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ORIGINAL RESEARCH

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The effects of Mediterranean diet on hepatic steatosis, oxidative stress, and inflammation in adolescents with non-alcoholic fatty liver disease: A randomized controlled trial

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Summary

Background: Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease in children and adolescents. The optimal dietary strategy to improve hepatic stetatosis and reduce oxidative stress and inflammation in adolescents is unknown.

Objective: This study was conducted to evaluate the effect of Mediterranean diet (MD) versus low-fat diet (LFD) on hepatic steatosis, inflammation, and oxidative stress in adolescents with obesity and NAFLD.

Methods: Adolescents diagnosed with NAFLD between the ages of 11–18 years were randomized to either a MD or conventional LFD (control diet) for 12 weeks. Dietary status, anthropometry, body composition, and biochemical parameters were evaluated. Hepatic steatosis was determined by ultrasonography.

Results: A total of 44 participants completed the study. At the end of the study, severity of hepatic steatosis, serum transaminase levels, and insulin resistance decreased significantly in both groups with no significant differences between groups except for aspartate aminotransferase (AST). The amount of decrease in AST levels in the MD group was greater than the LFD group ($p < 0.05$). In the MD group, serum total antioxidant capacity, paraoxanase-1, and glutathione peroxidase levels increased ($p < 0.05$); it did not change in the LFD group compared to baseline ($p > 0.05$). C-Reactive Protein (CRP) levels decreased only in the MD group ($p = 0.008$), interleukine-6 decreased only in the LFD group ($p = 0.031$).

Conclusion: Consumption of MD and LFD for 12 weeks in adolescents with obesity and NAFLD reduced BMI, fat mass, hepatic steatosis, and insulin resistance, improved high transaminase levels, and had positive effects on inflammation and oxidative stress.

Registered under ClinicalTrials.gov Identifier no. NCT04845373.

KEYWORDS
adolescent, inflammation, insulin resistance, Mediterranean diet, non-alcoholic fatty liver disease, oxidative stress

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1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in children and adolescents. NAFLD is closely associated with obesity, insulin resistance, and dyslipidaemia in children.[1] Due to the rapid increase in obesity worldwide in recent times, NAFLD has become the most common liver disease in children and adults.[1] Multiple complex interacting pathways including lipid deposition, lipotoxicity, inflammation, and oxidative stress lead to the development and progression of NAFLD.[2,3] If untreated, this condition can progress to cirrhosis, hepatocellular carcinoma, and end-stage liver disease.[4]

Treatment of NAFLD in adolescents is very important to reduce the risk of transition to advanced stages in adulthood.[5] In paediatric cases, the primary mode of treatment for NAFLD is based on nutritional therapy and increasing physical activity. Due to challenges with long-term adherence, it has been reported that weight loss as an effective and permanent treatment for NAFLD in children and adolescents is difficult to achieve.[6] In particular, recommending energy restriction for body weight loss may not be optimal in improving paediatric NAFLD.[5] Therefore, alternative strategies are needed to reduce hepatic fat and oxidative stress and inflammation, which play a role in pathogenesis in children and adolescents with NAFLD. In this context, there are a limited number of studies examining optimal dietary strategies in children and adolescents with NAFLD.[5,7]

The Mediterranean diet (MD) is characterized by a high intake of plant-based foods, including whole grains, fruits and vegetables, legumes, and olive oil, along with a moderate intake of fish, and poultry, and limited intake of meat and meat products.[8] Recently, it has been shown that the MD, which has been found to reduce the risk of diseases such as diabetes, cardiovascular diseases, and metabolic syndrome, can have positive effects in the prevention and treatment of NAFLD.[9,10] In a study conducted in children and adolescents with obesity, it was determined that the risk of diabetes and NAFLD increased in those with low adherence to the MD. In addition, it has been reported that the MD may be a safe and inexpensive therapeutic alternative in children with NAFLD.[11] In the literature, there are no studies examining the effectiveness of the MD and low-fat diet (LFD) on oxidative stress and inflammation in children and adolescents. The primary aim of this study was to compare the effects of MD versus a standard LFD on hepatic steatosis, liver enzymes, and inflammatory and oxidative stress markers in adolescents with NAFLD.

2 | MATERIALS AND METHODS

2.1 | Participants

Adolescents with obesity and NAFLD aged 11–18 years were recruited from İzmir Tepecik Training and Research Hospital Paediatric Gastroenterology Outpatient Clinic between January and November 2020. Inclusion criteria were age between 11 and 18 years old, body mass index (BMI) ≥95th percentile, and being diagnosed with grade ≥1 NAFLD. Exclusion criteria were having other hepatic diseases (such as hepatitis), alcohol consumption, history of type I or type II diabetes, using drugs that may affect the liver fat or lipid profile, dietary treatment for weight loss, presence of chronic inflammatory disease, cancer, thyroid dysfunction (hyperthyroidism and hypothyroidism), pregnancy or lactation, antibiotic treatment within 3 months prior to the study, and using nutritional supplements. Patients positive for chronic hepatitis other than NAFLD according to past medical data were excluded from the study.

This study was carried out in accordance with the guidelines laid down in the Declaration of Helsinki and written informed consent was obtained from all participants and their parents. The protocol study was approved by the Ethics Committee of Clinical Research at Gazi University (No: 16, 13/01/2020) and was registered at https://www.clinicaltrials.gov (NCT04845373).

2.2 | Study design

The study was designed as a single-blind, randomized, two-arm, parallel dietary intervention. Adolescents who met the study criteria and volunteered to participate were assigned to the MD or LFD group by the research assistant with an allocation ratio of 1:1, using a blocked randomization scheme stratified by sex. The random allocation sequence was provided by a statistician. Study personnel (radiologist, gastroenterologist, nurse, and laboratory technicians) involved in the analysis of outcomes were blinded to group assignment.

All adolescents were invited to a face-to-face interview at the start of study, and in the 2nd, 4th, 6th, 8th, 10th, and 12th weeks. Initially, the general characteristics, medical history, and nutritional habits of the adolescents were recorded using a questionnaire. Anthropometric measurements, body composition analysis, and a physical activity questionnaire were performed, and three-day food consumption records, and blood samples for measurement of biochemical parameters were taken. During the checkups performed every 2 weeks, physical activity and food consumption records of the adolescents were repeated and anthropometric measurements and body composition were analysed.

The primary outcomes were changes in liver enzymes, hepatic steatosis grades, and inflammatory and oxidative stress markers. Secondary outcomes included changes in anthropometric measurements, body composition, lipid profile, and insulin resistance.

2.3 | Dietary interventions

Both diets were designed to be non-calorie-restricted. The calories of the diets were prescribed by the dietitian based on the 85th percentile energy requirement values of adolescents with low physical activity levels of the same age and sex, according to the Turkey Dietary Guidelines-2015.[12] The two interventions were as follows:

MD: Target macronutrient energy contributions were 40% from carbohydrates, 35%–40% from fat (with <10% from saturated fat), and 20% from protein.[13] Participants in this group were instructed to

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consume fish and, legumes at least 2–3 times a week, and walnuts (20 g/day), and olive oil (30–45 g/day) every day, in accordance with the MD model.[13]

LFD: Target macronutrient energy contributions for the LFD were 50%–60% from carbohydrates, <30% from fat (with <10% from saturated fat), and 20% from protein. Participants in this group were especially recommended to consume low-fat foods.[14,15] Dietary principles to be followed in NAFLD such as restricting saturated fat intake, avoiding processed/packaged products, alcohol, instant beverages, carbonated/sugary drinks, and sweets containing high amounts of sugar and fat, were explained to adolescents in both diet groups by the same research dietitian.

2.4 | Compliance

In order to ensure that both study groups were able to apply the recommended diets correctly and appropriately, the dietitian had a phone call with the participants and their parents twice a week and continuous communication was established via social media. Daily meal plans were created in accordance with the energy intake of adolescents and sent to participants over social media for 12 weeks. Adolescents were invited to the research centre to be checked every 2 weeks, and 3-day dietary records were kept by all of the adolescents with the help of their parents. Dietary records were analysed using the BeBiS programme (Stuttgart, Germany; Nutrition Information System, Version 8). Adolescents and their parents were informed about the mistakes and/or deficiencies in their diet (skipping main and snack meals, not being able to adjust portion sizes) at each interview. Furthermore, compliance with the MD was evaluated at baseline and during follow-up visits using the Mediterranean Diet Quality Index (KIDMED).[16,17] The KIDMED was developed and validated by Serra-Majem et al.[17] to evaluate adherence to the MD for children and adolescents. The scores obtained from the index range from 0 to 12 points and are based on 16 statements. A total score of 0–3 is considered to be low adherence, a score of 4–7 indicates an improvement in the diet model (moderate compliance), and a score of ≥8 is considered the optimal MD (high compliance). The dietary compliance of the LFD group was evaluated using dietary records.

2.5 | Assessment of physical activity

Physical activity was assessed using a 24-h physical activity record form. Using the physical activity record form, the time spent on daily sleep, lying down activities, sitting activities, light activities, moderate activities, and heavy activities were recorded. The total energy cost was calculated by multiplying the physical activity rate (PAR) values determined for the physical activity types by the activity duration specified by the adolescents. Physical activity level (PAL) was found by dividing the total energy cost by 24 h.[18] Participants were advised to maintain their usual level of activity for the duration of the intervention.

2.6 | Anthropometric measurements and body composition assessment

Body weight and body composition analysis were performed using a bioelectrical impedance analysis device (TANITA, model SC 330). Height was measured with a stadiometer. BMI was calculated using the following formula: 'BMI = [body weight (kg)/(height [m])^2]'. Waist circumference (cm) was measured from the midpoint between the lowest rib and the iliac crest. Hip circumference (cm) was measured horizontally at the largest circumference of the hip. The waist to hip ratio was calculated by dividing the waist circumference by the hip circumference.

2.7 | Assessment of ultrasonographic measures

Ultrasonographic evaluation of the liver was performed at baseline and after the 12-week intervention by an expert radiologist using a B-mode ultrasonography (USG) method. NAFLD was graded as 0–3 according to the following criteria: echogenicity of liver parenchyma, echotexture, hepato-renal contrast, and visibility of the diaphragm and hepatic vessels.[2] If the texture was homogenous, exhibited fine-level echoes, was isoechoic compared to the renal cortex, and there was adequate visualization of the hepatic vessels and diaphragm, the liver was assessed as normal (Grade 0). Grade 1 was indicated by the presence of a minimal diffuse increase in hepatic echogenicity, normal diaphragmatic and intrahepatic vessel borders, mild steatosis; Grade 2 showed the presence of a moderately diffuse increase in hepatic echogenicity, mild deterioration in the appearance of the diaphragm and intra-hepatic vessels, and moderate steatosis. If there was a significant increase in hepatic echogenicity, the right lobe posterior segment of the liver was difficult to see, and the borders of the intra-hepatic vascular structures and diaphragm were unclear or not visible, steatosis was considered to be severe (Grade 3).[19,20]

2.8 | Biochemical analysis

Venous blood samples were collected at baseline and at the end of the study (12th week). Samples were centrifuged and stored at −80°C until further analysis. Serum fasting insulin, glucose, glycated haemoglobin (HbA1C), total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (γGT) were assayed in the Research Centre Hospital according to conventional laboratory standard methods. Normal ALT levels were defined as <40 U/L.[21] TNF-α, interleukin (IL)-1β, IL-6, IL-8, IL-10 levels were measured by enzyme-linked immunosorbent assay (ELISA) using standard kit (Elabscience). Total antioxidant status (TAS), Total oxidant status (TOS), Paraoxanase-1 (PON-1), catalase, and superoxide dismutase (SOD) analysis were performed by colorimetric method using the appropriate kit (Relassay,

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Turkey). TAS analysis was based on the bleaching of the characteristic colour of a more stable ABTS (2,2′-Azino-bis [3 ethylbenzothiazoline-6-sulfonic acid]) radical cation by antioxidants. The change in absorbance at 660 nm was associated with the total antioxidant level of the sample. In TOS analysis, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a coloured complex with xylenol orange in acidic medium. The colour intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated using hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per litre (μmol H2O2 equivalent/L). Paraoxon was used as a substrate for PON-1 measurement. The rate of paraoxon (diethylpnitrophenylphosphate) hydrolysis was measured by monitoring the increase in absorption at 412 nm at 37°C. The amount of p-nitrophenol generated was calculated from the molar absorption coefficient at pH 8.5, which was 18.290 M^{-1} cm^{-1}. Paraoxanase activity was expressed as U/L serum.For catalase, the colorimetric assay involved two steps. Samples were first incubated with a known amount of hydrogen peroxide, which was converted to water and oxygen. The ratio was proportional to the concentration of catalase. The enzyme was denatured and level of remaining hydrogen peroxide, following a fixed incubation period, was determined using a chromogen. The resulting absorbance was measured at 405 nm and the obtained results are expressed in U/L. In SOD analysis, xanthine and xanthine oxidase were used to generate superoxide radicals that react with 2-(4-) iodophenyl-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction. Glutathione levels were measured using the 'BT-lab' kit. Malondialdehyde (MDA) levels were measured using the thiobarbituric acid reactive substance (TBARs) method, and glutathione peroxidase (GSH-Px) levels were quantified using the method by Paglia and Valentine et al.[22] Homeostatic Model Assessment Insulin Resistance (HOMA-IR) index was calculated with the following formula = [(Fasting blood glucose (mg/dl) × Fasting insulin (μU/ml)/405). Calculated as the oxidative stress index (OSI) = TOS/TAS.

2.9 | Power calculation and statistical analysis

The sample size was determined based on a study conducted by Ramon-Krauel et al.[23] and a mean change in ALT levels of 10.5 U/L, with a 95% confidence interval ($\alpha = 0.05$) and 90% statistical power. On the basis of this, the sample size was estimated to be 17 per study group. After starting the study, more than 17 participants (41 participants) were included in each group, as it was thought that the number of participants who would not be able to adapt to the diet and could not come to the checkups regularly could be higher than expected due to the emergence of the COVID-19 pandemic.

The data of the study were analysed using the SPSS 22 (Statistical Package for the Social Sciences) program. Mean, standard deviation, median, and IQR values were used as descriptive statistical methods in evaluating the data. Categorical variables are given as frequency (*n*) and percentage (%). The compliance of the data to normal distribution was analysed with Shapiro–Wilk test. Mean and standard deviation were calculated for normally distributed data, and median and IQR values were calculated for non-normally distributed data. Chi-Square test for categorical variables, Student's *t* test for normally distributed variables, and Mann–Whitney *U* test for non-normally distributed variables were used for comparisons between groups. In repeated measurements, statistical differences within groups were analysed with the paired t-test or non-parametric wilcoxon paired two sample test or McNemar. Marginal Homogeneity was used to compare the differences in proportions of ordinal variables between two related groups. The ANCOVA test was used to eliminate the effect of confounding factors. In all analyzes, cases where the *p* value was less than 0.05 were considered statistically significant.

3 | RESULTS

A total of 96 adolescents were invited to the study, 14 of whom were excluded for various reasons. The remaining 82 adolescents were randomly divided into two groups. The study was completed with a total of 44 adolescents. The study flow is presented in Figure 1.

The MD group consisted of 22 adolescents (13 males, 9 females; age: 13.0 ± 1.99 year) and the LFD group consisted of 22 adolescents (13 males, 9 females; age: 13.9 ± 2.34 year). At baseline, there were no significant differences in the demographic, anthropometric, dietary and biochemical characteristics of the two groups, except for KIDMED index and GSH-Px (Tables 1–4).

3.1 | Dietary intake

After dietary intervention, changes in diet composition differed between groups as intended. The carbohydrate intake increased in the LFD group and decreased in the MD group, leading to a significant difference between the groups ($p < 0.001$). Protein intake significantly increased in both groups. Total fat (%E) and monosaturated fat (%E) intake increased in the MD group and decreased in the LFD group. The omega-3 intake significantly increased only in the MD group, whereas omega-6 decreased only in the LFD group. There was no statistically significant difference in the energy intake of the participants in the MD group at the beginning and end of the study [1906 kcal/day to 1787 kcal/day ($p = 0.721$)], while the LFD group significantly decreased their total energy intake from 1974 kcal/day to 1648 kcal/day ($p = 0.001$). Saturated fat, cholesterol, and sucrose intake significantly decreased in both groups compared with baseline. Physical activity levels did not change in either during the intervention. KIDMED scores increased significantly in both groups, but the MD group showed a greater increase than the LFD group (increase in KIDMED score during the

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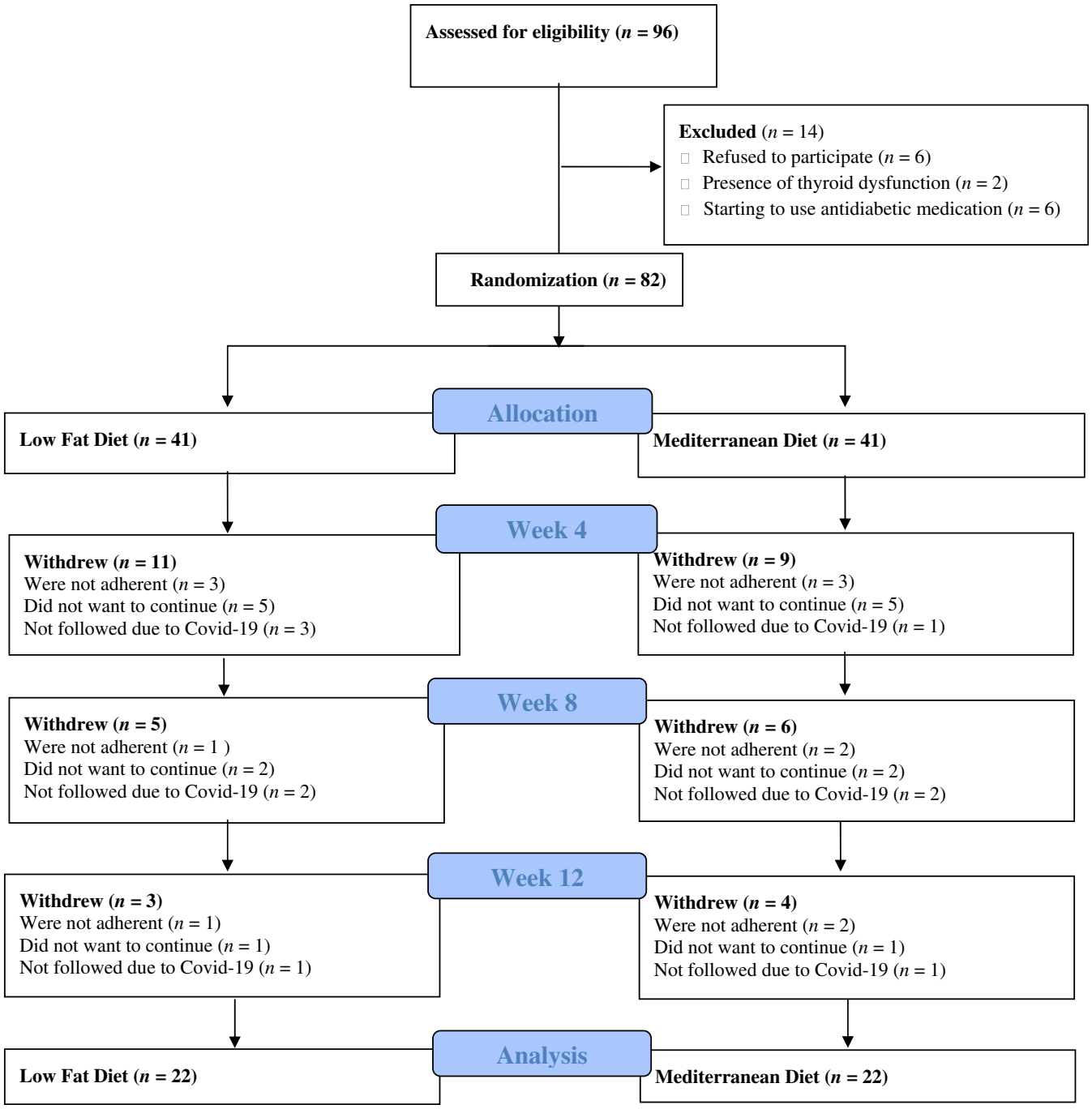


FIGURE 1 Study flow diagram

study 11.4 ± 1.18 vs. 6.3 ± 1.13, in MD and LFD, respectively; $p < 0.001$) (Table 2).

3.2 | Anthropometric outcomes

All anthropometric measurements and fat mass decreased in both groups and there was no significant difference between their changes. The mean body weight of participants significantly decreased in both groups (from 81.7 ± 21.05 kg to 76.6 ± 19.54 kg in the MD group; from 89.9 ± 18.75 kg to 84.2 ± 17.71 kg in the LFD group) (Table 3). Weight loss was not statistically different between groups (−5.6 ± 3.17 vs. -5.0 ± 3.18 kg; $p = 0.532$ in LFD and MD, respectively). Fat free mass (kg) decreased only in the LFD group (Table 3).

3.3 | Lipid profile and insulin resistance

At the 12th week, insulin and HOMA-IR levels decreased in both diet groups ($p < 0.05$). There was no significant difference in terms of TG,

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TABLE 1 Baseline demographics and characteristics by diet group

	MD ($n = 22$)	LFD ($n = 22$)	Total ($n = 44$)	p value
Age at screening, (mean ± SD), year	13.0 ± 1.99	13.9 ± 2.34	13.5 ± 2.19	0.220
Sex n (%)				
Male	13 (59.1)	13 (59.1)	26 (59.1)	1.000
Female	9 (40.9)	9 (40.9)	18 (40.9)	
Current smokers (%)	1 (4.5)	3 (13.6)	4 (9.1)	0.607
Comorbidities				
Hypertension	8 (36.4)	10 (45.5)	18 (40.9)	
Polycystic over syndrome 5 (22.7)		3 (13.6)	8 (18.2)	0.970
Asthma	8 (36.4)	9 (40.9)	17 (38.6)	
Grade of fatty liver (%) based on US				
Grade 1	8 (36.4)	10 (45.5)	18 (40.9)	
Grade 1–2	5 (22.7)	3 (13.6)	8 (18.2)	
Grade 2	8 (36.4)	9 (40.9)	17 (38.6)	0.619
Grade 2–3	1 (4.5)	– (–)	1 (2.3)	
Grade 3	8 (36.4)	10 (45.5)	18 (40.9)	

Abbreviation: US: ultrasound.

TABLE 2 Dietary intake and physical activity profile of participants in the LFD and the MD groups over time

	MD ($n = 22$)			LFD ($n = 22$)				
	Baseline	12 weeks[a]	p^1	Baseline	12 weeks[a]	p^1	p^2	p^3
Energy (kcal/day)	1899.5 ± 525.32	1858.8 ± 210.80	0.721	1909.8 ± 318.59	1689.8 ± 196.14	**0.001**	0.937	0.291
Carbohydrate (% E)	44.9 ± 6.55	41.7 ± 2.67	**0.047**	46.9 ± 6.42	52.5 ± 2.83	**0.001**	0.323	**<0.001**
Protein (% E)	14.9 ± 2.86	19.3 ± 1.51	**<0.001**	15.3 ± 2.53	20.7 ± 1.57	**<0.001**	0.782	0.057
Fat (% E)	40.2 ± 6.14	38.7 ± 2.34	0.391	37.8 ± 6.32	26.7 ± 2.60	**<0.001**	0.234	**<0.001**
Saturated fatty acid (% E)	15.3 ± 3.88	9.9 ± 0.86	**<0.001**	14.7 ± 3.25	9.6 ± 1.39	**<0.001**	0.707	0.756
Monosaturated fatty acid (% E)	12.7 ± 2.97	18.5 ± 2.10	**<0.001**	12.5 ± 2.44	8.2 ± 1.18	**<0.001**	0.830	**<0.001**
Polyunsaturated fatty acid (% E)	6.8 ± 3.06	8.1 ± 1.54	0.556	8.7 ± 2.31	6.3 ± 0.93	**0.010**	0.174	**0.048**
Omega 3 (g)	1.4 ± 1.06	3.1 ± 1.84	**0.009**	1.2 ± 0.41	0.9 ± 0.19	0.090	0.642	**0.003**
Omega 6 (g)	11.4 ± 7.96	12.6 ± 4.90	0.641	15.5 ± 5.15	9.39 ± 2.50	**0.009**	0.212	0.094
Omega 3/omega 6 (g)	0.14 ± 0.08	0.24 ± 0.38	**<0.001**	0.09 ± 0.43	0.09 ± 0.03	0.787	0.078	**<0.001**
Fibre (g)	19.6 ± 5.75	39.9 ± 6.59	**0.000**	18.8 ± 4.70	33.5 ± 5.07	**0.032**	0.592	0.125
Cholesterol (mg/day)	396.2 ± 192.81	219.1 ± 71.94	**<0.001**	363.2 ± 181.47	270.9 ± 83.68	**<0.001**	0.793	0.848
Diet antioxidant capacity (mmol)	1.9 ± 1.51	3.4 ± 2.07	**0.001**	1.4 ± 1.03	1.4 ± 0.96	0.983	0.162	**<0.001**
KIDMED score	4.5 ± 1.94	11.4 ± 1.18	**<0.001**	2.7 ± 1.72	6.3 ± 1.13	**<0.001**	**0.002**	**<0.001**
Physical activity level (PAL)	1.44 ± 0.15	1.47 ± 1.16	0.223	1.43 ± 0.16	1.44 ± 0.10	0.767	0.844	0.555

[a]These are the averages of the values in the 2nd, 4th, 6th, 8th, 10th, and 12th weeks, p^1 value for the intragroup comparison (baseline vs. week 12); p^2 value for the intergroup comparison (baseline values). p^3 value for the intergroup comparison (12 weeks values). The bold values are indicates significant at $p < 0.05$.

total cholesterol, LDL, and HDL cholesterol levels at the end of the 12th week (Table 3).

3.4 | Transaminases levels and the severity of steatosis

After 12 weeks of dietary intervention, a statistically significant decrease was found in the grade of hepatic steatosis in both diet groups ($p < 0.05$). In the MD group, 13.6% of adolescents did not have fatty liver, and 4.5% of them had Grade 0–1. In the LFD group, 18.2% of the adolescents did not have fatty liver. There was no statistically significant difference between the groups in terms of the amount of reduction in the degree of liver fat ($p = 0.875$). The ALT, AST, and GGT levels were significantly decreased in both diet groups. A greater decrease in ALT [−18.5 (37.50) vs. −13.0 (17.50) $p = 0.056$] and AST levels [−7.0 (22.75) vs. −4.5 (12.50) U/L; $p = 0.039$)] was seen with the MD than with the LFD (Table 3 and Figure 2). While the

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TABLE 3 Anthropometric and metabolic profile of participants in the LFD and the MD groups over time

Anthropometric measurements								
	MD ($n = 22$)			LFD ($n = 22$)				
	Baseline	Week 12	p^1	Baseline	Week 12	p^1	p^2	p^*
Body weight (kg)	81.7 ± 21.05	76.6 ± 19.54	**<0.001**	89.9 ± 18.75	84.2 ± 17.71	**<0.001**	0.181	0.532
Body mass index (kg/m^2)	30.9 ± 5.23	28.8 ± 5.03	**<0.001**	33.7 ± 5.49	31.4 ± 5.18	**<0.001**	0.095	0.737
Neck circumference (cm)	38.2 ± 3,61	35.9 ± 3.63	**<0.001**	38.6 ± 4.10	36.6 ± 3.64	**<0.001**	0.684	0.619
Hip circumference (cm)	109.0 ± 12,51	103.5 ± 12,08	**<0.001**	116.2 ± 1.51	110.7 ± 11.27	**<0.001**	0.051	0.929
Waist circumference (cm)	103.0 ± 14.07	94.9 ± 12,35	**<0.001**	109.0 ± 12,89	102.0 ± 11.86	**<0.001**	0.146	0.208
Waist to hip	0.94 ± 0.04	0.91 ± 0.04	**<0.001**	0.93 ± 0.06	0.92 ± 0.06	**0.009**	0,759	0.156
Body fat (%)	36.4 ± 6.13	32.5 ± 6.19	**<0.001**	38.5 ± 8.00	35.4 ± 8.75	**<0.001**	0.335	0.774
Fat free mass (kg)	51.1 ± 12,89	51.3 ± 13.16	0.671	54.6 ± 11.55	53.6 ± 10.82	**0.030**	0.374	0.564
Glycaemic control and lipid profile								
Glucose (mg/dl)	85.3 ± 8.17	86.4 ± 8.51	0.485	88.7 ± 7.15	85.6 ± 8.39	**0.049**	0.149	0.060
Insulin (IU/ml)	16.2 (16.88)	14.8 (7.10)	**0.010**	19.2 (11.52)	13.7 (11.87)	**0.007**	0.742	0.690
HbA1c (%)	5.5 ± 0.41	5.4 ± 0.43	0.065	5.6 ± 0.41	5.5 ± 0.31	**0.033**	0.450	0.409
HOMA-IR	3.6 (4.22)	3.1 (1.93)	**0.020**	4.4 (2.83)	2.8 (2.61)	**0.007**	0.742	0.622
Total cholesterol (mg/dl)	174.7 ± 41.65	168.1 ± 44.27	0.359	170.7 ± 53.90	177.7 ± 42.33	0.414	0.784	0.221
HDL cholesterol (mg/dl)	44.1 ± 5.85	43.5 ± 6.56	0.659	45.2 ± 8.39	47.5 ± 11.97	0.109	0.620	0.130
LDL cholesterol (mg/dl)	108.8 ± 53.02	98.1 ± 34.01	0.296	107.8 ± 36.29	105.1 ± 40.49	0.675	0.942	0.498
Triglycerides (mg/dl)	147.4 ± 83.14	127.5 ± 59.48	0.171	118.7 ± 64.65	122.1 ± 76.65	0.820	0.209	0.260
Aminotransferases levels								
ALT (U/L)	41.0 (45.50)	23.0 (14.75)	**<0.001**	30.5 (25.50)	21.0 (19.50)	**0.001**	0.149	0.057
AST (U/L)	33.0 (30.75)	23.0 (7.50)	**<0.001**	28.5 (13.50)	22.5 (8.25)	**0.008**	0.133	**0.039**
γGT (U/L)	23.5 (12.00)	17.5 (6.50)	**<0.001**	23.0 (12.75)	17.0 (14.25)	**0.026**	0.647	0.095
Steatosis *n* (%)								
Absent	– (–)	3 (13.6)		– (–)	4 (18.2)			
Grade 0–1	– (–)	1 (4.5)		– (–)	– (–)			
Grade 1	8 (36.4)	9 (40.9)	**0.008**	10 (45.5)	10 (45.4)	**0.006**	0.619	0.875
Grade 1–2	5 (22.7)	2 (9.1)		3 (13.6)	4 (18.2)			
Grade 2	8 (36.4)	6 (27.4)		9 (40.9)	4 (18.2)			
Grade 2–3	1 (4.5)	1 (4.5)		– (–)	– (–)			

Note: p^1 value for the intragroup comparison (baseline vs. week 12); p^2 value for the intergroup comparison (baseline values), p^* value for the intergroup comparison (changes from baseline between group). The bold values are indicates significant at $p < 0.05$.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; HOMA, homoeostasis model assessment; LDL, low-density lipoprotein; γGT, γ-glutamyltransferase.

proportion of adolescents with normal ALT levels increased significantly in the MD group, it did not change in the LFD group (Figure 3).

3.5 | Oxidative stress and inflammatory markers

TAS, PON-1, and GSH-Px levels increased significantly in the MD group ($p < 0.05$), but did not change in the LFD group. Malondialdehyde levels decreased significantly in both diet groups with no significant differences between groups (Table 4). Glutathione and GSH-Px levels increased in both diet groups ($p > 0.05$) and these increase was higher in the MD group compared to the LFD group ($p < 0.05$) (Figure 2). After adjustment for baseline, GSH-Px was not significantly different between groups.

The TNF-α, IL-6, IL-8, and IL-1β levels decreased and IL-10 levels increased at the end of the 12th week in both diet groups. However, the difference from baseline in these parameters was not statistically significant ($p > 0.05$), except IL-6 and CRP. Serum IL-6 levels were statistically significantly decreased only in the LFD group ($p = 0.031$), while CRP levels decreased only in the MD group ($p = 0.008$) (Table 4).

4 | DISCUSSION

To the best of our knowledge, the present study is the first to evaluate the effects of the MD and LFD on anthropometric measurements, body composition, insulin resistance, liver enzymes, severity of steatosis, oxidative stress, and inflammation in adolescents with

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TABLE 4 Oxidative stress and inflammatory markers at baseline and after 12 weeks of dietary intervention according to groups

	MD ($n = 22$)			LFD ($n = 22$)				
	Baseline	Week 12	p^1	Baseline	Week 12	p^1	p^2	p^*
Oxidative stress markers								
TAS (mmol/L)	1.8 ± 0.14	2.0 ± 0.26	**0.004**	1.8 ± 0.29	1.8 ± 0.29	0.991	0.293	0.061
TOS (μmol/L)	5.5 ± 3.14	5.4 ± 2.27	0.928	5.4 ± 3.01	5.0 ± 2.47	0.664	0.904	0.765
Oxidative stress index	0.30 ± 0.16	0.27 ± 0.10	0.295	0.29 ± 0.16	0.26 ± 0.12	0.599	0.777	0.859
PON-1 (U/L)	267.4 ± 194.96	299.8 ± 197.27	**0.038**	287.8 ± 170.85	284.0 ± 182.66	0.907	0.802	0.307
GSH-Px (U/L)	406.2 ± 86.90	506.9 ± 159.26	**0.004**	478.7 ± 130.05	482.7 ± 116.48	0.913	**0.036**	0.065[2]
SOD (U/ml)	220.4 ± 68.79	227.0 ± 99.7	0.753	197.9 ± 67.35	214.4 ± 76.19	0.286	0.280	0.701
Malondialdehyde(mmol/L)	10.1 (4.94)	5.5 (4.19)	**0.001**	7.6 (14.23)	5.3 (2.81)	**0.014**	0.630	0.544
Glutathione (mg/L)	62.5 (97.53)	87.0 (39.29)	0.217	82.0 (118.10)	81.5 (100.68)	0.200	0.080	**0.013**
Catalase (U/L)	115.7 ± 85.43	129.8 ± 111.92	0.638	151.4 ± 131.48	102.5 ± 67.06	0.293	0.280	0.144
Inflammatory markers								
TNF-α (pg/ml)	2.0 (5.13)	0.8 (3.91)	0.259	1.4 (1.82)	1.1 (2.18)	0.945	0.248	0.356
CRP (mg/dl)	2.9 (4.02)	2.7 (3.35)	**0.008**	2.1 (6.58)	3.0 (8.13)	0.673	0.503	0.152
IL-1β (pg/ml)	47.6 (12.21)	46.7 (10.49)	0.305	49.7 (10.50)	45.7 (10.35)	0.192	0.111	0.114
IL-6 (pg/ml)	3.3 (2.32)	3.2 (1.85)	0.520	4.2 (3.89)	3.7 (2.58)	**0.031**	0.189	0.138
IL-8 (pg/ml)	6.4 (7.87)	6.6 (6.44)	0.986	7.0 (10.58)	5.6 (6.78)	0.638	0.903	0.627
IL-10 (pg/ml)	6.4 (3.23)	6.9 (3.29)	0.131	6.9 (1.24)	7.7 (2.13)	0.054	0.082	0.627

Note: p^1 value for the intragroup comparison (baseline vs. week 12); p^2 value for the intergroup comparison (baseline values), p^* value for the intergroup comparison (changes from baseline between group).[2]*p* value adjusted for baseline measurement. The bold values are indicates significant at $p < 0.05$.
Abbreviations: GSH-Px, glutathione peroxidase; IL, Interleukin; OSİ, oxidative stress index; PON-1, paraoxanase-1; SOD, superoxide dismutase; TAS, total antioxidant status; TOS, total oxidant status.

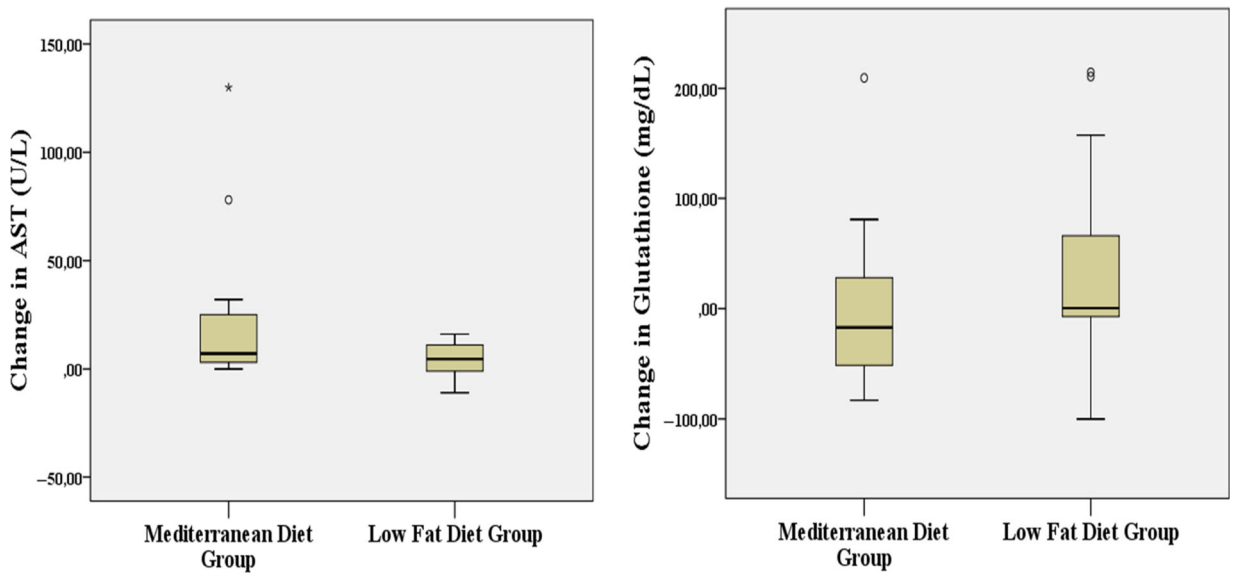


FIGURE 2 Changes in AST and Glutathione. The changes from baseline were calculated by subtracting the endpoint from the baseline value

obesity and NAFLD. This study demonstrated that a significant improvement in anthropometric measurements, body composition, insulin resistance, hepatic steatosis, liver enzymes oxidative stress and inflammatory parameters can be achieved in adolescents through consumption of the MD or LFD for 12 weeks, without prescribing energy restricted diet.

Both MD and LFD interventions yielded the beneficial effect of reducing body weight, BMI, fat mass, and waist, hip, and neck circumference. Consistent with these findings, Alvarez-Perez et al.[24] showed that there was a significant decrease in body weight, BMI, and waist circumference of individuals who followed MD and LFD treatment ad libutum. Another study indicated that consumption of a hypocaloric, high

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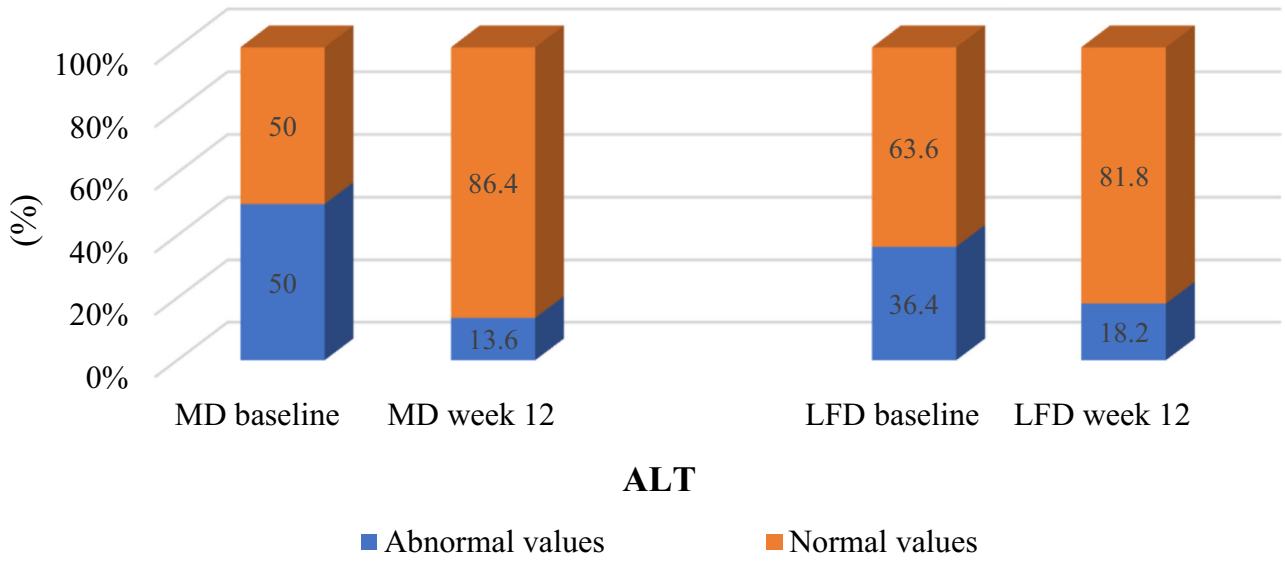


FIGURE 3 Effect of the dietary treatment on the normalization of ALT

monosaturated fat diet and an LFD for 1 year lead to decrease in body weight, BMI, fat mass and fat free mass to a similar degree.[25] In this study, the change in weight loss and other anthropometric parameters was similar in the two groups, but fat free mass significantly decreased only in the LFD group. This difference in terms of fat free mass change can be explained by the fact that, despite both diets being non-energy-restricted, participants showed a reduced energy intake on the LFD, whereas there was no change in energy intake on the MD.

This study showed that adherence to a MD or LFD for 12 weeks led to significant improvement in insulin resistance, hepatic steatosis, and liver enzymes level to a similar degree. Consistently, a 12-week ad libitum MD and LFD were reported to significantly reduce hepatic steatosis.[9] Gepner et al.[26] showed that in adults with NAFLD, insulin levels were significantly reduced by both the low-carbohydrate MD and LFD. In this study, no significant differences were seen in the changes between the MD and LFD groups, which may be explained by similar weight loss. Both groups achieved a level of body weight loss recommended by the current guidelines for the reduction of hepatic steatosis (3%–5% loss of baseline body weight).[27] Moreover, improvements in anthropometric and metabolic profiles can be attributed to the healthy diet of adolescents in both diet groups during the dietary intervention and accordingly to reduced intake of simple sugars and saturated fatty acids. In addition, increased dietary fibre intake in both diet groups may be associated with these outcomes. It has been shown that an increase in dietary fibre intake contributes to the reduction of hepatic steatosis and insulin resistance and may also affect the microbiota and therefore the intestinal-liver axis, which plays a role in the development and progression of NAFLD.[28] These results suggest that the recommendation of energy restriction may be unnecessary in effectively treating paediatric NAFLD.

As expected, this study showed that the MD was more effective in lowering ALT and AST levels. Similarly, Fraser et al.[29] showed that the MD was more effective than two different LFDs in reducing serum transaminase levels in diabetic patients. Another study indicated that ALT and AST levels were decreased more than the control diet group in individuals with NAFLD after 12 weeks of hypocaloric olive oil-based diet therapy.[30] In the current study, these results may be related to the fact that participants on the MD increased their intake of MUFA, omega-3 and antioxidant compounds such as polyphenols during the dietary intervention compared to the LFD group. These components of the MD have been shown to have wide-ranging beneficial effects, including inhibiting de novo hepatic lipogenesis, improving peripheral insulin sensitivity, and reducing the risk of cardiovascular disease.[28] Furthermore, the MD has been reported to be associated with a lower incidence of NAFLD due to its omega-3 fatty acid content, balanced omega-6/omega-3 ratio, and inflammatory neutrality.[28] Meta-analyses have also shown that diets rich in omega-3 fatty acids can reduce fatty liver and levels of hepatic enzymes.[31,32]

The MD has been reported to be associated with a lower incidence of cardiovascular disease and mortality.[33] However, the results of clinical studies with adults with NAFLD are inconsistent. In one study, it was found that total cholesterol levels decreased significantly after a 6-month MD intervention, and there was no difference in LDL and HDL cholesterol levels compared to baseline.[34] In another study, the MD and LFD were shown to have no effect on HDL cholesterol levels in individuals with NAFLD.[10] In this study, no significant difference was found in terms of the lipid profile in both diet groups. This result can be attributed to the relatively short duration and sample size of the study.

This study revealed that both the MD and LFD resulted in significant reductions in serum MDA, but the MD is more effective than the LFD in terms of increasing TAS, and especially increasing level of antioxidant enzymes (PON-1, glutathione, GSH-Px). Supporting this study,

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Urquiaga et al.[35] reported that plasma TAS levels were higher and oxidative damage was lower in healthy subjects on the MD for 3 months than the control diet group.[35] A significant increase was found in SOD and GSH-Px levels in individuals with metabolic syndrome after 3 months of the MD..[36] Üstündağ et al.[37] showed that the addition of soy isoflavones, a subgroup of flavonoids, to the diet in rat models with non-alcoholic steatohepatitis increased PON-1 enzyme activity.[37] In the MD group, it is thought that the increase in dietary antioxidant capacity due to the consumption of foods such as olive oil, vegetables and fruits with high antioxidant content is involved in these results. It has been reported that dietary antioxidant vitamins, minerals, and polyphenols comprise an exogenous defence mechanism against the development of oxidative stress, systemic inflammation, and non-communicable diseases.[38] Salehi-Sahlabadi et al.[39] found that the increase in dietary antioxidant capacity was associated with a reduced risk of NAFLD development. Taken together, these findings suggest that MD plays a protective role by reducing oxidative stress in NAFLD thanks to its antioxidant content.

With regard to inflammation, both diets were associated with improvement in most inflammatory parameters; in the case of the MD, a significant decrease in serum CRP level was observed, whereas in the case of the LFD, IL-6 levels were significantly reduced. It is well known that diet-induced body weight loss normalizes plasma levels and adipose tissue gene expression of many proteins derived from adipose tissue, and it has been reported that at least 5_10% loss of initial body weight is required to improve adipokine profile.[40,41] Moreover, the hypocaloric diet has been confirmed to have an anti-inflammatory effect independent of dietary composition, which plays an important role in preventing chronic diseases.[42] Imoya et al.[43] showed that IL-6 levels decreased significantly in women on an energy-restricted diet for 1 year. In addition, omega-6 fatty acids are known to have a pro-inflammatory profile and stimulate the production of TNF-α and IL-6 due to their metabolism directly related to the production of arachidonic acid, which is metabolized to produce pro-inflammatory interleukins and cytokines.[44] In this study, a significant reduction in energy and omega-6 fatty acid intake of adolescents in the LFD group compared to baseline may be associated with a significant decrease in IL-6 levels. In this study, although there was no difference between the groups in terms of body weight and fat mass loss, only the MD group showed a significant decrease in CRP levels. This result was supported by the finding in a recently published meta-analysis that showed that the MD was more effective in inducing long-term changes in inflammatory markers than LFDs.[45] In another meta-analysis, it was reported that higher fruit and vegetable consumption led to a decrease in proinflammatory markers.[46] In addition, many studies have shown that omega-3 fatty acids play an important role in inflammation control by reducing CRP, eicosanoid proinflammatory cytokines, chemokines, and other inflammatory mediators.[47,48] These results suggest that the effect of body weight loss on inflammatory markers may be more effective with the MD.

The present study has a few limitations. First, we used ultrasonography instead of liver biopsy, which is the gold standard method in the diagnosis and evaluation of hepatic steatosis. Second, the short duration of the study prevented the determination of the effectiveness of dietary intervention in reducing hepatic steatosis in the long term. Third, the number of participants withdrawn from the study due to the COVID-19 pandemic was high. Strengths of this study include a single blind randomized control design; a holistic evaluation of the effects of diets on hepatic steatosis, oxidative stress and inflammation markers using a range of complementary parameters; strict follow-up by a dietitian and good compliance to dietary advice.

5 | CONCLUSION

In conclusion, this study showed that both the MD and LFD reduce body weight, BMI, body fat, hepatic steatosis, and insulin resistance, improve high transaminase levels, and have positive effects on oxidative stress and inflammation in adolescents with obesity and NAFLD, in a short time without energy restriction. These dietary approaches should be tested in larger cohorts for longer periods to determine their long-term health benefits.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest to this work.

AUTHOR CONTRIBUTIONS

Conception and design: Gamze Yurtdaş, Gamze Akbulut; Data collection: Gamze Yurtdaş; Resources and formal analysis: Gamze Yurtdaş, Maşallah Baran, Canan Yılmaz, Supervision: Gamze Akbulut; Analysis, interpretation and drafting the manuscript: Gamze Yurtdaş; Critical revision of the manuscript: Gamze Akbulut. All authors read and approved the final version of the manuscript.

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