Original Article

Impact of a low-carbohydrate and high-fiber diet on nonalcoholic fatty liver disease

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Background and Objectives: To study the effects of a low-carbohydrate and high-fiber diet and education on patients with nonalcoholic fatty liver disease. **Methods and Study Design:** We randomly divided 44 patients with nonalcoholic fatty liver disease into two groups: low-carbohydrate and high-fiber diet and education (intervention group), and education alone (control group). Liver and kidney function, fasting plasma glucose, insulin resistance index, body composition, and controlled attenuation parameter were detected before and after the intervention. **Results:** After 2 months, the body fat, body weight, abdominal circumference, and visceral fat area, fasting plasma glucose, insulin resistance index, and levels of serum alanine aminotransferase, aspartate transaminase, uric acid, and insulin of the intervention group were significantly lower than before ($p<0.05$). In the female intervention group, the insulin resistance index and levels of serum alanine aminotransferase, uric acid, triglyceride, fasting plasma glucose, and C-peptide were lower and the level of serum high-density lipoprotein cholesterol was higher than in the female control group ($p<0.05$). In the male intervention group, the levels of serum alanine aminotransferase, triglyceride, and fasting plasma glucose were lower and the level of serum high-density lipoprotein cholesterol was higher compared with the male control group ($p<0.05$). **Conclusions:** A low-carbohydrate and high-fiber diet and education can effectively reduce the body weight and body fat of patients with nonalcoholic fatty liver disease and improve metabolic indicators such as liver enzymes, blood glucose, blood lipid, and uric acid. Our female patients showed significantly better improvement in the indicators than our male patients.

Key Words: NAFLD, low-carbohydrate diet, metabolism, inflammation, hepatic fatty infiltration

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is becoming a global public health problem.[1,2] The prevalence of NAFLD in European and American countries currently ranges from 24% to 42%, whereas its prevalence in Asian countries ranges from 5% to 40%. Moreover, a trend is occurring in which the disease is developing at younger ages.[3-5] The prevalence of NAFLD in adults was reported to reach 15% in Shanghai, Hong Kong, and other developed areas of China.[6] NAFLD is now considered the hepatic manifestation of metabolic syndrome, which is closely associated with dyslipidemia, obesity, insulin resistance, and hypertension.[7] However, consensus remains lacking regarding the appropriate medication for NAFLD. Several studies have revealed that personal behavior guidance including that related to changes in living habits, appropriate and regular physical exercise, and weight loss is beneficial to the alleviation of insulin resistance, thereby achieving the goals of treating metabolic syndrome and resolving NAFLD.[7] However, dietary recommendations for patients with NAFLD vary. According to the recommendations of the American Diabetes Association, reductions in energy, carbohydrate, and fat intake may be beneficial to resolving NAFLD. In addition to the intake amount, the quality or type of carbohydrates and lipids may also affect the development of NAFLD.[8] This paper discusses the possible effects of health education and a low-carbohydrate and high-fiber diet on clinical metabolic indicators, inflammatory status, and hepatic fat deposition in patients with NAFLD based on the low-carbohydrate dietary pattern.

METHODS

Reagents and apparatus

Nutrition bars, mixed protein drinks (each unit contained net cap 2 g, which had energy of 80 kcal), and dietary fiber (200 g/can) were purchased from Nutriease Health

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Technology Co. Ltd. (Zhejiang, China). We used the Mindray BC-6900 automatic hematology analyzer; Cobas 8000 automatic biochemical analyzer; MAGLUMI 4000 automated chemiluminescence immunoassay analyzer; Inbody 230 body composition analyzer (Inbody 720 model); human interleukin-1β kit, human interleukin-6 kit, and human interleukin-10 kit; and a quantitative tester of liver steatosis, the FibroScan 502 (M probe: 3.5 MHz).

Study subjects

Forty-four patients with NAFLD who were admitted to and examined at the clinic of Huadong Hospital affiliated to Fudan University between October 2015 and May 2017 were enrolled. We used the following inclusion criteria: having NAFLD and meeting the diagnostic criteria in the Guidelines for Diagnosis and Treatment of Nonalcoholic Fatty Liver Diseases (2010)[6]; age 8–60 years; and BMI ≥25 kg/m^2. All patients provided informed consent. The following exclusion criteria were used: secondary obesity, such as hypothyroid obesity, pituitary obesity, Cushing-syndrome-induced obesity, hypothalamic obesity, and hypogonadal obesity; diseases that require controlled protein intake, such as renal disease; psychiatric disease and malignancy; severe gastrointestinal disease; currently on a weight-loss diet or medical treatment or having undergone surgery in the preceding 3 months; weight fluctuations of more than 5 kg over the preceding 2 months; history of food allergy; in the gestation, preconception, or lactation period; perimenopausal or postmenopausal; and malformations or chronic infectious diseases. The study subjects were randomized to the intervention (diet intervention + education) or control (education) group by the investigators according to a random number table to ensure that all subjects had equal opportunities to be assigned to the treatment and control groups.

Study methods

The intervention group was given a diet intervention and education, whereas the control group was given education alone. The intervention lasted for 2 months. The study complied with the regulations outlined in the Declaration of Helsinki, and approval was obtained for the study protocol from the Ethics Committee of Huadong Hospital affiliated to Fudan University (Approval No.: 20150094).

Education

The main form of education was face-to-face counseling, which was divided into dietary guidance, physical activity guidance, and psychological behavioral counseling. Each patient received follow-ups in the form of a weekly phone call from a fixed and trained dietitian. The patients could obtain answers to questions as well as psychological and technical support on WeChat, and the dietitian provided prompt guidance based on the feedback from each patient.

Diet intervention

The low-carbohydrate and supplemental fiber dietary pattern was employed to limit the energy intake of patients during the weight reduction period of the intervention. The percentage of energy from carbohydrates was 20%–25%, and food with a low glycemic index was primarily selected. The percentage of energy from dietary protein was 40%–45%, and the main sources of protein were beans, bean products, nuts, soy milk, chicken, and fish. The percentage of energy from lipids was 30%–35%, and the lipids were mainly n-3 and n-6 polyunsaturated fatty acids. The intake of saturated fatty acids was low.

Nutrition bars were prepared for use as staple food substitutes by using food processing techniques. The daily staple food of the participants was substituted by the nutrition bars (Zhejiang Nuote Health Technology Co. Ltd.) provided by the researchers, and the patients were given 5 g of dietary fiber supplement (Zhejiang Nuote Health Technology Co. Ltd.) that was mixed in the protein drink (Zhejiang Nuote Health Technology Co. Ltd.) and consumed after stirring of the drink. Calcium and magnesium tablets and multivitamins (Zhejiang Nuote Health Technology Co. Ltd.) were taken by the participants twice a day. Subsidiary food, selected under the guidance of the dietitian, was defined as nonstaple food, such as chicken, duck, fish, fruits, and vegetables; it did not include staple food such as rice and noodles. In the diet in which energy intake was limited, the daily energy intake of a participant was limited to 1000-1200 kcal. Intake of 400 g of vegetables and 1800 mL of water a day was guaranteed.

Intervention phase

Subjects were given an adaptive period of approximately 1 week before the start of the intervention. During this period, irregular dietary behaviors were avoided and basic dietary habits were maintained. In the dietary intervention group, the subjects were given energy-limiting nutrition bars as a low-carbohydrate staple food substitute. The intake of nonstaple food was limited. Simultaneously, nutrition education was conducted, and the content and form of this education were the same as those in the education (control) group. The intervention lasted for 2 months. Relevant indicators were tested before and after the intervention, and the intervention effects and compliance indicators were tracked.

Observational indicators

The following indicators were tested at enrollment and after 2 months of the study.

Tests of blood, biochemistry, lipid metabolism, and other indicators

Routine blood work, complete biochemical analysis, and tests of lipid metabolism, insulin level, and other indicators were performed. The homeostasis model assessment–insulin resistance (HOMR-IR) index was calculated according to the formula HOMR-IR = Fasting insulin × Fasting plasma glucose / 22.5.

Test of inflammatory cytokines IL-1ß, IL-10, and IL-6

Interleukin-1β (IL-1ß), interleukin-10 (IL-10), and interleukin-6 (IL-6) levels were tested using human IL-1ß, IL-10, and IL-6 kits purchased from Maigeer Bioscience and Technology, Ltd. Co. (Wuhan, China).

Test of body composition

Body composition was tested using the Inbody 720 human body composition analyzer purchased from Biospace (South Korea).

Quantitative measurement of hepatic steatosis

The controlled attenuation parameter (CAP) was measured according to the standard protocol by using the FibroScan 502 M detector purchased from Echosens (France). The patient lay on his or her back with the right hand by the head to maximally stretch the intercostal space. The seventh, eighth, and ninth intercostal areas between the anterior axillary line and midaxillary line on the right side were examined. The detector was placed perpendicular to the skin surface in the intercostal space. The examination did not start until the pressure indicator appeared green, the intensity M wave was consistent and evenly distributed on the screen, and the A wave was linear. Each subject was guaranteed to be successfully examined more than 10 times. The CAP was displayed in dB/m, and the medians of the valid measurements were selected to represent the final results. The examiners were physicians with special training and FibroScan certifications. All the examinations followed the manufacturer's manual. The optimal thresholds were determined on the basis of the CAP and references of histopathological staging: S1 ≥237.7 (hepatic steatosis ≥11%), S2 ≥259.4 (hepatic steatosis ≥34%), and S3 ≥292.3 (hepatic steatosis ≥67%). The treatment effect was determined according to the two histopathological stages. If the first histopathological stage was greater than the second histopathological stage, improvement was considered to have occurred, and if not, improvement had not occurred.

Statistical analysis

The data analysis was performed using SPSS 22.0. Continuous data are represented as $\bar{x} \pm s$. The t test was used to compare the two groups regarding changes in their variables. The difference between before and after the intervention was examined using a paired t test. Nonnormally distributed data were statistically analyzed using the rank-sum test. Countable data are described by n (%) and examined using the chi-square test. Categorical data are displayed as n (%) and tested using the rank-sum test. The difference between before and after treatment was examined using the paired rank-sum test. We considered $p<0.05$ as statistically significant.

RESULTS

Baseline comparison

Altogether, 44 patients were included in our study, among which 28 were men and 16 were women. The average age was 38.1±9.4 years. No significant differences were observed in the white blood cell (WBC) count, hemoglobin count, platelet count, liver function, renal function, blood lipids, fasting blood glucose, fasting insulin, fasting C-peptide level, or HOMR-IR between the male control, male intervention, female control, and female intervention groups. Additionally, no significant differences in inflammatory cytokine levels, body composition, and liver CAP values were observed ($p>0.05$).

Comparative analysis of regular blood tests, liver and renal function, and glucose and lipid metabolism between the two groups of patients before and after treatment

After the 2-month intervention, the number of WBCs and fasting blood glucose level in the intervention group were significantly lower than those in the control group ($p<0.05$); however, no significant differences were observed in the rest of indicators (Table 1). The 2-month intervention significantly decreased the WBC and platelet counts and levels of aminotransferase (ALT), aspartate transaminase (AST), uric acid (UA), total cholesterol, triglyceride, and fasting plasma glucose (FPG) ($p<0.05$) but increased blood HDL level ($p<0.05$). No significant changes were observed in the rest of the indicators. Hemoglobin, ALT, and AST levels were significantly lower after treatment compared with before treatment in the control group ($p<0.05$), whereas the other indicators were not found to change significantly (Table 1).

After the 2-month intervention, the number of WBCs, FPG, HOMR-IR, and levels of ALT, creatinine, UA, triglyceride, insulin, and C-peptide were significantly lower in the female intervention group than in the female control group whereas the HDL level was significantly higher ($p<0.05$). The FPG and levels of hemoglobin, ALT, and triglyceride were significantly lower in the male intervention group than in the male control group, whereas the opposite was seen regarding the HDL level ($p<0.05$; Table 2).

In the female intervention group, the FPG, HOMR-IR, and levels of ALT, AST, UA, and insulin were significantly reduced after the intervention ($p<0.05$); in the male intervention group, the levels of hemoglobin, ALT, and AST were significantly reduced ($p<0.05$), whereas no significant differences were observed in the rest of the indicators (Table 3). The number of WBCs was significantly increased in the female control group ($p<0.05$). No significant changes were observed in the rest of the indicators. In the male control group, although changes were observed in blood lipid level, liver and renal function, and other indicators after treatment, the differences were nonsignificant ($p>0.05$, Table 3).

Changes in human body composition

After the 2-month intervention, both groups exhibited a significant reduction in body fat mass (BFM), body weight, skeletal muscle mass, BMI, abdominal circumference, and visceral fat area (VFA) ($p<0.05$). However, no significant differences were observed in body weight, skeletal muscle mass, BMI, abdominal circumference, BFM, and VFA between the intervention and control groups (Table 1).

The sex-specific comparisons showed that after the 2-month diet intervention and education, BFM, body weight, abdominal circumference, and VFA were significantly reduced in the female intervention group ($p<0.05$), whereas BFM, body weight, skeletal muscle mass, BMI, abdominal circumference and VFA were significantly reduced in the male intervention group, ($p<0.05$). In the control group, BFM, body weight, BMI, and abdominal circumference were significantly reduced in the female patients ($p<0.05$), whereas body weight, skeletal muscle mass, BMI, abdominal circumference, and VFA were significantly reduced in the male patients ($p<0.05$).

After the 2-month diet intervention and education, the female intervention group had lower BFM and body weight ($p<0.05$) but larger abdominal circumference

Table 1. Before- and after-treatment comparisons in the intervention and control groups (n=22)[†]

	Intervention group				Control group			
	Before treatment	After treatment	t	*p*	Before treatment	After treatment	t	*p*
WBC (10^9/L)	7.34±1.96	6.34±1.89	2.136	0.045	6.93±1.15	7.28±0.96	-1.220	0.236
HGB (g/L)	155±12.6	148±16.5	2.062	0.052	145±19.4	137±20.9	2.601	0.017
PLT (109/L)	256±39.2	237±49.3	2.273	0.034	264±45.8	257±49.9	1.161	0.259
ALT (U/L)	41.8±20.7	20.5±10.7	4.890	0.000	43.4±31.6	26.8±17.0	2.362	0.028
AST (U/L)	26.0±10.8	17.7±4.99	4.049	0.001	25.9±11.3	18.7±6.32	2.623	0.016
Total bilirubin (umol/L)	12.7±3.92	11.9±4.8	0.908	0.374	11.2±5.89	9.57±2.72	1.452	0.161
Direct bilirubin (umol/L)	3.62±1.06	4.02±1.6	-1.296	0.209	3.19±1.32	3.43±0.8	-0.797	0.434
Indirect bilirubin (umol/L)	9.1±3.05	7.79±3.48	1.889	0.073	8.05±4.67	6.15±2.26	2.120	0.046
Creatinine (umol/L)	75.1±15.6	69.7±12.4	2.000	0.059	74.7±15.0	72.9±9.48	0.573	0.573
UA (umol/L)	413±107	352±112	2.557	0.018	417±97.1	385±96.6	1.947	0.065
Total cholesterol (mmol/L)	5.15±0.9	4.84±0.8	2.662	0.015	5.02±1.18	4.79±0.84	1.041	0.310
Triglyceride (mmol/L)	1.64±0.81	1.19±0.91	2.493	0.021	1.78±1.22	1.59±1.01	0.779	0.445
LDL (mmol/L)	3.33±0.97	2.98±0.69	1.972	0.062	2.93±0.93	2.85±0.77	0.455	0.654
HDL (mmol/L)	1.29±0.26	1.41±0.27	-2.365	0.028	1.36±0.18	1.76±1.42	-1.328	0.198
Insulin (mIU/L)	15.7±7.46	16.6±38.4	-0.119	0.907	17.2±12.4	14.5±10.2	0.848	0.406
C-peptide (ng/mL)	2.44±0.84	2.09±1.4	1.157	0.260	2.63±1.01	2.48±0.93	0.713	0.484
FPG (mmol/L)	5.21±0.5	4.74±0.46	3.578	0.002	5.28±0.81	5.27±0.59	0.106	0.916
HOMR-IR	3.58±1.54	3.95±10.3	-0.174	0.864	4.19±3.45	3.35±2.26	1.064	0.299

WBC: white blood cell; HGB: handelsgesetzbuch; PLT: platelet; ALT: alanine aminotransferase; AST: aspartate transaminase; UA: uric acid; LDL: low-density lipoprotein in cholesterol; HDL: high-density lipoprotein in cholesterol; FPG: fasting plasma glucose; HOMR-IR: insulin resistance index.

[†]The difference between before and after the intervention was examined using a paired t test. The difference of Insulin and HOMR-IR between before and after the intervention was statistically analyzed using the rank-sum test; others were examined using a paired t test.

Table 2. Comparisons of the control and intervention groups after treatment†

	Female (n=16)			Male (n=28)		
	Control group	Intervention group	*p*	Control group	Intervention group	*p*
WBC (10^9/L)	7.2±0.97	5.46±1.72	0.026	7.32±0.99	6.84±1.85	0.399
HGB (g/L)	119±20.8	132±16.9	0.166	148±12.4	157±7.56	0.022
PLT (10^9/L)	283±71.2	227±61.0	0.112	242±25.3	242±42.7	0.986
ALT (U/L)	19.3±13.0	14.8±5.55	0.037	31.1±17.9	23.8±11.7	0.021
AST (U/L)	18.0±5.67	14.8±3.88	0.205	19.1±6.83	19.4±4.85	0.895
Total bilirubin (umol/L)	8.02±2.14	9.66±4.84	0.393	10.4±2.67	13.2±4.45	0.061
Direct bilirubin (umol/L)	3.23±0.54	3.39±1.59	0.798	3.54±0.92	4.38±1.55	0.091
Indirect bilirubin (umol/L)	4.8±1.74	6.28±3.54	0.307	6.92±2.21	8.66±3.25	0.109
Creatinine (umol/L)	64.0±7.83	56.9±4.44	0.041	77.9±6.07	77.2±8.79	0.773
UA (umol/L)	337±135	237±72.4	0.009	411±55.4	418±67.7	0.777
Total cholesterol (mmol/L)	4.6±0.84	4.74±0.59	0.705	4.91±0.86	4.9±0.91	0.992
Triglyceride (mmol/L)	0.87±0.36	0.69±0.27	0.028	2.01±1.03	1.47±1.03	0.018
LDL (mmol/L)	2.64±0.84	2.69±0.51	0.900	2.97±0.73	3.14±0.73	0.556
HDL (mmol/L)	1.51±0.23	1.65±0.34	0.033	1.91±1.78	2.41±4.43	0.007
Insulin (mIU/L)	14.4±9.43	8.2±1.93	0.009	14.5±11.0	21.4±48.0	0.607
C-peptide (ng/mL)	2.61±1.44	1.83±0.71	0.019	2.41±0.51	2.25±1.68	0.730
FPG (mmol/L)	5.23±0.51	4.63±0.38	0.019	5.30±0.64	4.80±0.51	0.033
HOMR-IR	3.35±2.13	1.69±0.39	0.048	3.36±2.41	5.25±2.94	0.596

WBC: white blood cell; HGB: handelsgesetzbuch; PLT: platelet; ALT: alanine aminotransferase; AST: aspartate transaminase; UA: uric acid; LDL: low-density lipoprotein in cholesterol; HDL: high-density lipoprotein in cholesterol; FPG: fasting plasma glucose; HOMR-IR: insulin resistance index.

†The difference of Insulin was statistically analyzed using the rank-sum test in the male population; others were examined using a t test

and VFA than the female control group ($p<0.05$); no significant differences were observed in the rest of the indicators. No significant differences were found in abdominal circumference, VFA, BFM, body weight, skeletal muscle mass, and BMI between the male intervention and control groups (Table 1).

Differences in the levels of inflammatory cytokines between the two groups before and after treatment

After the 2-month intervention, the plasma levels of inflammatory cytokines IL-1ß, IL-6, and IL-10 were not significantly different between the intervention and control groups ($p>0.05$). Additionally, no significant changes in plasma IL-1ß, IL-6, and IL-10 levels were observed in either group ($p>0.05$).

Changes in fatty infiltration of the liver

After the 2-month diet intervention and education, the intervention group showed a significantly different CAP distribution from the control group ($z=-2.31$, $p=0.021$, Table 3). The intervention group comprised 22 patients, among which 16 showed improvement (72.7%) and 6 showed no improvement (27.3%). Among the 22 patients of the control group, 8 patients showed improvement (36.4%), whereas 14 patients did not (63.6%). The percentage of patients showing improvement was significantly different between the two groups ($x^2=5.87$, $p=0.015$).

Among the male patients, 12 patients in total showed improvement (42.9%); 8 of the 14 male patients in the intervention group showed improvement (57.1%), whereas 4 of the 14 male patients in the control group showed improvement (28.6%). The difference between the groups was nonsignificant ($x^2=2.33$, $p=0.127$). Among the female patients, 12 in total showed improvement (42.9%). All eight female patients in the intervention group improved, whereas four of eight female patients in the control group showed improvement (50%). The difference between the two groups was significant ($x^2=5.33$, $p=0.021$).

DISCUSSION

Research on how a low-carbohydrate diet (LCD), in which carbohydrates provide 20%–40% of the daily energy supply, can reduce the body weight of patients with NAFLD and subsequently treat NAFLD has drawn substantial attention in recent years. LCD not only reduces body weight and the fat content of the liver but also improves metabolic indicators in patients with obesity.[9] Conversely, a high-fiber diet can decrease the absorption of carbohydrates, promote secretion of insulin and incretin, and thus alleviate insulin resistance and hepatic steatosis in patients with NAFLD.[10] In this study, we found that an LCD combined with high dietary fiber could significantly improve human body composition indicators - such as BFM, body weight, skeletal muscle mass, BMI, abdominal circumference, and VFA - as well as blood markers, such as the number of WBCs and platelets and levels of ALT, AST, UA, total cholesterol, triglyceride, and FPG in patients with NAFLD. Moreover, the combined diet significantly alleviated hepatic steatosis in the patients. These findings suggest that personal behavior changes are effective means of improving body composition and promoting the health of patients. Combining other methods with the LCD/high-fiber intervention could efficiently improve the body weight and BFM of patients with NAFLD. Moreover, sex-specific analysis showed that the female intervention group showed better improvement in HOMR-IR and levels of ALT, UA, creatinine, triglyceride, fasting blood glucose, C-peptide, and FPG than the female control group, whereas among the male patients, only the ALT, triglyceride, and FPG levels were significantly improved in the intervention group compared with the control group. This has three possible

Table 3. Sex-specific before- and after-treatment comparisons in the intervention and control groups[†]

	Intervention group						Control group					
	Female (n=8)			Male (n=14)			Female (n=8)			Male (n=14)		
	Before treatment	After treatment	*p*	Before treatment	After treatment	*p*	Before treatment	After treatment	*p*	Before treatment	After treatment	*p*
WBC (10^9/L)	6.68±1.01	5.46±1.72	0.129	7.72±2.29	6.84±1.85	0.189	6.05±0.9	7.2±0.97	0.042	7.43±0.98	7.32±0.99	0.721
HGB (g/L)	145±14.1	133±16.9	0.206	161±7.41	157±7.56	0.015	128±19.6	119±20.8	0.107	154±11.6	148±12.4	0.095
PLT (10^9/L)	261±37.6	227±61.0	0.129	253±41.2	242±42.7	0.119	290±64.7	283±71.2	0.519	250±22.1	242±25.3	0.381
ALT(U/L)	40.8±27.3	14.8±5.55	0.031	42.4±16.9	23.8±11.7	0.001	31±17.1	19.3±13.0	0.087	50.5±36.2	31.1±17.9	0.091
AST(U/L)	26.6±14.8	14.8±3.88	0.033	25.6±8.25	19.4±4.85	0.006	22.8±6.34	18.0±5.67	0.112	27.7±13.2	19.1±6.83	0.055
Total bilirubin (umol/L)	10.4±3.76	9.66±4.84	0.630	14.1±3.43	13.2±4.45	0.477	9.23±3.63	8.02±2.14	0.326	12.4±6.71	10.5±2.67	0.281
Direct bilirubin (umol/L)	2.99±1.1	3.39±1.59	0.441	3.98±0.88	4.38±1.55	0.347	2.8±0.9	3.23±0.54	0.162	3.42±1.49	3.54±0.92	0.787
Indirect bilirubin (umol/L)	7.36±2.71	6.28±3.54	0.312	10.1±2.85	8.66±3.25	0.156	6.44±2.79	4.8±1.74	0.109	8.98±5.33	6.92±2.21	0.149
Creatinine (umol/L)	64.1±11.3	56.9±4.44	0.086	81.4±14.4	77.1±8.79	0.269	61.3±5.51	64.0±7.83	0.445	82.3±13.2	77.9±6.07	0.337
UA (umol/L)	350±73.0	236.9±72.4	0.026	450±108	418±67.6	0.279	357±127	337±135	0.519	451±55.9	411.6±55.4	0.077
Total cholesterol (mmol/L)	5.14±0.68	4.74±0.59	0.059	5.15±1.03	4.9±0.91	0.122	4.75±0.97	4.6±0.84	0.284	5.18±1.3	4.91±0.86	0.440
Triglyceride (mmol/L)	1.36±0.66	0.69±0.27	0.052	1.79±0.87	1.47±1.03	0.188	1.08±0.42	0.87±0.36	0.106	2.18±1.35	2.01±1.03	0.657
LDL(mmol/L)	3.47±1.19	2.69±0.51	0.070	3.25±0.87	3.14±0.73	0.518	2.74±1.03	2.64±0.84	0.497	3.04±0.9	2.97±0.73	0.809
HDL(mmol/L)	1.46±0.24	1.65±0.34	0.347	1.2±0.23	2.41±4.43	0.322	1.51±0.17	1.51±0.23	0.967	1.27±0.13	1.91±1.78	0.200
Insulin(mIU/L)	13.1±4.39	8.2±1.93	0.028	17.1±8.55	21.4±48.0	0.733	16.5±10.8	14.4±9.43	0.464	17.5±13.6	14.5±11.0	0.542
C-peptide (ng/mL)	2.25±0.65	1.83±0.71	0.232	2.55±0.94	2.25±1.68	0.505	2.43±1.24	2.61±1.44	0.446	2.74±0.88	2.41±0.51	0.272
FPG (mmol/L)	5.36±0.47	4.63±0.38	0.003	5.13±0.51	4.80±0.51	0.087	5.36±0.77	5.23±0.51	0.627	5.24±0.86	5.30±0.64	0.682
HOMR-IR	3.13±1.10	1.69±0.39	0.016	3.84±1.73	5.25±2.94	0.681	4.13±3.10	3.35±2.13	0.221	4.23±3.75	3.36±2.41	0.487

WBC: white blood cell; HGB: handelsgesetzbuch; PLT: platelet; ALT: alanine aminotransferase; AST: aspartate transaminase; UA: uric acid; LDL: low-density lipoprotein in cholesterol; HDL: high-density lipoprotein in cholesterol; FPG: fasting plasma glucose; HOMR-IR: insulin resistance index.

[†]The difference of insulin between before and after the intervention was statistically analyzed using the rank-sum test in the male population; others were examined using a paired t test.

explanations: the incidence of NAFLD is higher in men than in women; the female participants in this study were mostly premenopausal, and the effects of estrogen on the regulation of blood lipid metabolism and fat distribution were not considered, possibly leading to biased results[11]; and better compliance of the female patients may also have led to biased results.

Inflammatory cytokines, such as tumor necrosis factor and transforming growth factor ß, are a group of small-molecule proteins secreted by immune cells, such as monocytes and macrophages, as well as nonimmune cells-such as endothelial cells, epithelial cells, and fibroblasts—during the body's inflammatory response. They regulate immune responses, cell differentiation, and proliferation and are categorized mainly into interleukins, chemokines, growth factors, colony-stimulating factors, and interferons. IL-1ß reportedly plays a role in the pathogenesis and progression of NAFLD. The pathogenesis may include lipid accumulation in hepatocytes, promotion of hepatic steatosis, and promotion of apoptosis-inducing gene Bax expression.[12,13] However, after a 2-month diet and education intervention, the levels of IL-1, IL-6, and IL-10 were nonsignificantly different between the two groups and between sexes. Standard values may not exist for plasma levels of inflammatory factors. Moreover, the variation among individuals is large and can be affected by multiple factors. Additionally, low-degree chronic inflammation is common in patients with NAFLD exhibiting increased expression of multiple inflammatory factors.[14] The relatively short intervention period in our study may not have been sufficient to induce significant changes in the expression of plasma inflammatory factors.

Previous large-scale clinical trials have shown that personal behavior intervention can improve hepatic histology. Promarat et al performed a randomized controlled trial of 31 participants with overweight and reported that a 48-week intervention led to an average weight loss of 9.3% in the experimental group and 0.2% in the control group (p=0.003). Significant histological improvement was observed in the participants with a weight loss of ≥7%.[15] Lazo et al. confirmed this with their study of hepatic steatosis in 96 patients with overweight and type II diabetes.[16] However, as the gold standard, liver biopsy is an invasive method of examination and has risks and complications. It is not well accepted by patients and is difficult to perform repeatedly for tracing and diagnosis. FibroScan, a novel device used to measure the stiffness of the liver, can dynamically monitor the progression of NAFLD.[17] The CAP can quantify the degree of human hepatic steatosis. As indicated by Sasso et al., CAP values measured from various degrees of hepatic steatosis are significantly different, and the change in CAP is significantly correlated with the degree of hepatic steatosis.[18] Chen et al observed that CAP values could be used to accurately evaluate various degrees of hepatic steatosis and are highly valuable in diagnosis.[19] The larger the CAP, the worse the steatosis. In this study, we used the FibroScan to quantitatively measure hepatic fat before and after an intervention in two groups of patients. We observed that after the intervention of an LCD combined with high fiber, 72.7% of the patients in the intervention group showed improvement in liver fat accumulation, whereas only 36.4% of the patients in the control group, who received only education, showed improvement. The difference was significant. All the patients in the female intervention group showed improvement, whereas only 50% of the patients in the female control group improved. The significant difference between the two groups suggested that the dietary intervention helped lessen liver fat accumulation in patients with NAFLD, especially in women.

Our study has limitations, such as being performed in a single center and having a small sample. In addition, we focused mainly on patients with overweight and NAFLD. For the minority of normal-weight patients with NAFLD, whether the intervention of an LCD combined with high fiber is suitable for improving NAFLD remains to be investigated. The effect of the intervention of an LCD combined with high fiber on NAFLD requires more multilayered studies with larger samples. However, our study has contributed to the theoretical basis of nonpharmacological interventions used to treat patients with NAFLD clinically and has provided new data, shedding light on the clinical treatment of NAFLD.

AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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REFERENCES

1. Fan J, Shi J. Epidemiology and noninvasive diagnosis of nonalcoholic steatohepatitis in 2011. Journal of Clinical Hepatology. 2012;15:81-3. doi: 10.3969/j.issn.1672-5069.2012.02.001.
2. National Workshop on Fatty L, Alcoholic Liver Disease CSoHCMA, Fatty Liver Expert Committee CMDA. [Guidelines of prevention and treatment for nonalcoholic fatty liver disease: a 2018 update]. Zhonghua Gan Zang Bing Za Zhi. 2018;26:195-203. doi: 10.3760/cma.j.issn.1007-3418.2018.03.008. (In Chinese)
3. Amarapurkar DN, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ, Goh KL et al. Asia-Pacific Working Party on NAFLD. How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? J Gastroenterol Hepatol. 2007;22:788-93. doi: 10.1111/j.1440-1746.2007.05042.x.
4. Fan JG, Saibara T, Chitturi S, Kim BI, Sung JJ, Chutaputti A; Asia-Pacific Working Party for NAFLD. What are the risk factors and settings for non-alcoholic fatty liver disease in Asia-Pacific? J Gastroenterol Hepatol. 2007;22:794-800. doi: 10.1111/j.1440-1746.2007.04952.x.
5. Caballeria L, Auladell MA, Toran P, Miranda D, Aznar J, Pera G et al. Prevalence and factors associated with the presence of non alcoholic fatty liver disease in an apparently healthy adult population in primary care units. BMC Gastroenterol. 2007;7:41. 4. doi: 10.1186/1471-230X-7-41.
6. National Workshop on Fatty Liver and Alcoholic Liver Disease CSoH, Chinese Medical Association. Guidelines of prevention and treatment for nonalcoholic fatty liver disease (2010 revision). Chinese Journal of Hepatology. 2010;18:

163-66. doi: 10.3760/cma.j.issn.1007-3418.2010.03.002. (In Chinese)
7. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement: Executive Summary. Crit Pathw Cardiol. 2005;4:198-203. doi: 0.1097/00132577-200512000-00018.
8. Hashemi Kani A, Alavian SM, Haghighatdoost F, Azadbakht L. Diet macronutrients composition in nonalcoholic Fatty liver disease: a review on the related documents. Hepat Mon. 2014;14:e10939. doi: 10.5812/hepatmon.10939.
9. Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. N Engl J Med. 2009;360:859-73.8. doi: 10.1056/NEJMoa0804748.
10. Zolfaghari H, Askari G, Siassi F, Feizi A, Sotoudeh G. Intake of nutrients, fiber, and sugar in patients with nonalcoholic fatty liver disease in comparison to healthy individuals. Int J Prev Med. 2016;7:98. doi: 10.4103/2008-7802.188083.
11. Chen KL, Madak-Erdogan Z. Estrogens and female liver health. Steroids. 2018;133:38-43. doi: 10.1016/j.steroids.2017.10.015.
12. Miura K, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H et al. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. Gastroenterology. 2010;139:323-334 e327.11. doi: 10.1053/j.gastro.2010.03.052.
13. Stienstra R, Saudale F, Duval C, Keshtkar S, Groener JEM, Rooijen N et al. Kupffer cells promote hepatic steatosis via interleukin-1beta-dependent suppression of peroxisome proliferator-activated receptor alpha activity. Hepatology. 2010;51:511-22. doi: 10.1002/hep.23337.
14. Bessone F, Razori MV, Roma MG. Molecular pathways of nonalcoholic fatty liver disease development and progression. Cell Mol Life Sci. 2019;76:99-128. doi: 10.1007/s00018-018-2947-0.
15. Promrat K, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, Fava J, Wing RR. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. Hepatology. 2010;51:121-9. doi: 10.1002/hep.23276.
16. Lazo M, Solga SF, Horska A, Bonekamp S, Diehl AM, Brancati FL et al. Effect of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. Diabetes Care. 2010;33:2156-63. doi: 10.2337/dc10-0856.
17. Wang Z, Yang Y, Huang M. Imaging techniques in diagnosis of non-alcoholic fatty liver disease. Chinese Journal of Gastroenterology & Hepatology. 2013;22:197-200. doi: 1006-5709 (2013) 03-0197-04.
18. Luo D, Xing G, Lao X, Lai C, Ye Y, Chen L. Analysis of factors affecting the implementation of controlled attenuation parameters in instantaneous elastic imaging. Guangdong Medical Journal. 2017;38:2980-7. (In Chinese)
19. Chen JN, Chen AP, Pan Q, Guo QY, Shen F, Zhen RD, Fan JG. Influential factors and clinical value of controlled attenuation parameters in the evaluation of hepatic steatosis using FibroScan. Chinese Hepatology. 2016;21:805-9. doi: 10.14000/j.cnki.issn.1008-1704.2016.10.001. (In Chinese)