) was associated with significantly higher levels of urinary α, α-diphenyl-β-picrylhydrazyl (adjusted odds ratio = 1.10; 95% CI: 1.02-1.20), as well as serum ferric reducing/antioxidant power (adjusted odds ratio = 1.05; 95% CI: 1.01-1.011) and α, α-diphenyl-β-picrylhydrazyl (adjusted odds ratio = 1.21; 95% CI: 1.11-1.33).

Conclusions: A greater compliance with a low-carbohydrate-style diet was related to lower levels of inflammatory biomarker and oxidative stress in healthy young women.

Keywords: Neutrophil, antioxidant, diet, inflammation

Introduction

Diet is an important determinant of health; different foods vary in their nutrient content, with multiple essential interactions and variable relationships.[1] Dietary carbohydrates are an important source of energy in the Iranian population and are mainly consumed in the form of rice and bread as well as foods containing simple sugars.[2]

A low-carbohydrate diet (LCD) is effective for weight loss by reducing the intake of calories in the diet.[3,4] Carbohydrate metabolism appears to play a critical role in the development of oxidative stress (OS), and an LCD diet has been reported to reduce OS.[5,6] Moreover, there are studies reporting that an LCD diet may reduce serum inflammatory indices such as high-sensitivity C-reactive protein (hsCRP), TNF-α, and IL-6. Low-grade inflammation and OS are important in the development of several human diseases.[7]

Reactive oxygen species (ROS), when generated in excess, lead to OS. It has been established that OS induces the oxidative modification of various biomolecules that include lipids, carbohydrates, proteins, and DNA, which ultimately leads to cellular and tissue injury.[8] Oxidative stress is connected with the development of cardiovascular, autoimmune, neurodegenerative, pulmonary, digestive system, and inflammatory diseases and plays a significant role in cellular aging.[9]

The hematological parameters derived from the routine complete blood count (CBC) are an inexpensive and reproducible method for assessing systemic inflammation. Moreover, changes in these markers are associated with various disorders.[10,11] For instance, increased

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platelet size is associated with higher thrombocyte reactivity and this is related to inflammatory response.[12] Mean platelet volume (MPV) is an indicator of platelet activation and is correlated with inflammatory conditions, disease intensity, and anti-inflammatory drugs' effectiveness in chronic inflammatory disorders. A high MPV is associated with an increased size of platelets, which contains more intracellular granules. Lymphocytes and platelets are associated with diverse innate and adaptive immune responses, while functional defects in lymphocyte signaling lead to autoimmune disease and activate platelets that enhance the inflammatory markers and cytokines secretion.[13] The red blood cell distribution width (RDW) reflects erythrocytic volume. Ineffective erythropoiesis results in higher RDW and is correlated with elevated secretion of different cytokines such as IL-1, IL-6, and TNF-α.[14]

Adherence to an LCD diet may has beneficial effects on OS and antioxidant status as well as the inflammatory response and that may be advantageous in different pathologies. Dietary carbohydrate restriction reduces ROS production by polymorphonuclear leukocytes, mononuclear cells, and inflammatory mediators, i.e., IL-6, hsCRP, and IL-8, and elevates the adiponectin levels as an anti-inflammatory factor.[15] As far as we know the relationship between LCD adherence with OS and the inflammatory response has not been studied in healthy individuals, so we aimed to explore the relationship between adherence to an LCD pattern with OS status and a panel of blood inflammatory parameters in healthy young women, which may aid our understanding of LCD regimen benefits.

Material and Methods

Study Design and Population

This study was conducted in Birjand, Iran, in January 2020. Apparently, healthy female university students aged 18-24 years were recruited. On initial screening, 330 females were initially identified from 5 different universities in Birjand. Since we aimed to perform our study on a homogeneous population, in order to control for potential confounders, only single, apparently healthy young women were recruited. We excluded those who have any severe complications and illnesses or those taking medication or those who were smokers. The final study sample population comprised 171 healthy young women. The study was approved by the Birjand University Research Ethical Committee (Date:11.3.2020, Decision No: IR.BUMS.REC.1398.402), and all subjects provided written informed consent.

Sampling and Storage

Blood and urine samples were collected after 12 hours of fasting. Samples were collected from participants in the luteal phase of the menstrual cycle (7-10 days prior to initiation of menstrual bleeding). Whole blood was collected into ethylenediamin tetra-acetic acid (EDTA) tubes and serum separator blood collection tubes. Urine samples were collected under aseptic procedures in a sterile container from the mid-stream portion of first morning urine. All the serum and urine samples were stored at −80°C before analysis.

Demographic, Biochemical, Clinical, and Anthropometric Data Collection

Height and weight were measured and body mass index (BMI) was calculated. Waist-to-hip ratio (WHR) was measured as the ratio of waist-to-hip circumference. Systolic (SBP) and diastolic (DBP) blood pressure levels in participants were obtained in resting condition and repeated during the same visit.

The serum levels of fasting blood glucose (FBG), urea, creatinine, alanine transaminase, aspartate transaminase, alkaline phosphatase, total and direct bilirubin, total protein, albumin, Ca, phosphate, Mg, and uric acid were measured using Pars Azmun commercial kits (Karaj, Iran) and an auto-analyzer (Prestige 24i, Japan).

Adherence to Low-Carbohydrate Diet Pattern

A validated 65-item semi-quantitative food frequency questionnaire (FFQ) was used to estimate the food intake of participants. Study participants were instructed to exactly record their food intake frequency. Food analysis was conducted using Diet Plan 6 software. We evaluated the LCD score of participants based on the total energy obtained from carbohydrates, proteins, and fats and then subjects were divided into 10 deciles. Participants with the lowest consumption of carbohydrate received 9 points and those in the second decile received a score of 8 and so forth. Individuals in the highest stratum received 0 point. The scoring procedure used for proteins and fats was similar, but the order was reversed. Finally, the total LCD score was identified by summing up the ratings contributed to deciles of carbohydrate, protein, and fat nutrients. The compliance to a defined LCD dietary pattern was defined based on the LCD score (ranged from 0 to 27). The higher scores represent greater adherence to the LCD pattern;[16,17] participants were classified into tertiles based on the LCD score.

Complete Blood Count

Various hematological parameters, blood cell counts, hemoglobin amounts, dimensional variables (MPV and RDW), and some combined parameters of blood cells, including NLR, PLR, and RDW, and platelet ratio (RPR), were evaluated using an automated blood cell counter (Sysmex K-800; Japan).

Measurement of High-Sensitivity C-Reactive Protein

Serum hsCRP value was determined using a commercial assay kit (Pars Azmun, Iran) on an auto-analyzer (Prestige 24i, Japan), based on the manufacturer's instructions.

Biochemical Assays

Ferric Reducing Antioxidant Power Assay: Ferric reducing antioxidant power was conducted based on the reduction reaction. About 10 µL of each sample was added to the 250 µL of the freshly prepared working FRAP solution and incubated for 10 minutes at 37°C. Finally, the absorption of the solution was measured at 593 nm. All obtained samples' absorbances were compared to the standard curve. All experiments were carried out in duplicate and the average of values was calculated.[18-20]

MAIN POINTS

- High adherence to a healthy low-carbohydrate diet is related to a high protein and fat intake and low consumption of fruit and whole and refined grains.
- Following of low-carbohydrate diet pattern was associated with lower levels of neutrophil, neutrophil:lymphocyte ratio, and higher levels of urinary α, α-diphenyl-β-picrylhydrazyl and serum ferric reducing/antioxidant power and α, α-diphenyl-β-picrylhydrazyl.
- Adherence to the low-carbohydrate diet style is related to lower inflammatory indicators and oxidative stress levels.

α, α-diphenyl-β-picrylhydrazyl Assay: A modified form of the established assay of Brand-Williams et al was used.[21] This method is based on the reduction of DPPH solution radicals to a steady state, which is recognized via color changes. The absorbance of remaining DPPH radicals is recorded at 515-520 nm wavelength.[22] About 50 µL of specimen was combined with 950 µL of DPPH reagent and incubated at room temperature for 15 minutes. The resulting mixture was centrifuged and the absorbance was read at 517 nm, against a blank of ethanolic DPPH solution. The percent (%) of DPPH scavenging activity was calculated as [(absorbance of the control – absorbance of the sample)/absorbance of the control] × 100.

Thiobarbituric Acid Reactive Substances Measurement: We employed the thiobarbituric acid reactive substances (TBARS) assay to evaluate the lipid peroxidation content in form of malondialdehyde (MDA) metabolite. This process is based on the reaction of thiobarbituric acid (TBA) with MDA, which is visible in form of a pink pigment (TBARS) in 532 nm.[23] For this procedure, 100 µL of each sample was combined with 1 mL of TBA/HCl mixture. The mixture was incubated in hot water for 20 minutes and then was immediately chilled. Finally, samples excitation and emission fluorescence peaks were measured at 515 nm and 553 nm, respectively, against a standard calibration curve, prepared using propane, 1,3,3, tetra methoxy.

Table 1. Baseline Clinical Characteristics of Study Participants (n = 171)

	Tertiles of LCD			
	T1 (n = 55)	T2 (n = 57)	T3 (n = 59)	*P*
LCD (score)	7.5 ± 3.1	14.0 ± 1.4	19.7 ± 2.4	
Age (year)	21.1 ± 2.0	21.1 ± 1.4	20.7 ± 1.7	.47
BMI (kg/m²)	21.5 ± 33.3	20.7 ± 2.3	20.9 ± 2.6	.39
WHR	0.73 ± 0.05	0.74 ± 0.04	0.73 ± 0.03	.37
SBP (mmHg)	106 ± 8.6	107 ± 9.4	108 ± 9.6	.57
DBP (mmHg)	71 ± 8.0	71 ± 7.2	71 ± 7.5	.92
HDL-C (mg/dL)	49.6 ± 6.4	51.3 ± 10.3	51.5 ± 9.5	.53
LDL-C (mg/dL)	69.0 ± 14.6	72.9 ± 15.8	71.5 ± 19.3	.79
TG (mg/dL)	73.4 ± 33.6	78.4 ± 51.4	73.6 ± 29.5	.81
TC (mg/dL)	150.5 ± 21.5	148.2 ± 22.2	157.1 ± 30.6	.25
FBG (mg/dL)	84.2 ± 6.5	82.6 ± 5.8	84.3 ± 7.9	.47
Urea (mg/dL)	28.3 ± 5.7	30.6 ± 5.7	30.9 ± 9.4	.19
Creatinine (mg/dL)	1.01 ± 0.46	1.04 ± 0.49	0.99 ± 0.13	.86
ALT (IU/L)	17.9 ± 3.3	22.2 ± 20.3	20.9 ± 11.1	.30
AST (IU/L)	16.2 ± 7.3	16.9 ± 13.7	16.9 ± 15.6	.95
ALP (IU/L)	180.4 ± 36.3	193.2 ± 41.0	191.3 ± 44.8	.30
Direct bilirubin (mg/dL)	0.33 ± 0.17	0.32 ± 0.13	0.25 ± 0.11	.023
Total bilirubin (mg/dL)	0.67 ± 0.33	0.66 ± 0.29	0.54 ± 0.25	.061
Total protein (g/d)	8.0 ± 0.49	7.9 ± 0.40	8.0 ± 0.42	.41
Albumin (g/dL)	5.05 ± 0.33	5.06 ± 0.23	5.05 ± 0.27	.98
Calcium (mg/dL)	10.1 ± 0.48	10.2 ± 0.43	10.1 ± 0.47	.46
Phosphate (mg/dL)	5.1 ± 0.65	5.1 ± 0.76	5.0 ± 0.56	.50
Magnesium (mg/dL)	2.3 ± 0.27	2.3 ± 0.32	2.4 ± 0.21	.76
Uric acid (mg/dL)	3.0 ± 0.54	3.3 ± 0.89	3.1 ± 0.77	.34

Data presented as mean ± SD or median (interquartile range). By using ANOVA or Kruskal–Wallis tests.
ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL-C, high-density cholesterol; LDL-C, low-density cholesterol; SBP, systolic blood pressure; SD, standard deviation; TG, Triglyceride; TC, total cholesterol; WHR, waist-to-hip ratio.

Pro-oxidant Antioxidant Balance Assay: The pro-oxidant antioxidant balance (PAB) method has previously been developed by Alamdari et al[24] For comparison of oxidant level and antioxidant capacity in each sample, standard and working solutions was prepared. The values of the measured samples were determined using the values obtained from a standard curve and were reported as Hamidi Koliakos units.

Statistical Analysis

The data normality assumption was judged using the Kolmogorov–Smirnov analysis. The participants were classified based on the LCD scores into tertiles. For comparison of quantitative variables between tertiles of LCD diet score, ANOVA test and Kruskal–Wallis test were used for normal distribution parameters and non-normal distribution parameters, respectively. For indication of the relationship between the LCD score and different factors, multivariate logistic regression was used and LCD tertiles were considered as dependent variable (reference category: first tertile) after adjustment for age, energy intake, and BMI. All statistical data analyses were undertaken using the IBM Statistical Package for Social Sciences software version 16.0. The differences were statistically significant at $P < .05$.

Results

The LCD diet score was used for classifying the participants into tertiles, with T1 set as the lowest tertile (minimum adherence; range: 15-22; n = 55), T2 (range: 22-25; n = 57), and T3 as the highest tertile (maximum adherence; range: 25-33; n = 59). There was no significant difference between the participants' LCD scores across tertiles for demographic, biochemical, clinical, and anthropometric variables ($P > .05$). However, mean serum levels of direct bilirubin were higher in the first tertile than in the third tertiles ($P = .023$; Table 1).

Table 2. Comparison of Dietary Intakes of Participants Between Tertiles of the Adherence to the Low-Carbohydrate Diet

	LCD Tertiles			
Variables	T1 (n = 55)	T2 (n = 57)	T3 (n = 59)	*P*†
Nutrient (per day)				
Energy (kcal)	2276 ± 794	1959 ± 622	2276 ± 868	.07
Protein (g)	55.9 ± 19.9	60.9 ± 18.7	69.1 ± 26.4	**.008**
Carbohydrate (g)	171.6 ± 44.3	114.3 ± 14.7	62.3 ± 46.6	**<.001**
Fat (g)	10.9 ± 21.1	31.5 ± 14.3	47.4 ± 24.9	**<.001**
Dietary fiber (g)	15.8 ± 6.7	12.7 ± 3.9	10.3 ± 5.0	**<.001**
Sodium (mg)	1596.9 ± 891.3	1489.4 ± 629.4	1306.6 ± 546.3	.110
Food groups (g/day)				
Vegetables	110 ± 124	128 ± 141	104 ± 103	.63
Fruits	230 ± 256	125 ± 119	93 ± 120	**<.001**
Nuts	12.2 ± 23.7	14.6 ± 18.2	28.1 ± 42.2	**.017**
Legumes	18.7 ± 25.1	27.6 ± 25.6	22.3 ± 14.1	**.033**
Red and processed meat	28.7 ± 30.3	39.8 ± 27.1	57.2 ± 53.0	**.001**
Whole grains	47.1 ± 58.3	24.1 ± 32.8	25.3 ± 35.8	**.015**
Refined grains	247 ± 216	170.0 ± 74.0	106 ± 79.4	**<.001**
Poultry	18.7 ± 15.8	27.0 ± 26.1	39.8 ± 37.2	**<.001**
Dairy products	192 ± 124	215 ± 123	201 ± 105	.64
Egg	12.9 ± 12.5	16.3 ± 14.6	19.3 ± 16.4	**.05**
Fish	12.7 ± 24.5	15.3 ± 10.4	18.1 ± 18.7	**.017**

Data presented as Mean ± SD and adjusted for energy intake. T1 represents low compliance and T3 high compliance with an LCD.
†*P*-value obtained from ANOVA test.
ANOVA, analysis of variance; LCD, Low-carbohydrate diet; SD, standard deviation.

Table 3. Inflammatory Profile and Antioxidant Status of Individuals Across Low-Carbohydrate Diet Tertiles Categories

	Tertiles of LCD			
Variables	**T1 (n = 55)**	**T2 (n = 57)**	**T3 (n = 59)**	***P***
Inflammatory parameters				
WBC (10^9 cells/L)	7.0 ± 1.4	6.9 ± 1.9	6.7 ± 1.9	.75
Neutrophil (%)	56.8 ± 11.7	53.5 ± 8.9	50.0 ± 8.7	**.009**α
RDW (%)	13.1 ± 0.9	13.3 ± 1.38	13.2 ± 1.08	.91
MPV (fL)	10.3 ± 1.1	10.4 ± 0.86	10.3 ± 0.85	.87
NLR	1.9 ± 0.90	1.5 ± 0.70	1.3 ± 0.86	**.017**α
PLR	8.2 ± 3.0	7.5 ± 2.9	7.0 ± 3.0	.21
RPR	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	.81
hsCRP (mg/L)	0.2 (0.01-1.3)	0.1 (0.01-1.1)	0.2 (0.01-1.1)	.76
Antioxidant status				
Urinary FRAP (μmol TAC/mg Cr)	9.1 ± 11.2	7.2 ± 6.6	9.0 ± 6.4	.58
Urinary DPPH (mmol trolox equivalent/mg Cr)	1.9 ± 0.66	2.0 ± 2.1	2.8 ± 1.9	**.032**α
Urinary MDA (μmol TBARs/mg Cr)	0.67 ± 0.20	0.62 ± 0.46	0.71 ± 0.55	.67
Serum FRAP (μmol TAC/L)	683 ± 101	681 ± 142	739 ± 113	**.043**α,β
Serum DPPH (mmol trolox equivalent/L)	85.8 ± 57.4	109.0 ± 76.2	134.1 ± 88.1	**.038**α
Serum MDA (μmol TBARs/L)	0.58 ± 0.34	0.78 ± 0.54	0.66 ± 0.30	.35
Serum PAB (HK)	235 ± 50	223 ± 53	230 ± 47	.57

Data presented as Mean ± SD or median (IQR).
Cr, creatinine; DPPH, α, α-diphenyl-β-picrylhydrazyl; FRAP, ferric reducing/antioxidant power; hdCRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; MPV, mean platelet volume; NLR, neutrophil:lymphocyte ratio; PAB, pro-oxidant antioxidant balance; PLR, platelet:lymphocyte ratio; RDW, red blood cell distribution width; RPR, red blood cell distribution width:platelet ratio; TAC, total antioxidant capacity; TBAR, thiobarbituric acid reactive substances.
†Obtained from ANOVA test and post hoc Tukey.
αSignificant value between tertile 1 and 3.
βSignificant value between tertile 2 and 3.

A significant difference was observed across tertiles of LCD scores in terms of protein, carbohydrate, fat, and dietary fiber ($P < .05$). The subjects with the highest adherence to LCD pattern (upper tertile) had greater eating of poultry, fish, red and processed meat, egg, legumes, and nuts compared to those with the lowest adherence (undermost tertile: $P < .05$). However, those in the third tertile significantly consumed lesser fruits, as well as whole and refined grains, versus those in the first tertile ($P < .05$; Table 2).

The hematological inflammatory parameters assessment showed that those who were in the first tertile for the LCD score had significantly higher levels of blood neutrophils and NLR ($P < .05$). However, the mean level of other hematological inflammatory factors did not show any statistically significant difference between different tertiles ($P > .05$; Table 3).

Women with the highest adherence to the LCD dietary pattern showed significantly higher concentrations of urinary DPPH, as well as serum DPPH and FRAP levels, compared to the participants in the lower tertiles ($P < .05$; Table 3).

In unadjusted and multivariable-adjusted regression analysis for calculating odd ratios (ORs), after controlling of age, WHR, and energy intake, third tertile versus first tertile was correlated with lower levels of neutrophil (OR = 0.82; 95% CI: 0.78-0.87; adjusted OR (OR_{adj}) = 0.83; 95% CI: 0.78-0.88), NLR (OR = 0.51; 95% CI: 0.27-0.98; OR_{adj} = 0.54; 95% CI: 0.28-0.99; Table 4), and higher levels of urinary DPPH (OR = 1.11; 95% CI: 1.02-1.19; OR_{adj} = 1.10; 95% CI: 1.02-1.20), as well as serum FRAP (OR = 1.06; 95% CI: 1.02-1.12; OR_{adj} = 1.05; 95% CI: 1.01-1.011) and DPPH (OR = 1.23; 95% CI: 1.12-1.35; OR_{adj} = 1.21; 95% CI: 1.11-1.33; Table 4).

Discussion

In this study, we evaluated the relationship between adherence to the LCD dietary pattern and measures of antioxidant status and

Table 4. Adjusted Odds Ratio and 95% Confidence Interval for Inflammatory, Antioxidant, and Oxidative Parameters Between Tertiles of Low-Carbohydrate Diet Scores

	Crude			Adjusted		
Parameters	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**
Neutrophil	Ref.	0.87(0.72-1.01)	0.82(0.78-0.87)**	Ref.	0.81(0.76-1.02)	0.83(0.78-0.88)**
NLR	Ref.	0.74(0.44-1.3)	0.51(0.27-0.98)*	Ref.	0.68(0.38-1.2)	0.54(0.28-0.99)*
Urinary DPPH	Ref.	1.05(0.75-1.48)	1.11(1.02-1.19)*	Ref.	1.08(0.78-1.53)	1.10(1.02-1.20)*
Serum FRAP	Ref.	1.00(0.99-1.01)	1.06(1.02-1.12)*	Ref.	1.00(0.99-1.01)	1.05(1.01-1.011)*
Serum DPPH	Ref.	1.01(0.98-1.02)	1.23(1.12-1.35)*	Ref.	1.002(0.99-1.04)	1.21(1.11-1.33)*

Tertile 1 was considered as a reference group. Adjusted for age, waist-to-hip ratio (WHR), and energy intake.
DPPH, α, α-diphenyl-β-picrylhydrazyl; FRAP, ferric reducing/antioxidant power; NLR, neutrophil:lymphocyte ratio. *$P < .05$; **$P < .01$.

inflammation in this population of young healthy women. A higher LCD score indicated higher intakes of protein and fat and lower intakes of carbohydrate and fiber. Women with higher adherence to the LCD dietary pattern showed significantly higher concentrations of urinary DPPH, serum DPPH and FRAP levels, and lower blood inflammatory biomarkers (neutrophils and NLR) compared to the participants in lower tertiles.

Dietary intake has an important impact on health status, with multiple essential interactions and variable associations.[1] Accordingly, it may be better to evaluate and consider the role of foods in combination, as dietary patterns, on the pathogenesis of disease since diets comprise a combination of various foods, and adherence to a special dietary regimen could have different impacts on consumption of other types of food.[25]

If we apply an analytical model that contains all food items altogether, it may be somewhat challenging to understand the interactions and relationships between individual food consumption.[26] Due to the increasing understanding of the complications of dietary intake and its relationships with health status, investigation of the impacts of dietary patterns on health status should be based on individual nutrient intake.[27] Dietary patterns examine the complicate network among different foods and nutrients considering all parts, which is contribute with more facts and details about the association between nutrients and disease pathogenesis.[1] Notably, individuals are more adherent to their dietary patterns over time than specific nutrients, and considering the impacts of dietary patterns on health status is better than considering nutrients.[26] As expected, there was a significant difference across tertiles of LCD scores in terms of protein, carbohydrate, fat, and dietary fiber. Subjects with the highest adherence to LCD pattern had a diet containing more poultry, fish, red and processed meat, egg, legumes, and nuts compared to those with the lowest adherence.

The results of our study revealed that there was a significant relationship between adherence to an LDC dietary pattern with neutrophil counts and NLR level. However, no statistically significant difference was observed regarding the serum hsCRP and other hematological inflammatory factors between the tertiles. Neutrophil:lymphocyte ratio is an inflammatory index that is useful as a prognostic marker in different diseases because it is cheap and easy in assessing inflammation.[28] Low-carbohydrate diet induces a reduction of glucose supply in different organs and consequently restricts glucose availability. Therefore, gluconeogenesis that maintains the level of glucose is increased. However, gluconeogenesis may not produce sufficient glucose, and ketone bodies are used as an energy source, which are associated with decrease in insulin secretion and fat and glucose storage. It should be noted that increased glucose content could trigger innate immune cell activity and pro-inflammatory cytokines production.[29] Furthermore, adiponectin, as an anti-inflammatory factor, was reported to be inversely associated with the glycemic load and carbohydrate amount of the diet.[30] Therefore, the LCD diet may decrease inflammation by lowering the level of glucose and ameliorating chronic inflammation. However, in the current study, the overall inflammatory data may not provide sufficient information about the effects of LCD diet on inflammation status of participants because it is unknown that when anti-inflammatory mediators lead to a decreased inflammation process during the dieting time period.

Multiple lines of evidence have indicated that ROS are produced during different pathophysiological conditions, and excess ROS could lead to toxic effects that cause cell damage, hence, enzymatic and non-enzymatic antioxidative mechanisms are adopted by cells to protect them against this toxic effect. It has been shown that the LCD pattern is associated with increasing antioxidant capacity.[31] Our findings showed that there is a significant correlation between adherence to LCD pattern and a higher level of serum FRAP and DPPH, and urinary DPPH, which serves as antioxidant biomarkers. In the present study, women who were in the highest tertile (T3) consumed more nuts, legumes, eggs, and fish. There have been reports that these nutrients have antioxidant activity and enhance the antioxidant defense potential system. Moreover, similar findings reported that a high carbohydrate diet could result in OS.[32] For instance, Gregersen et al[33] (2012) have shown that a high-carbohydrate and high-fat dietary pattern may exacerbate inflammation and decrease the antioxidant capacity in healthy individuals. Dietary weight loss patterns have a blood pressure–controlling impact.[34] In contrast to the study of Phillips et al[35], our findings did not show any relationship between blood pressure values and adherence to an LCD regimen, which may be due to the long-term LCD dieting in the study mentioned above. Moreover, our participant's blood pressure was within normal ranges.

This study has several number of limitations. Assessment of diet based on self-reported information is susceptible to potential recall bias. Furthermore, we did not assess the physical activity in participants. Due to the cross-sectional nature, causality cannot be provided, only association was captured. It is noteworthy that the neutrophil count and NLR may be influenced by several conditions such as dehydration.[36] Our population is a 12-hour fast so dehydration may have affected the results. This matter should be considered for future studies.

Conclusion

Taken together, the results of the current study revealed a significant association between adherence to LCD patterns and increased antioxidant factors such as DPPH and FRAP and decreased inflammatory markers including NLR and neutrophils in young women. So, adherence to LCD dietary patterns could enhance the antioxidant and anti-inflammatory capacity. Future interventional studies need to extend public health knowledge on whether diet modification interventions may be an advantageous dietary regimen for ameliorating inflammatory and oxidative status.

Data Availability: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Committee Approval: Ethical approval was obtained from the Birjand University of Medical Sciences and informed written consent was completed by all participants (Date:11.3.2020, Decision No: IR.BUMS.REC.1398.402).

Informed Consent: Written informed consent was obtained from all individual participants included in the study.

Peer-review: Externally peer-reviewed.

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