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The effect of the Dietary Approaches to Stop Hypertension (DASH) diet on body composition, complete blood count, prothrombin time, inflammation and liver function in haemophilic adolescents

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Abstract

There is no dietary strategy that has yet been specifically advocated for haemophilia. Therefore, we sought to assess the effect of the Dietary Approaches to Stop Hypertension (DASH) diet in adolescents with haemophilia. In this parallel trial, forty male adolescents with haemophilia were dichotomised into the DASH group or control group for 10 weeks. The serum high sensitivity C-reactive protein, IL-6, complete blood count (CBC), serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, partial thromboplastin time (PTT), waist circumference (WC), percentage of body fat, fat-free mass and liver steatosis were measured at the beginning and end of the study. Serum vitamin C was measured as a biomarker of compliance with the DASH diet. The DASH diet was designed to include high amounts of whole grains, fruits, vegetables and low-fat dairy products, as well as low amounts of saturated fats, cholesterol, refined grains, sweets and red meat. Serum vitamin C in the DASH group was significantly increased compared with the control ($P = 0{\cdot}001$). There was a significant reduction in WC ($P = 0{\cdot}005$), fat mass ($P = 0{\cdot}006$), hepatic fibrosis ($P = 0{\cdot}02$) and PTT ($P = 0{\cdot}008$) in the DASH group, compared with the control. However, there were no significant differences regarding other selected outcomes between groups. Patients in the DASH group had significantly greater increase in the levels of erythrocyte, Hb and haematocrit, as compared with the control. Adherence to the DASH diet in children with haemophilia yielded significant beneficial effects on body composition, CBC, inflammation and liver function.

Key words: Haemophilia: Dietary Approach to Stop Hypertension: Body composition: Complete blood count: Inflammation: Liver function: Adolescents

Haemophilia is a hereditary haemorrhagic disorder caused by a deficient or defective clotting factor VIII (type A haemophilia) or clotting factor IX (type B haemophilia)[1]. The inheritance pattern of this disorder is X-linked recessive; therefore, haemophilia is exclusively transmitted through female carriers and affects only males[2]. Recent evidence has shown that more than 1 125 000 men suffer from haemophilia around the world[3]. The World Federation of Hemophilia has announced that Iran is among the top ten countries with the highest prevalence of haemophilia, globally[4].

When a male patient manifests unusual haemorrhagic episodes, with an elevated partial thromboplastin time (PTT) but normal prothrombin time and platelet count, the diagnosis of haemophilia is suspected[2]. The extent of coagulation factor VIII or IX deficiency determines the probability and severity of internal and external bleeding[1]. Depending on age, the predominant sites of haemorrhage are also varied; for instance, haemophilic newborns and toddlers are usually affected by head bleeding, while haemophilic adolescents typically experience intra-articular bleeding, known as haemarthrosis[5]. Therefore,

Abbreviations DASH, Dietary Approaches to Stop Hypertension; FFM, fat-free mass; HCT, haematocrit; hs-CRP, high sensitivity C-reactive protein; ITT, intention-to-treat; NAFLD, non-alcoholic fatty liver disease; PTT, partial thromboplastin time; WC, waist circumference.

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management of the disease becomes increasingly important in these subjects.

Prolonged haemorrhage, as evidenced by an elevated PTT, is a major concern in haemophilic patients[2]. Due to recurrent bleeding and haemolysis, mild anaemia is also a common complication in subjects with haemophilia[6]. Moreover, recent evidence has suggested that haemophilic individuals have chronic low-grade inflammation, originating from increased levels of lipopolysaccharide in their blood circulation[7]. Furthermore, haemarthrosis and subsequent synovitis and arthropathy make haemophilia sufferers less physically active in comparison with others[8]. Therefore, overweight, obesity and non-alcoholic fatty liver disease (NAFLD) are prevalent co-morbidities in this population[9]. Unfortunately, no dietary approach has yet been designed or advocated to specifically manage the aforementioned problems in patients with haemophilia.

The Dietary Approaches to Stop Hypertension (DASH) diet is a well-known eating pattern, with multiple, documented, beneficial effects on weight management, body composition, liver function, inflammatory biomarkers and some other aspects of human health[10–12]. Adherence to this multifunctional diet creates a unique balance between the consumption of plant-based foods, including whole grains, fruits, vegetables, legumes and nuts, and animal-based foods, including poultry, fish and dairy products. In addition, it limits the intake of salt, red meat, fatty foods and sugar-sweetened beverages and foods[13]. To the best of our knowledge, no study has yet been conducted to evaluate the efficacy of the DASH diet in haemophilic children and adolescents. Therefore, we sought to conduct a randomised controlled trial to assess the effect of the DASH diet on haematological parameters, inflammatory biomarkers, anthropometric indices, body composition and liver function in adolescents with haemophilia.

Method

Participants

The present parallel randomised clinical trial was conducted from March to June 2020. A total of forty adolescents with haemophilia were recruited from Omid Hospital, Isfahan, Iran. Volunteers were eligible if they: (1) were male, (2) were aged between 10 and 18 years old, (3) had clotting factor (VII, VIII and IX) deficiency, (4) had not used antioxidant supplements within the preceding 3 months and (5) were not on a specific diet. Also, incidence of a new chronic disease during the study has been considered as a premature withdrawal. The sample size was calculated by $n\ 2((Z_{1-\alpha/2}+Z_{1-\beta})^2\times S^2)/\Delta^2$, where $\alpha=0{\cdot}05$ (type one error) and $\beta=20\,\%$ (type two error). BMI was considered as the main variable. A previous study showed that the standard deviation of BMI in haemophilic patients was 2·08 kg/m^2[14], and the minimal detectable difference of BMI was 1·34 kg/m^2. Accordingly, forty haemophilia adolescents were recruited for the current clinical trial. An introductory session was set up to clarify plans and details regarding the study. Written consent was completed by parents and adolescents, and the study was ethically approved by The Research Council and Ethical Committee of Isfahan University of Medical Sciences, Isfahan, Iran, and Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran (Code: IR.MUI.RESEARCH.REC.1399.098). This randomised clinical trial was registered at IRCT.ir (IRCT20130903014551N6).

Study procedure and dietary intervention

In this current study, participants were randomly dichotomised into the DASH group (n 20) or control group (n 20) for 10 weeks. Each subject received a code and randomisation was run using select random number in SPSS 20. As is was a dietary intervention, patients and their parents could not be blinded. In DASH group, energy requirements of each participant were calculated individually based on the Harris–Benedict equation[15]. The DASH diet was determined based on the previously modified DASH diet for adolescents[16], where the macronutrient composition was as follows: 50–55 % of total energy from carbohydrates, 16–18 % of total energy from protein and 27–30 % of energy from fat. The DASH diet was designed to include high amounts of whole grains, fruits, vegetables and low-fat dairy products, as well as low amounts of saturated fats, cholesterol, refined grains, legumes, nuts, sweets and red meat. A sample of 1-d menu is demonstrated in Table 1. Also, the consumption of red meat and Na was limited. In this way, limiting red meat was applied by reducing the amount of red meat per serving in the diet and replacing red meat with white meat and poultry. It is recommended to consume a maximum of 180 g of meat/d with emphasis on the consumption of fish and white meat. Following recommendations were used to limit Na intake: (1) avoid using of table salt, (2) cooking low-salt food, (3) limit using Na-rich foods such as pickles and processed foods and (4) use of low-salt cheese and breads. A maximum Na intake of 2300 mg/d was allowed. All adolescents and their parents attended the meetings to learn daily food menus. Adolescents in the control group received nutritional recommendations based on healthy eating behaviours, including chewing food completely, using low-volume frequent meals, using healthy snacks, drinking adequate water, avoiding deep frying, limiting added fat and sugar. Serum vitamin C was defined as a biochemical indicator

Table 1. A sample menu of the prescribed diet to the Dietary Approaches to Stop Hypertension group (6276 kJ (1500 kcal), 55 % from carbohydrate, 17 % from protein, 28 % from fats)

Food groups	Serving size/d	Food item (g/d)
Grains	6	Whole bread (60)
Vegetables	5	Whole cereals (150)
Fruits	5	Cooked peas (34)
Dairy	2	Cooked green beans (42)
Meat	2·5	Cabbage (93)
Nuts/seeds	1	Tomato (60)
Fats/oils	6	Green pepper (108)
		Cooked zucchini (78)
		Apple (200)
		Plum (65)
		Pear (170)
		Low-fat milk (230)
		Low-fat yogurt (230)
		Low-fat cheese (15)
		Cooked fish (45)

of compliance with DASH diet[17]. Accordingly, compliance with the DASH diet was assessed by measuring serum vitamin C at baseline and after 10 weeks of intervention. Serum vitamin C was measured using biochemical colorimetric analysis. All patients and their parents participated in meetings programmed at baseline and 2, 4, 6 and 8 weeks. Parents were required to complete a 1-d food record in the 1st, 5th and 10th week of the study. So, three, 1-d food records (two weekdays and one weekend day) were completed by each participant, and a researcher investigated the completeness of food diaries with parents. All collected food records were analysed using the United States Department of Agriculture (USDA) database.

Measurement of biochemical variables

Blood samples were drawn after 12 h of fasting, in the early morning, from the antecubital vein, and all of the related concentrations were prepared on the day of blood sampling. Samples were centrifuged at 3000 × ***g*** for 10 min to separate the serum. High sensitivity C-reactive protein (hs-CRP) was measured using the Inm.turbid method by Audit kit (Delta treatment), and IL-6 was measured using the commercially available ELISA kits (Siemens) by CLIA. Complete blood counts were conducted using an automated procedure (Mindray apparatus). Serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase were measured by the enzymatic system with a Pars Azmon kit (Pars Azmon). PTT tests were performed using the turbidometry method by IL kits (Instrument Lab). In the present study, serum vitamin C was measured using vials previously treated with meta-phosphoric acid based on a biochemical colorimetric analysis.

Measurement of anthropometric variables and body composition

Weight was measured to the nearest 100 g, with participants minimally clothed and unshod, while height was measured using a standard stadiometer according to standard protocols. BMI was calculated as weight in kg divided by the square of height in metres. Waist circumference (WC) was measured at the narrowest level, over light clothing, using a non-stretchable tape measure, without any pressure to the body surface. Weight, height and BMI percentiles were calculated based on Centers for Disease Control and Prevention growth charts for children and adolescents, aged 2–19 years[18]. Body fat and fat-free mass (FFM) were measured using bioelectrical impedance analysis (Bodyvis A_1c), which is highly correlated with dual X-ray absorptiometry (r 0·88)[19]. All measurements were conducted at baseline and at the end of the trial.

Measurement of fibrosis and steatosis

Liver fat was measured using FibroScan and accepted as the reference standard. All biopsy specimens were investigated by a circulatory expert who specialised in liver diseases. The probe transducer tip was located on the skin between the ribs and the level of the right lobe of the liver. The depth was between 25 and 65 mm under the skin exterior[20]. Stages were recognised according to the NASH Clinical Research Network scoring system[21].

Statistical analysis

The analyses were performed on the basis of an intention-to-treat (ITT) approach. Missing values were treated according to linear regression method. To evaluate the distribution of variables, we used the Kolmogorov–Smirnov test, which demonstrated that body fat, serum glutamic pyruvic transaminase and CRP were not normally distributed. Therefore, log transformation was applied. Quantitative variables were analysed between two groups utilising an independent Student's t test, while an ANCOVA was conducted to set confounding variables, especially baseline values. Data were presented as means and standard deviations, unless otherwise stated. All statistical analyses were performed using SPSS (version 20) statistical software.

Results

Among the forty subjects with haemophilia who enrolled in the study, three patients in the DASH diet group (due to medical conditions and personal reasons) and four patients in the control group (due to medical conditions and low adherence to intervention) were excluded from follow-up data collection (Fig. 1). During the study process, two subjects had low adherence and they did not want to continue participation. Therefore, they excluded according to their tendency and researchers had no role in excluding these subjects. Nevertheless, baseline measurements were performed for these patients and we run ITT analysis according to the baseline measurements.

We excluded two subjects from the control group because they stated that they did not start being on the prescribed diet. Therefore, we did not follow them because they had no compliance with prescribed diet. At the end of the study, we included baseline data of all subjects randomly assigned to the DASH or control groups using an ITT method.

The analyses were performed according to ITT approach; therefore, all forty participants were enrolled in the final analyses. There were no side effects following the DASH diet among the participants.

Table 2 indicates baseline characteristics of study participants in each group. There were no significant differences between the groups in terms of age, BMI percentile and WC. However, at baseline, individuals in the DASH diet group had higher weight and height percentiles, compared with those in the control group.

Dietary intake of the study participants as a sum of the three 1-d food records is presented in Table 3. Based on food diaries, the mean intakes of energy, carbohydrate, protein, fat, vitamin A, vitamin E, vitamin C, vitamin K, vitamin B_1, vitamin B_2, Se, Zn, Fe and dietary fibre during the trial were not significantly different between groups. As expected, dietary intakes of Na were significantly lower (1172·35 *v.* 1580·94 mg/d, $P = 0·002$), while Ca intakes were higher (754·26 *v.* 545·28 mg/d, $P = 0·017$), in the DASH *v.* control group. Individuals in the DASH group had higher intakes of K (1987·52 *v.* 1624·36 mg/d, $P = 0·040$) as compared with those in the control group.

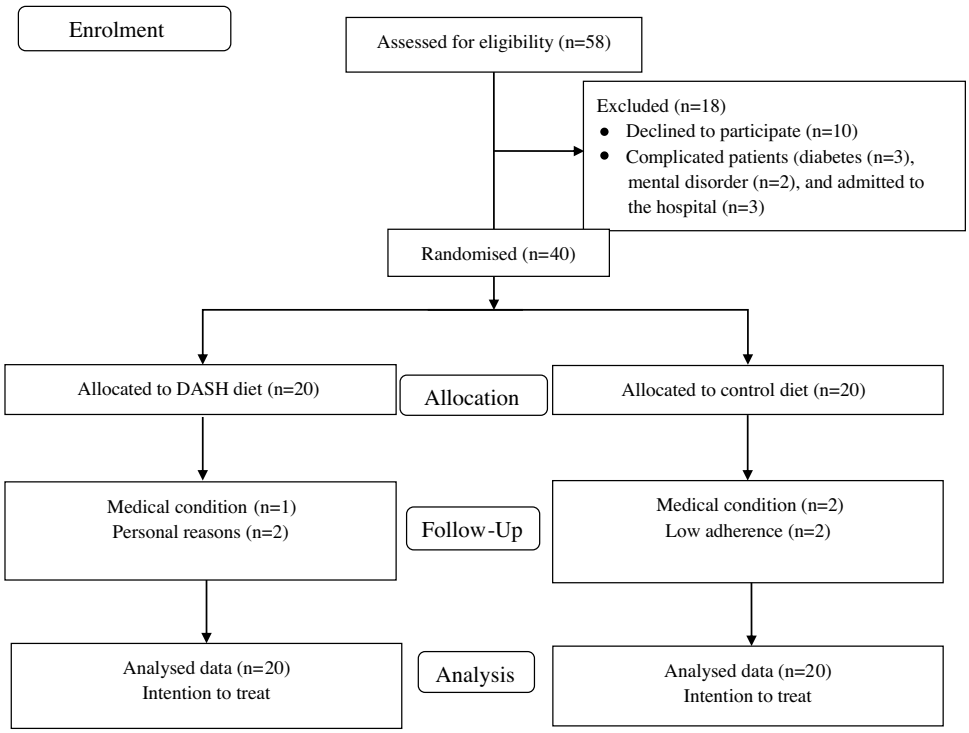

Fig. 1 CONSORT flow diagram of intervention.

Presents serum vitamin C levels at baseline and at the end of trial in each group. At the end of the trial, serum vitamin C levels were significantly increased in the DASH diet group (0·28 mg/dl) and control group (0·10 mg/dl) compared with baseline values. Between group comparison indicated a significant increase in serum vitamin C levels in the DASH group compared with the control group ($P = 0·001$), suggesting a relatively good compliance of the participants to the DASH diet.

Table 4 details the effect of the DASH diet on anthropometric indices, inflammation and liver histology and enzymes. Within group comparison showed a significant reduction in WC (-2·25 cm), FAT (-0·52 kg) and PTT (-8·48), and a significant rise in FFM (1·25 kg), in the DASH diet group. Also, a significant increase was seen regarding FAT (0·88 kg), CRP (2·22 mg/l) and hepatic fibrosis (0·15 *k*Pa) in the control group. Compared with the control group, adherence to the DASH diet led to significant reductions in WC ($P = 0·005$), FAT ($P = 0·006$), hepatic fibrosis ($P = 0·02$) and PTT ($P = 0·008$). There were no significant differences regarding other selected outcomes between groups. To attenuate the difference in baseline values, the effect of baseline measurements was adjusted.

The effects of DASH diet on complete blood count profile are presented in Table 5. Within group analysis showed that leucocyte, erythrocyte, Hb, haematocrit (HCT) and mean corpuscular Hb were significantly increased after using DASH diet. Patients in the DASH group had significantly greater increase in the levels of erythrocyte ($P = 0·02$), Hb ($P = 0·005$) and HCT ($P = 0·006$), compared with the control group. To attenuate the difference in baseline values, the effect of baseline measurements was adjusted.

Discussion

In the current study, adherence to the DASH diet, for 10 weeks, among haemophilic adolescents resulted in a significant reduction in WC, fat mass, fibrosis and PTT, in comparison with the control group. In addition, erythrocyte, Hb and HCT significantly increased, while steatosis and CRP levels were marginally reduced, in response to following the DASH diet, compared with the control group. However, changes in weight, BMI, FFM, IL-6, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, mean corpuscular volume, mean corpuscular Hb and mean corpuscular Hb concentration were not significantly different between the two groups. To the best of our knowledge, this is the first study investigating the effects of the DASH diet on body composition, complete blood count, inflammatory markers and liver function in haemophilic adolescents. It has previously been demonstrated that overweight and obesity are major issues when concomitant to haemophilia and are associated with several metabolic abnormalities[9]. With regard to the difficulties of these patients to be physically active[22,23], following a healthy diet and decreasing energy intake are suggested as key factors in the prevention or reduction of overweight and obesity among these patients[9]. However, currently, no dietary recommendation has been specifically advocated for these patients. Therefore, our findings, which indicated the beneficial effects of the DASH diet on several health aspects of haemophilia, might be useful in clinical settings to prevent and treat health complications of these patients.

The results of our study showed that serum vitamin C level significantly increased following the DASH diet, compared with the control diet. In addition, intake of soluble fibre and Ca was

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Table 2. Baseline characteristic of study subjects*
(Mean values and standard deviations)

Variable	DASH diet (*n* 20) Mean	SD	Control diet (*n* 20) Mean	SD	*P*†
Age (year)	13·85	2·64	14·8	2·74	0·30
Weight (percentile)	76·02	25·66	73·98	16·04	0·03
Height (percentile)	50·75	34·48	39·46	23·69	0·006
Percentile BMI	85·45	14·17	80·76	15·29	0·88
WC (cm)	83·25	15·1	79·2	9·11	0·07
Serum Vitamin C (mg/dl)	0·42	0·21	0·36	0·21	<0·001

DASH, Dietary Approaches to Stop Hypertension; WC; waist circumference.
*Variables are expressed as mean values and standard deviations.
† *P*-values resulted from independent *t* tests for quantitative and χ^2 for qualitative variables between the two groups.

Table 3. Dietary intake of the study participants as a sum of the three 1-d food records*
(Mean values and standard deviations)

Variable	DASH diet (*n* 20) Mean	SD	Control diet (*n* 20) Mean	SD	*P*†
Energy (kcal/d)	1144·58	154·65	1293·71	310·73	0·067
Carbohydrate (g/d)	167·75	32·89	173·84	32·89	0·989
Protein (g/d)	49·74	18·59	50·71	18·59	0·882
Fat (g/d)	41·08	10·41	41·11	10·41	0·885
Na (mg/d)	1172·35	398·36	1580·94	398·36	0·002
Vitamin A (re/d)	613·74	383·34	501·35	383·34	0·386
Vitamin E (mg/d)	14·69	6·74	12·46	6·74	0·287
Vitamin C (mg/d)	72·55	38·66	54·43	38·66	0·140
Vitamin K (ug/d)	35·08	17·96	26·89	17·96	0·219
Vitamin B_1 (mg/d)	0·9	0·22	0·83	0·22	0·355
Vitamin B_2 (mg/d)	1·38	0·35	1·23	0·35	0·261
Vitamin D (ug/d)	1·91	1·38	0·75	1·38	0·021
K (mg/d)	1987·52	574·08	1642·36	574·08	0·040
Ca (mg/d)	754·26	246·96	545·28	246·96	0·017
Se (mg/d)	0·08	0·08	0·07	0·08	0·683
Zn (mg/d)	6·11	1·78	5·52	1·78	0·261
Fe (mg/d)	8·48	3·08	8·66	3·08	0·717
Dietary fibre (g/d)	14·43	4·42	12·10	4·42	0·139
Soluble fibre (g/d)	0·52	0·17	0·38	0·17	0·022

DASH, Dietary Approaches to Stop Hypertension.
*Variables are expressed as mean values and standard deviations.
† Obtained from ANCOVA adjusted for energy intake.

significantly higher, and Na intake was notably lower, in the DASH group *v.* the control group. Currently, serum vitamin C level and dietary records represent the best tools to assess the compliance of study participants to the DASH diet[17]. Concordant with our results, in a previous study conducted on children with the metabolic syndrome, serum vitamin C significantly increased following the DASH diet, compared with the control group[17]. Indeed, it has been suggested that the consumption of vitamin C may be associated with lower blood pressure, by enhancing nitric oxide synthase activity[24,25]. Therefore, one of the possible explanations for the anti-hypertension effects of the DASH diet might be due to the high fruits and vegetable content of this diet. Indeed, the focus of the DASH diet is on vegetables, fruits and low-fat dairy foods[26]; thus, a significant increase in the soluble fibre and Ca intake is predictable among intervention participants, as compared with controls.

In the present study, WC and fat mass were significantly decreased in response to adherence to the DASH diet in comparison with the control diet. However, the differences between weight, BMI and FFM did not significantly differ between groups. It has previously suggested that overweight and obesity, which are highly prevalent among haemophilia patients, are associated with annual joint bleeding rate and influence the joint health of these patients[27–29]. It has also been widely demonstrated that overweight and obesity contribute to the reduction in motion of joints, acceleration of loss of joint mobility and increasing chronic pain[29]. Given that obesity in haemophilia patients is associated with an increasing prevalence of anxiety and depression and several other negative health consequences[29], prevention and treatment of obesity are necessary in these patients. However, generally, weight reduction during childhood is not recommended and the aim of the diet therapy should be the maintenance of weight among children and adolescents. Therefore, our results, which indicate that adherence to the DASH diet, for 10 weeks, can maintain BMI and FFM, in addition to facilitating reductions in WC and fat mass, in adolescents with haemophilia, suggest that the DASH diet is a practical and safe dietary approach to help prevent obesity and its related complications among children and adolescents with haemophilia. Indeed, the results of a systematic review and meta-analysis showed that, in comparison with the control diet, adherence to the DASH diet elicited a significant reduction in weight, BMI and WC among adults[30]. Moreover, the results of a cohort study showed that, after 3-year follow-up, the DASH diet had an inverse association with central and general obesity among adolescents[31]. In another previous study, it was reported that, after 6 weeks adherence to the DASH diet, weight, BMI and WC did not significantly change compared with usual dietary habits among children with the metabolic syndrome[17]. In Saneei *et al.*, WC was significantly reduced in the DASH diet group compared with baseline; however, although the mean change of WC was higher in the DASH diet compared with control diet, it was not statistically significant[17]. Thus, discordance in findings of some previous work and our study, in terms of WC, might be attributable to the difference in compliance and longer treatment time of our study (10 weeks *v.* 6 weeks). Overall, the favourable effects of the DASH diet on WC and body composition in our sample might be due to the fact that the DASH diet is rich in fruits and vegetables, legumes and low-fat dairy products, as well as healthy vegetable oils, which provide a low-energy dietary pattern and simultaneously provide suitable amounts of nutrients for the growth and development of children and adolescents.

In our study, fibrosis and steatosis were markedly decreased in the DASH diet group compared with the controls. Although NAFLD is often associated with adulthood, recent studies have shown that the risk of developing NAFLD in adolescents has doubled in the past 20 years[32]. Recent studies have presented NAFLD as one of the two most important complications of obesity in children and adolescents[33]. Also, it should be noted that children with haemophilia have limited physical activity due to the risk of bleeding, which increases the risk of NAFLD. In a previous randomised controlled trial study, in comparison with controls, following the DASH diet for 8 weeks among adult NAFLD patients resulted in improvements in several metabolic

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Table 4. The effects of Dietary Approach to Stop Hypertension (DASH) diet on anthropometric indices, inflammation and liver histology and enzymes*
(Mean values and standard deviations)

	DASH diet (*n* 20)						Control diet (*n* 20)							
	Baseline		End of trial		Change		Baseline		End of trial		Change			
	Mean	SD	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	*P*†	*P*‡	*P*§
Weight (percentile)	76·02	25·66	79·46	20·23	3·44	7·78	0·06	73·98	16·04	75·45	15·64	1·47	10·28	0·52	0·527	0·32
BMI (percentile)	85·45	14·17	85·56	11·50	0·11	4·44	0·91	80·76	15·29	81·94	16·08	1·18	16·61	0·75	0·404	0·72
WC (cm)	83·25	15·10	81	13·06	-2·25	3·32	0·007	79·2	9·11	79·61	8·91	0·41	1·89	0·33	0·659	0·005
FFM (kg)	49·68	12·69	50·94	13·04	1·25	1·01	<0·001	48·67	9·13	49·04	9	0·73	2·69	0·26	0·708	0·43
Fat (kg)	14·45	7·08	13·92	6·55	-0·52	0·99	0·03	12·12	4·16	12·73	4·41	0·88	1·33	0·02		0·006
IL-6 (pg/ml)	3·06	1·48	2·59	1·48	-0·46	1·28	0·09	2·15	0·56	2·3	0·56	0·15	0·68	0·32	0·512	0·30
CRP (mg/l)	2·72	2·54	2·26	2·38	-0·45	1·73	0·24	1·33	0·89	3·55	6·71	2·22	0·19	0·04	0·372	0·08
Fibrosis (*k*Pa)	4·25	1·04	4·13	0·78	-0·11	0·69	0·45	4·54	0·95	4·69	0·92	0·15	0·24	0·01	0·520	0·02
Steatosis	204·20	55·25	187·11	47·98	-17·08	46·22	0·11	200·10	53·83	203·36	56·64	3·25	9·47	0·15	0·079	0·06
SGOT (U/l)	19·60	4·44	19·67	3·28	0·07	3·47	0·92	24·3	11·36	24·59	11·2	0·29	3·63	0·72	0·061	0·47
SGPT (U/l)	16·58	8·85	17·23	6·93	0·38	4·33	0·38	21·2	8·19	22·06	8·92	0·86	4·91	0·48	0·056	0·43
PTT (1000/μl)	80·59	25·1	72·09	19·06	-8·49	14·31	0·01	72·86	25·07	76·92	22·43	4·05	12·91	0·17	0·547	0·008

WC, waist circumference; FFM, fat-free mass; CRP, C-reactive protein; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; PTT, partial thromboplastin time.
*Variables are expressed as mean values and standard deviations.
† It shows the comparison baseline and final values in each group obtained from paired *t* test.
‡ It shows the comparison final values between DASH and control groups obtained from independent *t* test comparing endpoint measurements.
§ It shows the comparison final values between two groups after adjusting for baseline measurements obtained from ANCOVA, adjusted for baseline value.

factors, such as weight, BMI, TAG, markers of insulin metabolism, inflammatory and stress oxidative markers of these patients[11]. Similarly, observational studies have reported that the consumption of the DASH diet has an inverse correlation with risk of NAFLD[34,35]. As mentioned above, haemophilia patients have several hindrances to being physically active and they are at high risk for obesity and related metabolic diseases. One the most important undesirable outcomes of being physically inactive and gaining weight, particularly enhancing visceral fat and central obesity, in both adolescents and adults is insulin resistance, which has a bilateral association with fatty liver[36,37]. The beneficial effects of the DASH diet on the metabolic status of patients with haemophilia might be explained by the low amounts of simple sugar and higher fibre, Mg and calcium of this dietary pattern, which may reduce markers of insulin metabolism, TAG and VLDL-cholesterol levels[38]. It has recently been shown that the consumption of sugar-sweetened soda has a direct correlation with increased risk of NAFLD, since they possess high amounts of fructose and energy content which both have a substantial role in the aetiology of fatty liver[39,40]. Animal studies have shown that high Ca and Mg diets, as exists in the DASH eating pattern, have a salient role in stimulating microsomal TAG transfer protein in the liver[41], suppression of endothelial injury, reduction in the peroxidation of lipids and enhancing the antioxidant capacity in both serum and tissues(42).

The findings of the present study demonstrated that the consumption of the DASH diet yielded, albeit marginal, significantly reduced serum CRP levels compared with the control group. In accordance with our results, it has been shown that following the DASH diet significantly reduced hs-CRP among adolescents with the metabolic syndrome[17], adult NAFLD patients[11] and polycystic ovary syndrome[43] patients compared with control groups.

However, adhering to the DASH diet for 4 weeks did not lead to reductions in hs-CRP in women with gestational diabetes, while it significantly increased plasma total antioxidant capacity and total glutathione[44]. Several potential mechanisms have been posited regarding the anti-inflammatory effects of DASH diet, including high amounts of antioxidants, such as vitamin C, and high amounts of Ca, Mg and fibre present within this diet[45–47]. It has previously been shown that Mg inhibits nuclear factor kappa-light-chain-enhancer of activated B cells and has a role in the down-regulation of the inflammatory response[48,49].

One the most valuable findings of our study is that PTT significantly decreased after the consumption of the DASH diet compared with the control diet. A prolongation in the PTT is occurrent in patients with haemophilia, even in some cases with severe haemophilia the PTT is 2–3 times longer than the normal range[50]. However, to our knowledge, there is no study that has assessed the relationship between healthy dietary patterns and PTT; thus, more preclinical and clinical trials are needed to clarify our results and its underlying mechanisms. The DASH diet is rich in vegetables that are full of vitamin K. As a result, following this diet increases vitamin K intake[51]. In addition, DASH diet is a rich source of dietary fibre which can lead to more vitamin K production by altering gut microbiota[52]. These suggested mechanisms should be investigated by additional studies. Similarly, our

Table 5. The effects of Dietary Approach to Stop Hypertension (DASH) diet on complete blood count*

	DASH diet (*n* 20)							Control diet (*n* 20)								
	Baseline		End of trial		Change			Baseline		End of trial		Change				
	Mean	SD	Mean	SD	Mean	SD	*P*†	Mean	SD	Mean	SD	Mean	SD	*P*†	*P*‡	*P*§
Leucocyte (1000/μl)	6·32	1·49	6·84	1·40	0·51	0·86	0·01	6·06	1·04	6·34	1·34	0·27	0·77	0·12	0·342	0·30
Erythrocyte (mil/μl)	5·25	0·5	5·58	0·44	0·32	0·36	0·001	5·2	0·52	5·29	0·5	0·08	0·44	0·40	0·010	0·02
Hb (g/dl)	14·49	1·53	14·94	1·01	0·45	0·80	0·02	14·21	1·80	14·16	1·57	-0·04	0·77	0·8	0·054	0·005
HCT (%)	42·24	4·10	43·56	3·27	1·32	2·36	0·02	42·16	4·34	41·93	3·89	-0·22	1·47	0·49	0·130	0·006
MCV (fl)	80·97	3·68	81·43	4·39	0·46	2·12	0·34	78·3	7·13	78·26	7·30	-0·03	4·39	0·97	0·131	0·46
MCH (pg)	28·08	1·57	28·98	2·57	0·9	2·26	0·09	27·23	3·45	27·96	3·63	0·73	1·98	0·11	0·387	0·69
MCHC (g/dl)	34·5	0·94	34·29	1·27	-0·21	1·28	0·46	33·75	1·46	33·85	1·65	0·1	1·06	0·66	0·324	0·71

HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular Hb; MCHC, mean corpuscular Hb concentration.
*Variables are expressed as mean values and standard deviations.
† It shows the comparison baseline and final values in each group obtained from paired *t* test.
‡ It shows the comparison final values between DASH and control groups obtained from independent *t*-test comparing endpoint measurements.
§ It shows the comparison final values between two groups after adjusting for baseline measurements obtained from ANCOVA, adjusted for baseline value.

results indicated that adherence to the DASH diet significantly increased erythrocyte, Hb and HCT compared with the control group, which might due to the high antioxidant levels of the DASH diet, which are necessary in haematopoiesis[53].

The average age of the subjects in our study was 13–14 years. According to the WHO height-for-age percentile[54], the rate of height growth is about 6–7 cm/year (0·11–0·12 cm/week) in this age. The duration of the present study was 10 weeks. Therefore, the subjects had a maximum height growth of 1·1–1·3 cm. It should be kept in mind that the impaired growth is prevalent among children with chronic disease such as haemophilia[55]. Therefore, it is unlikely that our findings on body composition were affected by the height growth of the subjects.

Strengths and limitations

The strengths of our study included that we utilised serum levels of vitamin C as a valid biomarker of diet compliance, and we applied the ITT approach for the analysis data, which permitted all of the study participants who participated in our study into data analysis. Evidence determined that the concentration of Na and K in a 24-h urine sample can be considered as a biomarker of compliance with the DASH diet. Although, collecting 24-h urine samples is difficult, especially for children and adolescents. Additionally, day to day variation of 24-h urinary Na is high, and multiple samplings are required to attenuate day to day variation[41]. Since it was not suitable for adolescents, we utilised vitamin C because it does not have these limitations. Moreover, to the authors' knowledge, this is the first study investigating the effects of the DASH diet, as one of the most well-known healthy dietary patterns, on liver fibrosis and steatosis using a FibroScan, inflammatory factors, anthropometry and body composition, as well as complete blood count among haemophilic adolescents. Nevertheless, as a main limitation of our study, blinding was not applicable due to the nature of our intervention. Dietary intakes were not assessed before the intervention, and all the nutritional assessments were performed during the study. Also, dietary intake has been investigated during the study using three 1-d food records. Due to the difficulty of obtaining food records from children with specific disease conditions, we could not convince participants to increase the number of food records. However, due to the fact that the compliance with the intervention has been evaluated by biomarkers (serum vitamin C), these limitations had no serious unfavourable effect on the validity of the study.

Conclusion

Our findings showed that the DASH diet, which was primarily designed to control cardiovascular risk factors in healthy or unhealthy adults, has several beneficial effects on various health aspects of adolescents with haemophilia. Indeed, adherence to the DASH diet significantly reduced WC and fat mass, in addition to eliciting beneficial effects on CRP, liver fibrosis and steatosis, as well as RBS, Hb and HCT, in haemophilic adolescents. However, more, well-designed and well-conducted, studies are needed to confirm the results of the present study.

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There are no conflicts of interest.

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