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Association Between Protein Intake From Different Animal and Plant Origins and the Risk of Non-Alcoholic Fatty Liver Disease: A Case-Control Study

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ABSTRACT

Previous studies have frequently reviewed how different macronutrients affect liver health. Still, no study centered around protein intake and the non-alcoholic fatty liver disease (NAFLD) risk relationship. This study aimed to examine the association between the consumption of total and different sources of protein and NAFLD risk. We allocated 243 eligible subjects to the case and control groups, including 121 incidence cases of NAFLD, and 122 healthy controls. Two groups were matched in age, body mass index, and sex. We evaluated the usual food intake of participants using FFQ. Binary logistic regression was conducted to estimate the risk of NAFLD in relation to different sources of protein intake. The age of participants was 42.7 years on average, and 53.1% were male. We found Higher intake of protein in total (odds ratio [OR], 0.24; 95% confidence interval [CI], 0.11–0.52) was significantly associated with a lower risk of NAFLD, despite adjusting for multiple confounders. in detail, higher tendency to the vegetables (OR, 0.28; 95% CI, 0.13–0.59), grains (OR, 0.24; 95% CI, 0.11–0.52), and nuts (OR, 0.25; 95% CI, 0.12–0.52) as the main sources of protein, were remarkably correlated with lower NAFLD risk. In contrary, increased intake of meat protein (OR, 3.15; 95% CI, 1.46–6.81) was positively associated with a higher risk. Totally, more calorie intake from proteins was inversely associated with lower NAFLD risk. This was more likely when the protein sources were selected less from meats and more from plants. Accordingly, increasing the consumption of proteins, particularly from plants, may be a good recommendation to manage and prevent NAFLD.

Keywords: NAFLD; Non alcoholic fatty liver disease; Protein intake; Plant protein; Animal protein

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Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

Conceptualization: Ebrahimi Mousavi S, Nazari M, Salamat S, Asbaghi O, Mansoori A; Data curation: Ebrahimi Mousavi S, Nazari M, Salamat S, Asbaghi O, Mansoori A; Formal analysis: Khazaei Y, Dehghanseresht N, Ebrahimi Mousavi S, Nazari M, Salamat S, Asbaghi O, Mansoori A; Investigation: Khazaei Y, Dehghanseresht N; Writing - original draft: Khazaei Y, Dehghanseresht N; Writing - review & editing: Khazaei Y, Dehghanseresht N, Ebrahimi Mousavi S, Nazari M, Salamat S, Asbaghi O, Mansoori A.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), as the most common liver disease in the world, is considered a pathological condition in which accumulated hepatic fat exceeds 5% of liver weight [1]. This condition covers a range of liver diseases, starting with simple steatosis as the first stage, that may eventually lead to non-alcoholic steatohepatitis (NASH) cirrhosis or hepatocellular carcinoma [2,3]. Over the past decades, NAFLD has become increasingly common with an estimation of 25% global prevalence and even more in South America and Asia [4]. It is important to consider the possibility of NAFLD in any patient with liver enzymes (alanine aminotransferase and/or aspartate transaminase) higher than normal ranges [5].

NAFLD is characterized by a strong associated with metabolic syndrome features like obesity, dyslipidemia, type 2 diabetes mellitus (T2DM), hypertension, and other cardiovascular diseases [6]. Insulin resistance, which is common in T2DM, and higher fasting insulin levels lead to increased hepatic fat accumulation and developing NAFLD [3]. Therefore, there is no pharmacological therapy for NAFLD. It is obvious that lifestyle modification including diet and exercise are golden keys to fatty liver managements [7-10]. Regardless of calorie-related aspects of diet and its correlation with weight control, studies have less addressed the effects of dietary composition on liver health [11]. Most existing articles have focused on the relationship of different dietary patterns like Western, DASH, Mediterranean, and etc., with NAFLD incidence. They mostly address to calorie contend, alcohol usage, food groups choices, food processing, and sedentary status [12] as possible factors on NAFLD pathogenesis. The majority of research on macronutrients component of diet and fatty liver outcomes mentioned that lowering carbohydrates and modifying lipid content of dietary intake are positively effective on weight management, liver enzymes, and fat accumulation of liver [13]. But we missed finding a clear data about the exact association of protein intake and its different sources with the risk of NAFLD.

Therefore, in this case-control study, we evaluated the association between intake of total protein, and separately different sources of animal and plant proteins, in an Iranian adult population, and their NAFLD incidence.

MATERIALS AND METHODS

Study population

This study registered a total of 243 eligible clients who had been referred to a gastroenterology outpatient health center in Ahvaz, Iran, between November 2018 to May 2019.

One hundred twenty-two incidence cases of NAFLD and 121 controls were allocated to our study groups based on convenience sampling method, relying on inclusion criteria. Subjected entered to our study if they were between 19–70 years of age, and underwent to an abdominal ultrasonography check-up. Also, they were excluded if: 1) they had a history of alcohol abuse (more than 20 grams/d for men and 10 grams/d for women), 2) physical or mental abnormalities, 3) alcoholic fatty liver, 4) viral hepatitis, 5) hepatic cancer, 6) diabetes or other chronic conditions or malignancies, 7) immunodeficiency viruses, and 8) contraceptive or hepatotoxic drugs intake. The consent forms were signed by all participants after explaining the details of the study to them.

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NAFLD assessment

A registered gastroenterologist confirmed the diagnosed NAFLD cases based on a chronic rise in liver enzymes in lab tests and evidence of steatosis using NAFLD-compliant liver ultrasound sonography as a high sensitivity method, if all other causes of liver abnormalities were rejected. We defined the control group as individuals with no history of hepatic steatosis. Both group members underwent an ultrasound examination. matching individuals to selecting the case and control groups members were based on age (5-year groups), body mass index (BMI; 18–24.9, 25–29.9, ≥ 30 kg/m^2), and sex (male/female). The bioethics committee of Isfahan University of Medical Sciences confirmed the protocol of this study.

Dietary intake and exposure assessment

In addition to the demographic characteristics questionnaire-including information on gender, age, marriage, education, job, medical history, and smoking status, a validated and reliable semi-quantitative 147-item food frequency questionnaire (FFQ) [14,15] were completed by our interviewer after asking all items from participants one by one. This willet-format-designed questionnaire was following the usual dietary habits of Iranians. to determine the annual intake of total protein we estimated the amount of protein intake from primary plant and animal sources according to the frequency of participants' food intake during the past year considering their household activities.

We made a Korea National Health and Nutrition Examination Survey (KNHANES)-classification of 19 food groups from all consumed foods by people [16]. According to the 19 food groups, we considered meats, poultry, fish, dairies, and eggs as primary animal sources, and vegetables, fruit, grains, legumes, nuts, and seeds as primary plant sources [17]. To estimate the exact grams of protein in each source, we used U.S. Department of Agriculture food composition databases. Total protein was considered as the sum of grams of all the above sources (animal protein and plant protein) [18].

Assessment of other covariates

Weight and height of participants were measured by the digital Seca scale with an accuracy of 0.5 kg with least clothes and without shoes, and a tape-meter with 0.1 cm accuracy while they were standing to the wall, looking ahead, respectively. We used International Physical Activity Questionnaire (IPAQ) to check the physical activity status, based on metabolic equivalent-h/d (MET-h/d), which was also completed by the interviewer [19]. BMI was estimated by dividing weight (kg) by the square of the height (m^2). Waist circumference [20] was measured while persons were upright with usual breathing by putting a tape-meter on the middle point of the lower rib and the iliac crest. Waist-to-height ratio (WHtR) and waist-to-hip ratio (WHR) also were estimated [21,22]. The blood pressure was measured twice with a 10-minute rest time, while they were sitting on a chair calmly, using a standard sphygmomanometer. The average value of two measurements was considered as the right blood pressure of the person.

Statistical analysis

Categorizing of participants was performed based on all tertiles of total protein, animal, dairy, meat, vegetable, fruit, grain, legumes, and nuts protein. We used an independent t-test to compare the case and the control groups for continuous variables and χ^2 for stratified variables, respectively. The comparisons among tertiles of all protein sources were performed using one-way analysis of variance and χ^2 test. To evaluate the differences in dietary intakes of cases and controls, as well as across tertiles of each protein source, we applied analysis of covariance (ANCOVA) with adjusting total energy, sex, and age. A multivariable logistic

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regression test was performed to estimate the odds ratio of NAFLD and mentioned different sources of protein intake and their details in the crude and adjusted models. The first model was adjusted for sex (male/female), age (continuous), and energy intakes (continuous). about the second model, additional adjustments were considered for physical activity (continuous), smoking (smoker/nonsmoker), marital status (married/single), education (university graduated/non-university education), supplement use (yes/no), drug use (yes/no) fat intakes, and carbohydrate intakes. Eventually, in the third model, adjustments for BMI were done. The first tertiles of each protein source were marked as the reference category. All analyses were applied using Statistical Package for Social Sciences (version 23; SPSS Corp, Chicago, IL, USA). The p values < 0.05 were considered as statistically significant.

Ethics approval and consent to participates

All individuals were informed about the study procedure and goals. They were also required to sign informed consent forms to enter the study. The study protocol was confirmed by the Ethics Committee of Jundishapur University of Ahvaz based on the ethical guidelines of the 1975 declaration of Helsinki (IR.AJUMS.REC.1397.939).

RESULTS

The main characteristics of study participants by cases and controls are shown in **Table 1**. Cases were more likely to be smokers and have higher educational levels and physical activity than control subjects. NAFLD patients had greater height, waist circumference [20], WHtR and WHR. Other variables (including age [p = 0.76], sex [p = 0.84], weight [p = 0.31], BMI [p = 0.05], hip circumference [p = 0.89], marital status [p = 0.48], drug use [p = 0.48], and supplement use [p = 0.19]) were not significantly different between case and control groups.

Dietary intakes of nutrients and food groups of participants by case and control groups are summarized in **Table 2**. Compared with controls, cases had higher consumption of plant protein (p = 0.002), total fiber (p = 0.03), vegetables (p = 0.001), fruits (p < 0.001), meats (p < 0.001), and grains (p = 0.001). Consumption of other nutrients and food groups were not significantly different between the two groups. Multivariable-adjusted odds ratios (ORs) and

Table 1. General characteristics of study participants separately by case and control groups

Baseline characteristics	Cases (n = 121)	Controls (n = 122)	p*
Age (yr)	42.95 ± 11.46	42.52 ± 11.52	0.769
Male (%)	46.3	47.5	0.840
Weight (kg)	83.17 ± 14.72	81.28 ± 14.66	0.316
Hight (cm)	167.05 ± 9.75	164.45 ± 9.44	0.035
BMI (kg/m^2)	30.53 ± 5.04	29.32 ± 4.49	0.050
Hip circumference (cm)	106.25 ± 7.84	106.10 ± 9.41	0.893
Waist circumference (cm)	102.86 ± 10.78	98.12 ± 10.51	0.001
WHR	0.96 ± 0.07	0.92 ± 0.07	< 0.001
WHtR	0.62 ± 0.07	0.59 ± 0.06	< 0.001
Married (%)	86.8	83.6	0.480
University graduated (%)	48.4	30.6	0.005
Smoker (%)	9.9	3.3	0.037
Drug use (%)	46.3	21.3	< 0.001
Supplement use (%)	17.4	11.5	0.192
Physical activity (MET hours/week)	34.11 ± 5.87	35.94 ± 7.87	0.040

Data are expressed as mean ± standard deviation.
BMI, body mass index; WHtR, waist/height ratio; WHR, waist/hip ratio; MET, metabolic equivalents.
*p values were obtained from independent Student's t-test.

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Table 2. Dietary and nutrient intakes of study participants separately by case and control groups

Dietary and nutrient intakes	Cases (n = 121)	Controls (n = 122)	p*
Energy intake (kcal)	3,052.71 ± 590.71	2,901.79 ± 626.49	0.89
Carbohydrate (g/d)	428.39 ± 99.35	404.44 ± 94.29	0.91
Protein (g/d)	111.94 ± 28.63	105.38 ± 26.06	0.64
Animal protein (g/d)	50.60 ± 23.60	42.84 ± 19.87	0.08
Plant protein (g/d)	117.48 ± 55.48	91.85 ± 40.24	0.002
Dairy protein (g/d)	15.44 ± 7.90	15.18 ± 10.03	0.33
Fat (g/d)	109.08 ± 9.75	105.38 ± 32.41	0.70
Total fiber (g/d)	69.49 ± 28.69	63.06 ± 22.77	0.03
Cholesterol (mg/d)	258.56 ± 102.82	257.41 ± 130.74	0.20
Food groups	106.25 ± 7.84	106.10 ± 9.41	0.893
Vegetables (g/d)	151.51 ± 92.64	142.71 ± 78.37	0.001
Fruits (g/d)	0.96 ± 0.07	0.92 ± 0.07	< 0.001
Meats (g/d)	131.19 ± 87.07	103.28 ± 53.28	< 0.001
Nuts and seeds(g/d)	21.71 ± 24.17	15.28 ± 17.58	0.06
legumes (g/d)	86.45 ± 52.31	99.44 ± 58.01	0.79
Dairies (g/d)	284.68 ± 153.42	277.94 ± 179.83	0.35
Grains (g/d)	712.18 ± 384.81	639.89 ± 292.51	0.01

Data are expressed as mean ± standard deviation. All values were adjusted for age, sex and energy, except for dietary energy intake, which was only adjusted for age and sex using ANCOVA.
ANCOVA, analysis of covariance.

95% confidence intervals (CIs) for NAFLD by tertiles of dietary protein intake are presented in **Table 3**. For total protein intake, the association was significant both in crude and adjusted models. In the crude model, participants in the highest tertiles of total protein intake had decreased chance of having NAFLD compared with those in the lowest tertile (OR, 0.29; 95% CI, 0.15–0.56; p-trend < 0.001). After adjustment for several potential confounders, the same association was found (OR, 0.23; 95% CI, 0.11–0.51; p-trend < 0.001). In the last model, by further adjustment for BMI, the findings remained unchanged (OR, 0.24; 95% CI, 0.11–0.52; p-trend < 0.001). A significant positive association was reported between animal protein intake and NAFLD risk in both crude and adjusted models. participants in the highest tertiles of animal protein intake reported higher risk of NAFLD compared with those in the lowest tertile (OR, 2.48; 95% CI, 1.31–4.69, p-trend = 0.004). after adjustment of confounders findings remained unchanged (OR, 2.92; 95% CI, 1.36–6.27; p-trend = 0.007). additional adjustment for BMI did not differ the results (OR, 2.80; 95% CI, 1.29–6.04; p-trend = 0.005). the association between plants protein and NAFLD risk was not significant. In crude model, individuals of the highest tertile comparing lowest tertile of plant protein had no significant associated with NAFLD risk (OR, 3.58; 95% CI, 0.87–7.86; p-trend = 0.732). by adjustment of potential confounders still the findings were unchanged (OR, 3.26; 95% CI, 0.83–5.98; p-trend = 0.081) further adjustment of BMI in the third model, didn't change the results (OR, 3.24; 95% CI, 0.81–5.96; p-trend = 0.080). The non-significant association between protein intake from dairy and the chance of NAFLD was identified both in crude and adjusted models. In the crude model, individuals in the highest tertile compared to lowest tertile of dairy protein had not been associated with NAFLD (OR, 1.56; 95% CI, 0.84–2.9; p-trend = 0.15). After adjusting for several potential confounders, the same non-significant association was seen (OR, 1.44; 95% CI, 0.72–2.90; p-trend = 0.29). In the third model by additional adjustment for BMI, the findings remained non-significant (OR, 1.39; 95% CI, 0.69–1.80; p-trend = 0.35). For protein intake from meat, there was a significant association with a chance of NAFLD in crude models and controlled models. In the crude model, individuals in the top tertile of protein intake from meat had 2.78 times higher odds of NAFLD than those in the bottom tertile (OR, 2.78; 95% CI, 1.46–5.27; p-trend = 0.002). After controlling for potential confounders, this association remained significant but slightly strengthened (OR,

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Table 3. Multivariable-adjusted ratios for NAFLD across tertiles of dietary protein intake (odds ratios and 95% confidence intervals)

Variables	Tertiles of protein intake			
	T1	T2	T3	p-trend
Total protein (g/day)				
Crude	1.00	0.28 (0.15, 0.52)	0.29 (0.15, 0.56)	< 0.001
Model 1	1.00	0.25 (0.12, 0.49)	0.26 (0.13, 0.52)	< 0.001
Model 2	1.00	0.18 (0.08, 0.40)	0.23 (0.11, 0.51)	< 0.001
Model 3	1.00	0.18 (0.08, 0.40)	0.24 (0.11, 0.52)	< 0.001
Animal protein (g/day)				
Crude	1.00	2.61 (1.38, 4.94)	2.48 (1.31, 4.69)	0.004
Model 1	1.00	2.40 (1.25, 4.61)	2.39 (1.24, 4.59)	0.004
Model 2	1.00	2.94 (1.40, 6.15)	2.92 (1.36, 6.27)	0.007
Model 3	1.00	2.88 (1.37, 6.04)	2.80 (1.29, 6.04)	0.005
Plant protein (g/day)				
Crude	1.00	1.58 (0.84, 2.97)	3.58 (0.87,7.86)	0.732
Model 1	1.00	1.56 (0.77, 3.13)	4.07 (0.99, 8.69)	0.733
Model 2	1.00	1.16 (0.52, 2.70)	3.26 (0.83, 5.98)	0.081
Model 3	1.00	1.16 (0.51, 2.60)	3.24 (0.81, 5.96)	0.080
Dairy protein (g/day)				
Crude	1.00	1.91 (1.02, 3.56)	1.56 (0.84, 2.91)	0.158
Model 1	1.00	1.70 (0.91, 3.27)	1.48 (0.78, 2.80)	0.227
Model 2	1.00	1.71 (0.85, 3.41)	1.44 (0.72, 2.90)	0.299
Model 3	1.00	1.70 (0.85, 3.41)	1.39 (0.69, 2.80)	0.354
Meat protein (g/day)				
Crude	1.00	3.23 (1.69, 6.17)	2.78 (1.46, 5.27)	0.002
Model 1	1.00	3.01 (1.56, 5.82)	2.70 (1.39, 5.23)	0.004
Model 2	1.00	3.78 (1.79, 7.97)	3.32 (1.55, 7.13)	0.002
Model 3	1.00	3.83 (1.81, 8.10)	3.15 (1.46, 6.81)	0.003
Fruit protein (g/day)				
Crude	1.00	1.22 (0.65, 2.26)	1.81 (0.97, 3.38)	0.060
Model 1	1.00	1.14 (0.60, 2.16)	1.75 (0.91, 3.39)	0.092
Model 2	1.00	1.00 (0.50, 2.01)	1.54 (0.75, 3.17)	0.238
Model 3	1.00	1.01 (0.50, 2.04)	1.52 (0.73, 3.14)	0.257
Vegetable protein (g/day)				
Crude	1.00	0.30 (0.15, 0.58)	0.24 (0.12, 0.47)	< 0.001
Model 1	1.00	0.28 (0.14, 0.54)	0.24 (0.12, 0.47)	< 0.001
Model 2	1.00	0.34 (0.16, 0.71)	0.27 (0.13, 0.56)	0.001
Model 3	1.00	0.34 (0.16, 0.72)	0.28 (0.13, 0.59)	0.001
Nuts and seeds protein (g/day)				
Crude	1.00	0.32 (0.16, 0.61)	0.23 (0.11, 0.45)	< 0.001
Model 1	1.00	0.31 (0.16, 0.60)	0.22 (0.11, 0.43)	< 0.001
Model 2	1.00	0.41 (0.20, 0.83)	0.24 (0.12, 0.51)	< 0.001
Model 3	1.00	0.42 (0.20, 0.86)	0.25 (0.12, 0.52)	< 0.001
Grain protein (g/day)				
Crude	1.00	0.28 (0.15, 0.54)	0.29 (0.15, 0.56)	< 0.001
Model 1	1.00	0.25 (0.12, 0.49)	0.26 (0.13, 0.52)	< 0.001
Model 2	1.00	0.18 (0.09, 0.40)	0.23 (0.11, 0.51)	< 0.001
Model 3	1.00	0.19 (0.08, 0.40)	0.24 (0.11, 0.52)	< 0.001
Legumes protein (g/day)				
Crude	1.00	1.05 (0.56, 1.94)	1.16 (0.62, 2.14)	0.637
Model 1	1.00	1.05 (0.56, 1.98)	1.11 (0.59, 2.08)	0.738
Model 2	1.00	1.05 (0.52, 2.13)	1.28 (0.63, 2.61)	0.477
Model 3	1.00	1.02 (0.50, 2.07)	1.30 (0.64, 2.65)	0.451

Model 1: Adjusted for age (continuous), sex (male/female) and energy intake (kcal/d).
Model 2: Additional adjusted for physical activity, marital status (married/single), education (university graduated/non-university education), supplement use (yes/no), drug use (yes/no), smoking status (smoker/nonsmoker), fat intakes (continuous), and carbohydrate intakes (continuous).
Model 3: Further adjustments were conducted for BMI.
NAFLD, non-alcoholic fatty liver disease; BMI, body mass index.
*The analysis of binary logistic regression was used to determine the odds ratio and 95% confidence interval. Considering the mean differences among the quartiles of dietary vitamin C intakes determined p for trends.

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3.32; 95% CI, 1.55–7.13; p-trend = 0.002). When BMI was taken into account, this association remained significant (OR, 3.15; 95% CI, 1.46–6.81; p-trend = 0.003). For protein intake from vegetables, we found a negative significant association with NAFLD in crude model and adjusted models (OR, 0.24; 95% CI, 0.12–0.48; p-trend < 0.001). In the fully adjusted model, this association remained significant (OR, 0.28; 95% CI, 0.13–0.59; p-trend = 0.001). A significant inverse association was seen between protein intake from nuts and NAFLD in all models. Subjects in the highest tertile of nut protein had a 75% decreased chance of NAFLD than those in the lowest tertile (OR, 0.23; 95% CI, 0.11–0.45; p-trend < 0.001). When potential confounders were considered in the second model, the effect measure was attenuated (OR, 0.24; 95% CI, 0.12–0.51; p-trend < 0.001). Additional adjustment for BMI had no effect on the observed association (OR, 0.25; 95% CI, 0.12–0.52; p-trend < 0.001). The significant association between protein intake from grain and the risk of NAFLD was identified both in the crude model and all models. In the crude and fully adjusted model, a negative significant association was observed between protein intake from grain and risk of NAFLD (OR for crude model, 0.29; 95% CI, 0.15–0.56; p-trend < 0.001, and OR for fully adjusted model, 0.24; 95% CI, 0.11–0.52; p-trend < 0.001). For protein intake from legumes, the association was not significant either in crude or all models. The full-adjusted OR of NAFLD was 1.30 (95% CI, 0·64–2.65) for the highest versus the lowest tertile of protein intake from legumes.

DISCUSSION

In this present case-control study, we aimed to investigate a possible association between dietary protein intake and the risk of developing NAFLD. With regards to our investigation, we found an inverse association between total protein and other sources like vegetable protein, nut protein, and grain protein with the odds of NAFLD. Our findings also indicated that higher consumption of meat protein increased the probability of hepatic fat accumulation.

Healthy eating habits, weight loss, and increased physical activity are the foundation of NAFLD management [23]. Studies have shown that diet therapy and weight loss could be beneficial in case of managing fatty liver disease progression. In such a scenario, some studies suggested a possible role of macronutrients in the early stages of NAFLD and their preventive characteristics on progression to severe levels [7]. In this regard, evidence collected from both animal and human studies has demonstrated that diets with low protein led to hepatic fat accumulation [2,24]. Whereas a high-protein diet showed a reduction in liver fat content compared to diets with the same calorie intake but high in carbohydrates in healthy human adults. Dietary protein intake has been indicated to be worth considering with insulin resistance [25]. Therefore, there might be an advantage to increase dietary protein intake by targeting different protein sources in terms of preventing hepatic lipid accumulation [24].

The effects of protein intake on hepatic fat accumulation have not been clear yet; since mechanistic studies are interested in the role of fat and carbohydrates, predominantly, rather than dietary protein [7]. Therefore, it is difficult to propose an underlying mechanism on how dietary proteins act in terms of developing NAFLD [26]. Nevertheless, increasing protein consumption has shown to be beneficial during weight loss and weight maintenance, and could be helpful in hepatic fat reduction [27]. This may happen due to the downregulation of lipogenesis along with liver lipid oxidation and utilization [3]. Confirmation of our results, a prospective study conducted by Markova et al. showed that adherence to a higher protein diet, regardless its source, animal or plant, had a remarkable reduction of hepatic

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fat independent of one's anthropometric changes [28]. On the other hand, some previous studies declared that a higher dietary protein intake, in particular specific amino acids, was associated with increased liver fat accumulation, disease prevalence, and severity [20].

In recent years, many studies investigated the possible influence of different dietary protein sources on the development of cancer and cardiovascular disease [29]. Alferink et al.'s study [30] revealed that adherence to dietary patterns high in vegetable protein, dietary fiber, nuts, and grains like the Mediterranean diet had an inverse association with the risk of NAFLD and insulin resistance. It would be more helpful if studies address the source, type, and quality of ingested protein. Based on previous observations, a vegetable protein-rich diet with an abundant amount of branched-chain amino acids (BCAA) content, but low in methionine and aromatic amino acids (AAA) has been shown to enhance the health of cirrhotic patients [31,32]. Plant proteins also seemed to reduce the risk of mortality, improve the plasma glucose level, and enhance hormonal responses to the meal in diabetic patients who suffer from cirrhosis [24,31]. Animal data suggest that soy protein may decrease liver fat synthesis and enhance insulin sensitivity [33]. Meanwhile, based on these research findings, higher intake of proteins originated from nuts and grains showed to be associated with a lower risk of hepatic fat accumulation.

Certain dietary amino acids may alter some critical biological processes [23]. There are studies proposing that specific dietary amino acids consumption may affect liver status in the case of the pathogenesis of NAFLD and glucose metabolism. Therefore, a healthy dietary pattern characterized by balanced dietary amino acids might be on some level helpful for the management of NAFLD [34]. Emerging evidence suggests that meat protein contains a high level of methionine, homocysteine, and cysteine. Since the liver is the ultimate place of metabolism of this amino acid, dysregulation of their metabolic pathway may cause accumulation of them in the liver and plasma, which are a significant risk factor for NAFLD [31]. A study conducted in the Brazilian population revealed that higher consumption of red meat is associated with plenty of unhealthy conditions like the occurrence of metabolic syndrome, obesity, insulin resistance, and especially NAFLD [35]. In particular, higher consumption of red meat and saturated fat containing red meat had a significant association with hepatic fat [36].

A case-control study on dietary patterns among Lebanese NAFLD patients revealed that all types of meat were significantly associated with an increase in insulin resistance and liver fat storage [37]. The data are consistent with our finding of a significant association between high consumption of meat protein and increased risk of developing NAFLD.

This study had multiple strengths. First, this was the first investigation to examined the association between protein intake from different animal and plant origins and NAFLD. Second, we controlled major confounders that could possibly affect the associations. However, our study has some limitations. Although we adjusted our assessments to a wide range of potentially confounding variables, uncontrolled confounder variables including diet and other factors could not be ruled out. Furthermore, like all epidemiological studies, misclassification of study subjects owing to the use of FFQ is inevitable.

In conclusion, these findings replicating some of the previous studies suggest that the reduction of meat consumption along with higher intake of foods rich in vegetable, nut, and grain protein may decrease the risk of developing NAFLD.

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REFERENCES

1. Barbier-Torres L, Fortner KA, Iruzubieta P, Delgado TC, Giddings E, Chen Y, Champagne D, Fernández-Ramos D, Mestre D, Gomez-Santos B, Varela-Rey M, de Juan VG, Fernández-Tussy P, Zubiete-Franco I, García-Monzón C, González-Rodríguez Á, Oza D, Valença-Pereira F, Fang Q, Crespo J, Aspichueta P, Tremblay F, Christensen BC, Anguita J, Martínez-Chantar ML, Rincón M. Silencing hepatic MCJ attenuates non-alcoholic fatty liver disease (NAFLD) by increasing mitochondrial fatty acid oxidation. Nat Commun 2020;11:3360.
PUBMED | CROSSREF
2. Recena Aydos L, Aparecida do Amaral L, Serafim de Souza R, Jacobowski AC, Freitas Dos Santos E, Rodrigues Macedo ML. Nonalcoholic fatty liver disease induced by high-fat diet in C57bl/6 models. Nutrients 2019;11:3067.
PUBMED | CROSSREF
3. Drummen M, Dorenbos E, Vreugdenhil AC, Raben A, Fogelholm M, Westerterp-Plantenga MS, Adam TC. Long-term effects of increased protein intake after weight loss on intrahepatic lipid content and implications for insulin sensitivity: a PREVIEW study. Am J Physiol Endocrinol Metab 2018;315:E885-91.
PUBMED | CROSSREF
4. Zhou F, Zhou J, Wang W, Zhang XJ, Ji YX, Zhang P, She ZG, Zhu L, Cai J, Li H. Unexpected rapid increase in the burden of NAFLD in China from 2008 to 2018: a systematic review and meta-analysis. Hepatology 2019;70:1119-33.
PUBMED | CROSSREF
5. Jeznach-Steinhagen A, Ostrowska J, Czerwonogrodzka-Senczyna A, Boniecka I, Shahnazaryan U, Kuryłowicz A. Dietary and pharmacological treatment of nonalcoholic fatty liver disease. Medicina (Kaunas) 2019;55:166.
PUBMED | CROSSREF
6. Yki-Järvinen H, Luukkonen PK, Hodson L, Moore JB. Dietary carbohydrates and fats in nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 2021;18:770-86.
PUBMED | CROSSREF
7. Berná G, Romero-Gomez M. The role of nutrition in non-alcoholic fatty liver disease: pathophysiology and management. Liver Int 2020;40 Suppl 1:102-8.
PUBMED | CROSSREF
8. Glass O, Filozof C, Noureddin M, Berner-Hansen M, Schabel E, Omokaro SO, Schattenberg JM, Barradas K, Miller V, Francque S, Abdelmalek MF; Liver Forum Standard of Care Working Group. Standardisation of diet and exercise in clinical trials of NAFLD-NASH: recommendations from the liver forum. J Hepatol 2020;73:680-93.
PUBMED | CROSSREF
9. Ebrahimi Mousavi S, Dehghanseresht N, Dashti F, Khazaei Y, Salamat S, Asbaghi O, Mansoori A. The association between Dietary Diversity Score and odds of nonalcoholic fatty liver disease: a case-control study. Eur J Gastroenterol Hepatol 2022;34:678-85.
PUBMED | CROSSREF
10. Ebrahimi-Mousavi S, Alavian SM, Sohrabpour AA, Dashti F, Djafarian K, Esmaillzadeh A. The effect of daily consumption of probiotic yogurt on liver enzymes, steatosis and fibrosis in patients with nonalcoholic fatty liver disease (NAFLD): study protocol for a randomized clinical trial. BMC Gastroenterol 2022;22:102.
PUBMED | CROSSREF
11. Solga S, Alkhuraishe AR, Clark JM, Torbenson M, Greenwald A, Diehl AM, Magnuson T. Dietary composition and nonalcoholic fatty liver disease. Dig Dis Sci 2004;49:1578-83.
PUBMED | CROSSREF
12. Riazi K, Raman M, Taylor L, Swain MG, Shaheen AA. Dietary patterns and components in nonalcoholic fatty liver disease (NAFLD): what key messages can health care providers offer? Nutrients 2019;11:2878.
PUBMED | CROSSREF
13. Jang EC, Jun DW, Lee SM, Cho YK, Ahn SB. Comparison of efficacy of low-carbohydrate and low-fat diet education programs in non-alcoholic fatty liver disease: a randomized controlled study. Hepatol Res 2018;48:E22-9.
PUBMED | CROSSREF
14. Asghari G, Rezazadeh A, Hosseini-Esfahani F, Mehrabi Y, Mirmiran P, Azizi F. Reliability, comparative validity and stability of dietary patterns derived from an FFQ in the Tehran Lipid and Glucose Study. Br J Nutr 2012;108:1109-17.
PUBMED | CROSSREF

https://doi.org/10.7762/cnr.2023.12.1.29

15. Ebrahimi-Mameghani M, Behroozi-Fared-Mogaddam A, Asghari-Jafarabadi M. Assessing the reliability and reproducibility of food frequency questionnaire and identify major dietary patterns in overweight and obese adults in Tabriz, Iran. J Mazandaran Univ Med Sci 2014;23:46-57.
16. Kweon S, Kim Y, Jang MJ, Kim Y, Kim K, Choi S, Chun C, Khang YH, Oh K. Data resource profile: the Korea National Health and Nutrition Examination Survey (KNHANES). Int J Epidemiol 2014;43:69-77.
PUBMED | CROSSREF
17. Huang J, Liao LM, Weinstein SJ, Sinha R, Graubard BI, Albanes D. Association between plant and animal protein intake and overall and cause-specific mortality. JAMA Intern Med 2020;180:1173-84.
PUBMED | CROSSREF
18. Pasiakos SM, Agarwal S, Lieberman HR, Fulgoni VL 3rd. Sources and amounts of animal, dairy, and plant protein intake of US adults in 2007–2010. Nutrients 2015;7:7058-69.
PUBMED | CROSSREF
19. Aadahl M, Jørgensen T. Validation of a new self-report instrument for measuring physical activity. Med Sci Sports Exerc 2003;35:1196-202.
PUBMED | CROSSREF
20. Lang S, Martin A, Farowski F, Wisplinghoff H, Vehreschild MJGT, Liu J, Krawczyk M, Nowag A, Kretzschmar A, Herweg J, Schnabl B, Tu XM, Lammert F, Goeser T, Tacke F, Heinzer K, Kasper P, Steffen HM, Demir M. High protein intake is associated with histological disease activity in patients with NAFLD. Hepatol Commun 2020;4:681-95.
CROSSREF
21. Ashwell M, Hsieh SD. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. Int J Food Sci Nutr 2005;56:303-7.
PUBMED | CROSSREF
22. World Health Organization. Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008. Geneva: World Health Organization; 2011.
23. Chakravarthy MV, Waddell T, Banerjee R, Guess N. Nutrition and nonalcoholic fatty liver disease: current perspectives. Gastroenterol Clin North Am 2020;49:63-94.
PUBMED | CROSSREF
24. Ampong I, Watkins A, Gutierrez-Merino J, Ikwuobe J, Griffiths HR. Dietary protein insufficiency: an important consideration in fatty liver disease? Br J Nutr 2020;123:601-9.
PUBMED | CROSSREF
25. Charatcharoenwitthaya P, Tansakul E, Chaiyasoot K, Bandidniyamanon W, Charatcharoenwitthaya N. Dietary composition and its association with newly diagnosed nonalcoholic fatty liver disease and insulin resistance. Nutrients 2021;13:4438.
PUBMED | CROSSREF
26. Zhang S, Gu Y, Bian S, Górska MJ, Zhang Q, Liu L, Meng G, Yao Z, Wu H, Wang Y, Zhang T, Wang X, Sun S, Wang X, Zhou M, Jia Q, Song K, Qi L, Niu K. Dietary patterns and risk of non-alcoholic fatty liver disease in adults: a prospective cohort study. Clin Nutr 2021;40:5373-82.
PUBMED | CROSSREF
27. Tricò D, Biancalana E, Solini A. Protein and amino acids in nonalcoholic fatty liver disease. Curr Opin Clin Nutr Metab Care 2021;24:96-101.
PUBMED | CROSSREF
28. Markova M, Pivovarova O, Hornemann S, Sucher S, Frahnow T, Wegner K, Machann J, Petzke KJ, Hierholzer J, Lichtinghagen R, Herder C, Carstensen-Kirberg M, Roden M, Rudovich N, Klaus S, Thomann R, Schneeweiss R, Rohn S, Pfeiffer AF. Isocaloric diets high in animal or plant protein reduce liver fat and inflammation in individuals with type 2 diabetes. Gastroenterology 2017;152:571-585.e8.
PUBMED | CROSSREF
29. Jia Q, Xia Y, Zhang Q, Wu H, Du H, Liu L, Wang C, Shi H, Guo X, Liu X, Li C, Sun S, Wang X, Zhao H, Song K, Huang G, Wu Y, Cui N, Niu K. Dietary patterns are associated with prevalence of fatty liver disease in adults. Eur J Clin Nutr 2015;69:914-21.
PUBMED | CROSSREF
30. Alferink LJ, Erler NS, de Knegt RJ, Janssen HL, Metselaar HJ, Darwish Murad S, Kiefte-de Jong JC. Adherence to a plant-based, high-fibre dietary pattern is related to regression of non-alcoholic fatty liver disease in an elderly population. Eur J Epidemiol 2020;35:1069-85.
PUBMED | CROSSREF
31. De Chiara F, Ureta Checcllo C, Ramón Azcón J. High protein diet and metabolic plasticity in non-alcoholic fatty liver disease: myths and truths. Nutrients 2019;11:2985.
PUBMED | CROSSREF

https://doi.org/10.7762/cnr.2023.12.1.29

32. Okita M, Watanabe A, Nagashima H. A vegetable protein-rich diet for the treatment of liver cirrhosis. Acta Med Okayama 1985;39:59-65.
PUBMED

33. McCarthy EM, Rinella ME. The role of diet and nutrient composition in nonalcoholic fatty liver disease. J Acad Nutr Diet 2012;112:401-9.
PUBMED | CROSSREF

34. Galarregui C, Cantero I, Marin-Alejandre BA, Monreal JI, Elorz M, Benito-Boillos A, Herrero JI, de la O V, Ruiz-Canela M, Hermsdorff HH, Bressan J, Tur JA, Martínez JA, Zulet MA, Abete I. Dietary intake of specific amino acids and liver status in subjects with nonalcoholic fatty liver disease: Fatty Liver in Obesity (FLiO) study. Eur J Nutr 2021;60:1769-80.
PUBMED | CROSSREF

35. Cocate PG, Natali AJ, de Oliveira A, Alfenas RC, Peluzio MC, Longo GZ, dos Santos EC, Buthers JM, de Oliveira LL, Hermsdorff HH. Red but not white meat consumption is associated with metabolic syndrome, insulin resistance and lipid peroxidation in Brazilian middle-aged men. Eur J Prev Cardiol 2015;22:223-30.
PUBMED | CROSSREF

36. Kaenkumchorn TK, Merritt MA, Lim U, Le Marchand L, Boushey CJ, Shepherd JA, Wilkens LR, Ernst T, Lampe JW. Diet and liver adiposity in older adults: the multiethnic cohort adiposity phenotype study. J Nutr 2021;151:3579-87.
PUBMED | CROSSREF

37. Fakhoury-Sayegh N, Younes H, Heraoui GN, Sayegh R. Nutritional profile and dietary patterns of lebanese non-alcoholic fatty liver disease patients: a case-control study. Nutrients 2017;9:1245.
PUBMED | CROSSREF

https://doi.org/10.7762/cnr.2023.12.1.29