Eur J Nutr
DOI 10.1007/s00394-014-0679-y

ORIGINAL CONTRIBUTION

The impact of cereal grain consumption on the development and severity of non-alcoholic fatty liver disease

Michael Georgoulis · Meropi D. Kontogianni · Nafsika Tileli · Aikaterini Margariti · Elisabeth Fragopoulou · Dina Tiniakos · Rodessa Zafiropoulou · George Papatheodoridis

Received: 2 September 2013 / Accepted: 26 February 2014

Abstract
Purpose There is evidence that dietary habits contribute to the presence and severity of non-alcoholic fatty liver disease (NAFLD). The aim of the present study was to explore any associations between consumption of grains and the development and severity of NAFLD.
Methods Seventy-three consecutive NAFLD patients were enrolled. Additionally, 58 controls matched for age, sex and body mass index with 58 patients were also included. Consumption of grains was estimated through a semi-quantitative food frequency questionnaire. Medical history, anthropometric indices, body composition analysis, physical activity data, biochemical and inflammatory markers were available for all the participants. Liver stiffness measurement by transient elastography was performed in 58 and liver biopsy in 34 patients.
Results In patients, consumption of whole grains was associated with lower abdominal fat level ($\beta = -0.24$, $p = 0.02$) and lower levels of insulin resistance index ($\beta = -0.28$, $p = 0.009$), while it also correlated inversely with interleukin-6 levels ($\rho = -0.23$, $p = 0.05$). Consumption of whole grains was associated with lower likelihood of having histological steatohepatitis (OR 0.97, 95 % CI 0.94–1.000), after adjusting for sex and energy intake, but the association became weaker after further adjusting for abdominal fat or interleukin-6 levels. In the case–control analysis, consumption of refined grains was associated with higher odds of having NAFLD (OR 1.021, 95 % CI 1.001–1.042), after adjusting for age, sex, energy intake, abdominal fat level, HOMA-IR, LDL, adiponectin and TNF-α.
Conclusions Although refined grain consumption increased the likelihood of having NAFLD, whole-grain consumption favorably affected clinical characteristics of patients with NAFLD and tended to be associated with less severe disease.

Keywords Non-alcoholic fatty liver · Cereals · Inflammation · Insulin resistance · Case–control

M. Georgoulis · M. D. Kontogianni (✉) · N. Tileli · E. Fragopoulou
Department of Nutrition and Dietetics, Harokopio University, El. Venizelou 70, Kallithea, 17671 Athens, Greece
e-mail: mkont@hua.gr

A. Margariti · R. Zafiropoulou · G. Papatheodoridis
2nd Department of Internal Medicine, Hippokration Hospital of Athens, University of Athens Medical School, Athens, Greece

D. Tiniakos
Laboratory of Histology & Embryology, Medical School, National & Kapodistrian University of Athens, Athens, Greece

G. Papatheodoridis
Department of Gastroenterology, Laiko Hospital of Athens, University of Athens Medical School, Athens, Greece

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a pathological entity characterized by triglyceride accumulation in the liver, which is not due to excess alcohol consumption [1]. Non-alcoholic steatohepatitis (NASH) represents the necroinflammatory phase of the disease, which can lead to advanced liver fibrosis, cirrhosis and hepatocellular carcinoma [1]. NAFLD is considered as the hepatic manifestation of the metabolic syndrome, with insulin resistance being the most prevailing pathogenetic mechanism, while oxidative stress, inflammatory cytokines and mitochondrial dysfunction appear to contribute to the development of

Published online: 07 March 2014

NASH [1–4]. While previously considered as a mild condition, NAFLD is currently recognized as the most common cause of elevated liver enzymes and a major cause of liver-related mortality in Western countries [1, 5].

The pathogenesis of NAFLD is multifactorial, involving a complex interaction of genetic factors, metabolic disorders and lifestyle characteristics [1]. Nutrition and physical activity consist the most important modifiable environmental factors associated with both the development and severity of the liver disease. Overweight has been consistently shown to be an independent risk factor for NAFLD [6]. Moreover, the role of diet's composition in the development and progression of NAFLD is currently under investigation. Although several epidemiological studies have reported associations between dietary intake of specific nutrients and risk for NAFLD [7], evidence regarding the role of diet's composition, in terms of foods or food groups, in the development of the disease is still sparse.

Cereal grains constitute a significant part of the human diet and an important source of essential macro- and micronutrients. The association between consumption of grains and health outcomes has been extensively investigated, and it is noteworthy that refined and whole grains appear to have different effects on health indices, probably due to the effect of processing on their nutritional value. Although high refined grain intake has been cross-sectionally linked to several metabolic abnormalities such as dyslipidemia, insulin resistance, arterial hypertension and increased body weight and/or abdominal fat level, the majority of the available data from prospective cohort studies show no significant associations between refined grain intake and the risk of cardiovascular and metabolic diseases or overall mortality [8]. On the other hand, whole-grain intake has been consistently linked to favorable health outcomes, such as reduced risk of developing obesity, type 2 diabetes and cardiovascular disease, as well as blood lipid improvement, increased insulin sensitivity and decreased inflammatory biomarkers [9, 10].

To our knowledge, there is little evidence regarding the association between consumption of grains and development or severity of NAFLD. In a recent review of the evidence supporting that whole-grain intake can protect against several risk factors and comorbidities associated with NAFLD, Ross et al. [11] concluded that the replacement of refined grains with whole grains should be included in the dietary guidelines for patients with NAFLD and individuals at risk for developing the disease. However, there is currently no direct evidence that a diet rich in whole grains may help to prevent NAFLD and/or be a part of its treatment. Hence, the aim of the present study was to explore any potential associations between consumption of cereal grains and the clinical or histological characteristics of patients with NAFLD. A control group was also stratified to test any impact of cereal grain consumption on the development of NAFLD.

Materials and methods

Seventy-three consecutive adult (18–65 years old) patients with newly diagnosed (within 6 months) NAFLD, who visited the outpatient liver clinics of the second Academic Department of Internal Medicine at Hippokration General Hospital of Athens between May 2009 and December 2010, were enrolled. NAFLD diagnosis was based on the following criteria: elevated alanine aminotransferase (ALT) and/or gamma-glutamyl transpeptidase (GGT) levels, evidence of hepatic steatosis on ultrasound and/or compatible liver histology and no other cause of liver injury or steatosis. In particular, all patients should have had negative serological markers for hepatitis B (HBsAg), hepatitis C (anti-HCV) and human immunodeficiency virus (anti-HIV), weekly alcohol consumption less than 210 g) for men or 140 g) for women, no use of potentially hepatotoxic agents, no evidence of metabolic or autoimmune liver disease, and absence of any known systemic disease with potential liver involvement. In addition, patients who had changed their eating habits since NAFLD diagnosis, those following a weight loss diet and those with diabetes mellitus or any diagnosed malignancy were excluded from the study. Medical records were thoroughly reviewed, and laboratory data were recorded, namely complete blood count, prothrombin time, uric acid, urea, creatinine, liver enzymes [ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), GGT, total protein, albumin], serum copper, ceruloplasmin, iron and ferritin, as well as detection of HBsAg, anti-HBc, anti-HBs, anti-HCV, anti-HIV and liver autoantibodies (anti-nuclear, anti-smooth muscles, anti-microsomal, anti-mitochondrial). The history of alcohol use was taken from the patients and was confirmed by the patients' relatives or friends. Blood pressure was measured in a sitting position, and hypertension was defined as systolic blood pressure $\geq$140 mmHg and or diastolic blood pressure $\geq$90 mmHg.

Fifty-eight control subjects matched for age, sex and body mass index (BMI) with 58 of the aforementioned 73 patients were also enrolled. Controls were either subjects who visited the outpatients departments of the same hospital and at the same period for routine examinations or people working at the hospital and the universities conducted the study. Controls had normal glucose metabolism and liver enzymes, no evidence of hepatic steatosis at ultrasonography and stable dietary and exercise habits during the last year.

The study was approved by the Ethics Committee of the Hippokration General Hospital of Athens and by the Ethics

Committee of Harokopio University and was carried out in accordance with the Declaration of Helsinki [12]. All participants were informed about the aims and procedures of the study and gave their written consent.

Lifestyle assessment

Participants' (both cases and controls) habits such as smoking, alcohol consumption and reception of any medication were recorded.

Habitual dietary intake of the participants over the last 12 months was assessed through a semi-quantitative food frequency questionnaire (FFQ) enriched with foods and beverages commonly consumed in Greece [13]. Based on the FFQ data, dietary intake of cereal grains (total, refined and whole grains) was expressed as servings per week, using serving sizes provided in the dietary guidelines for Greek adults (e.g., one serving was equal to a 30-g) slice of bread, 2 rusks, 1/2 cup of breakfast cereals, 1/2 cup of cooked pasta or rice) [14]. Given that grains typically consumed in Greece are wheat mainly in the form of bread (but also flour, pastas, pitas, crackers and breakfast cereals) and rice [14, 15], refined grains assessed included white bread, pita bread, white crisp bread and rusks, white pasta, white rice, refined breakfast cereals and pastries, while whole grains assessed included whole-grain bread, whole-grain crisp bread and rusks, whole-grain pasta, brown rice and whole-grain breakfast cereals. Whole-grain definition was based on that developed by the HEALTHGRAIN Consortium and presented in the 2010 Final HEALTHGRAIN Conference in Lund (http://lund2010.healthgrain.org), which is similar to the prior definition of the American Association of Cereal Chemists in 1999, but is more in line with current industrial production practices. Percentages of both refined and whole-grain intakes were also calculated (e.g., whole grain:total grain intake $\times$ 100), as well as gram(s) of daily whole-grain products consumption, after multiplying the number of food servings consumed with the amount of gram(s) each food serving contained. Daily energy intake of the participants was also estimated through three non-consecutive 24-h dietary recalls, including 2 weekdays and 1 weekend day. Each dietary recall was analyzed using Nutritionist Five (Axxya Systems, USA).

Physical activity level of the participants was assessed through a questionnaire that has been previously used in adults [16]. Briefly, the questionnaire recalls previous day's physical activities by recording the duration, the type and the intensity of each activity, as well as time spent daily in television viewing or computer use. Based on this information, daily time spent in moderate and vigorous activities (structured or not) and in sedentary activities was calculated. Participants were also given a pedometer (Digi-Walker, Yamax, SW-200, Japan) for a 7-day period (including weekend days), and each participant was asked to record his/her daily steps according to the measurements appearing on the pedometer screen.

Anthropometric and body composition assessment

Body weight of participants was measured with digital scale (Seca Robusta 813, Hamburg, Germany), to the nearest 100 g and height to the nearest 0.5 cm. BMI was calculated as weight (kg) divided by height squared (m^2). Waist circumference (WC) was tape measured to the nearest 0.1 cm. Increased WC was defined as >102 cm for men and >88 cm for women. Abdominal fat compartments, namely trunk fat% and abdominal fat level, were estimated by abdominal bioelectrical impedance analysis (Tanita Viscan AB140, Japan) [17].

Biochemical and inflammatory markers

Fasting blood samples were collected following a 12-h fast, and the obtained serum and plasma samples were immediately frozen at −80 °C. Glucose, total cholesterol and high-density lipoprotein (HDL) cholesterol were measured using enzymatic colorimetric method (analyzer Cobas 8000, Roche), and low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [18]. Triglycerides were measured with chromatometric enzymatic method (analyzer Cobas 8000, Roche) and insulin with chemiluminescence (Centaur analyzer, Siemens). Patients were considered to have dyslipidemia, if they met at least one of the following criteria: (1) total cholesterol >5.2 mmol/L, (2) LDL cholesterol >3.1 mmol/L, (3) triglycerides >1.7 mmol/L at the time of diagnosis or during follow up or (4) treated with lipid lowering medication. The insulin resistance index HOMA-IR (Homeostasis Model of Assessment-Insulin Resistance) was calculated using the formula by Matthews et al. [19]. ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP) and GGT were retrieved from patients' medical records and measured for the controls with routine commercially available assays. High-sensitivity CRP (hsCRP) was measured using a nephelometric assay (BN II® nephelometer, Siemens). Tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), interleukin 8 (IL-8) and adiponectin were measured by sensitivity enzyme-linked immunosorbent assay (ELISA, Quantikine/immunoassay kit, R&D Systems, Minneapolis, MN, USA). The intra-assay coefficient of variation was <7 % for TNF-α and IL-8 and <5 % for IL-6 and adiponectin. The inter-assay coefficient of variation was <8 % for TNF-α, IL-6 and adiponectin and <10 % for IL-8.

Transient elastography

Reliable liver stiffness measurements (LSM) (in kPa) by transient elastography (FibroScan®, Echosens, France)

were available in 58 of the 73 patients. The examination was considered to be reliable, if 10 successful measurements were obtained, with a success rate >60 % and a ratio of interquartile range (IQR) to mean stiffness <30 %. For patients who underwent both transient elastography and liver biopsy, LSM was performed a few hours before liver biopsy in most or within 4 weeks before or after liver biopsy in some patients. In any case, LSM was performed within the first 4 weeks from the diagnosis of NAFLD.

Liver histology

Adequate liver biopsies were available in 34 of the 73 patients who consented to undergo liver biopsy. They were evaluated by a single hepatopathologist (D.T.), who was blinded to the clinical data. A liver biopsy was considered to be adequate, if at least 6 portal tracts were identified and the specimen length was ≥1.5 cm. The diagnosis of NASH was based on the overall pattern of injury and the criteria of Brunt et al. [20] modified by Kleiner et al. [21]. Global grade of necroinflammatory activity and stage of fibrosis were assessed according to Brunt et al. [20]. Severity of steatosis and NAFLD activity score (NAS) were evaluated according to Kleiner et al. [21].

Statistical analysis

Continuous variables are presented as mean values ± standard deviation and categorical variables as absolute frequencies. The normality of the data was assessed graphically using histog. Correlations between consumption of grains and anthropometric, biochemical and histological parameters were tested using the Spearman correlation coefficient. Contingency tables and the calculation of Chi-squared test, two-sample *t* test or Mann–Whitney *U* test were used appropriate to test differences between patients and controls. Mann–Whitney *U* test for 2 samples was used to test differences among patients with NASH and simple fatty liver. Multiple linear regression analysis was applied to explore the association between consumption of grains and degree of insulin resistance or abdominal fat level, after controlling for several potential confounders. HOMA-IR index was log-transformed to become normal and fit in the multiple linear regression model. Standardized residuals were used to test model's goodness of fit. Logistic regression analysis was used to estimate the association between consumption of grains and likelihood of obesity, increased sex-specific WC values, NAFLD and NASH. All reported *p* values were based on two-sided tests and compared to a significance level of 5 %. Statistical Package for Social Sciences software (version 18.0, SPSS 2009, Chicago, Illinois, USA) was used for all the statistical calculations.

Table 1 Descriptive characteristics of 73 patients with NAFLD

Gender—males, *n* (%)	50 (68.5)
Age (years)	45.4 ± 11.3
Smoking habits—current smokers, *n* (%)	20 (27.4)
Body mass index (kg/m^2)	29.7 ± 4.2
Waist circumference (cm)	
Males	103 ± 9
Females	102 ± 12
Increased waist circumference[a], *n* (%)	45 (61.6)
Trunk fat (%)	39.6 ± 7.7
Abdominal fat level (1–59)	16.4 ± 5.1
Number of steps per day	6957 ± 3409
Sedentary activities (h/day)	3.07 ± 1.70
Cereal grain intake	
Total grains, servings/week	62.9 ± 31.9
Refined grains, servings/week	55.4 ± 32.1
Refined grains, % of total	84.6 ± 20.6
Whole grains, servings/week	7.5 ± 10.5
Whole grains (g/day)	31.2 ± 44.0
Whole grains, % of total	15.4 ± 20.6
Clinical characteristics	
Systolic blood pressure (mmHg)	129 ± 15
Diastolic blood pressure (mmHg)	84.9 ± 11
Hypertension, *n* (%)	17 (23.3)
Total cholesterol (mmol/L)	5.38 ± 1.13
LDL cholesterol (mmol/L)	3.79 ± 1.07
HDL cholesterol (mmol/L)	0.97 ± 0.46
Triglycerides (mmol/L)	1.34 ± 0.64
Dyslipidemia, *n* (%)	56 (76.7)
ALT (IU/L)	77.3 ± 47.6
AST (IU/L)	42.3 ± 21.1
GGT (IU/L)	94.1 ± 116.3

Data are presented as mean ± SD or otherwise mentioned

NAFLD non-alcoholic fatty liver disease, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma-glutamyl transferase

[a] >102 cm for males and >88 cm for females

Results

Patients' characteristics

Patients' main characteristics are presented in Table 1. Their mean age was 45 years, whereas the majority (69 %) of the patients was males. Mean total cereal grain consumption was 63 servings per week, 88 % of which was refined. Mean dietary fiber intake was 17 g)/day. The mean liver stiffness in 58 patients who underwent transient elastography was 7.6 ± 4.9 kPa. In the 34 patients with available liver biopsies, the mean grade of necroinflammation was 1.1 ± 0.9, the mean stage of fibrosis 1.4 ± 1.2

and mean NAFLD activity score 4.0 ± 2.1. Of these 34 patients, 11 (32 %) cases were diagnosed to have simple fatty liver and 23 (68 %) cases to have NASH.

Associations between patients' cereal grain consumption and anthropometric indices

Consumption of whole-grain products expressed either as g/day or servings/week correlated negatively with BMI ($\rho = -0.26$ and $\rho = -0.25$, respectively, both $p = 0.03$) and WC values ($\rho = -0.30$, $p = 0.009$ and $\rho = -0.31$, $p = 0.008$, respectively). The association between consumption of grains and the likelihood of being obese (BMI $\geq$ 30 kg/m^2) was further explored using logistic regression analysis. According to the results, consumption of whole-grain products (g/day) was associated with lower likelihood of being obese (OR 0.97, 95 % CI 0.95–0.99, $p = 0.02$), after adjusting for age, sex, daily energy intake and daily time spent in sedentary activities. This association remained unchanged when whole grains were expressed either as servings/week or % of total grain intake. Consumption of whole-grain products (g/day) was also negatively associated with the likelihood of increased sex-specific WC values, after adjusting for the same confounding factors (OR 0.98, 95 % CI 0.96–0.99, $p = 0.04$).

Regarding abdominal fat level, % of refined grains correlated positively ($\rho = 0.24$ and 0.25, both $p = 0.04$) while % of whole-grain consumption correlated negatively with abdominal fat level ($\rho = -0.25$, $p = 0.04$). In a multiple linear regression model with abdominal fat level as the dependent variable, whole-grain products consumption (g)/day) was a significant predictor (standardized beta coefficient = -0.24, $p = 0.02$) after adjusting for age, sex, daily energy intake and daily time spent in sedentary activities, whereas total grains or refined grains were not significantly associated with abdominal fat level ($p > 0.45$).

Associations between patients' cereal grain consumption and laboratory parameters

Regarding biochemical variables, total consumption of grains (servings/week) correlated negatively with GGT ($\rho = -0.24$, $p = 0.05$), total cholesterol ($\rho = -0.32$, $p = 0.007$) and LDL cholesterol levels ($\rho = -0.34$, $p = 0.004$). Consumption of refined grains (servings/week) also correlated negatively with total cholesterol ($\rho = -0.31$, $p = 0.008$) and LDL cholesterol ($\rho = -0.34$, $p = 0.004$), while it correlated positively with insulin levels ($\rho = 0.32$, $p = 0.007$) and HOMA-IR ($\rho = 0.29$, $p = 0.01$). Consumption of whole grains (servings/week) correlated negatively with glucose levels ($\rho = -0.32$, $p = 0.006$), insulin levels ($\rho = -0.43$, $p < 0.001$) and HOMA-IR ($\rho = -0.47$, $p < 0.001$), while it tended to correlate negatively with TG levels ($\rho = -0.21$, $p = 0.08$), and these correlations remained significant when whole-grain intake was expressed as g/day.

Table 2 Multiple regression analysis model, exploring the association between consumption of cereal grains and insulin resistance ($N = 73$)

	logHOMA-IR		
	R^2 adjusted[a]	Standardized beta coefficient	p
Total grains (servings/week)	0.307	0.04	0.73
Refined grains (servings/week)	0.324	0.15	0.21
Whole grains (g/day)	0.387	−0.28	0.009

HOMA-IR homeostasis model of assessment—insulin resistance

[a] After adjustment for age, sex, daily energy intake, abdominal fat level and adiponectin levels

Regarding the inflammatory markers assessed in the present study, there were inverse correlations between total grain or refined grain consumption (servings/week) and adiponectin levels ($\rho = -0.33$ or -0.34, $p \leq 0.005$) and between consumption of whole grains expressed either as servings/week or g/day and IL-6 levels ($\rho = -0.24$ and $\rho = -0.23$, respectively, all $p < 0.05$), while consumption of refined grains (servings/week) correlated positively with IL-6 levels ($\rho = 0.25$, $p = 0.03$).

Given the involvement of insulin resistance in NAFLD pathogenesis, the association between consumption of cereal grains and HOMA-IR was also explored using multiple linear regression analysis. Whole-grain products consumption (g/day) was negatively associated with log-HOMA-IR after adjusting for factors that also correlated with HOMA-IR, namely age, sex, daily energy intake, abdominal fat level and adiponectin levels (Table 2). Abdominal fat and adiponectin levels were additional significant predictors (standardized beta coefficient = 0.52, $p < 0.001$ and standardized beta coefficient = -0.27, $p = 0.02$, respectively) in this model. When whole-grain consumption was expressed as servings/week, results remained practically unchanged (standardized beta coefficient = -0.29, $p = 0.006$).

Associations between patients' cereal grain consumption and liver stiffness or histological parameters

No significant correlations were observed between consumption of grains (total, refined or whole grains) and liver stiffness measurement or histological parameters in patients with available transient elastography and/or liver

Table 3 Logistic regression analysis models, exploring the association between consumption of cereal grains and the likelihood of NASH, based on liver biopsy data ($N = 34$)

	Model 1[a]			Model 2[b]			Model 3[c]		
	OR	95 % CI	*p*	OR	95 % CI	*p*	OR	95 % CI	*p*
Total grains (servings/week)	0.999	0.970–1.029	0.95	1.019	0.982–1.057	0.31	0.975	0.939–1.013	0.19
Refined grains (servings/week)	1.018	0.986–1.050	0.27	1.029	0.993–1.067	0.12	1.000	0.965–1.037	0.99
Whole grains (g/day)	0.969	0.938–1.000	0.05	0.969	0.932–1.008	0.113	0.973	0.943–1.004	0.08

NASH non-alcoholic steatohepatitis, *OR* odds ratio, *CI* confidence interval

[a] Model 1: adjusted for sex and daily energy intake

[b] Model 2: adjusted for sex, daily energy intake and abdominal fat level

[c] Model 3: adjusted for sex, daily energy intake and IL-6 levels

biopsy data (data not shown). Among the 34 patients with liver biopsies, cases with NASH compared to those with simple fatty liver reported lower consumption of whole grains (2.83 ± 5.27 vs. 13.59 ± 15.93 servings/week, $p = 0.03$ or 12.1 ± 22.5 vs. 52.8 ± 66.5 g/day, $p = 0.04$), but there were no significant differences between the two groups in the total consumption of grains (64.86 ± 29.32 vs. 59.95 ± 35.48 servings/week, $p = 0.51$), consumption of refined grains (62.03 ± 31.37 vs. 46.36 ± 32.09 servings/week, $p = 0.15$) or consumption of other food groups assessed (data not shown).

According to logistic regression analysis, a one gram per day increase in the consumption of whole-grain products was associated with 3 % lower likelihood of having NASH, after adjusting for sex and daily energy intake (Table 3). Results were similar when whole-grain intake was expressed as servings/week (OR 0.86, 95 % CI 0.74–0.99, $p = 0.05$). After further adjusting for abdominal fat level (Model 2, Table 3) or IL-6 levels (Model 3, Table 3), the aforementioned associations showed only a trend toward significance.

Comparisons between participants with NAFLD and matched controls

Comparisons in several descriptive characteristics between the 58 patients matched with 58 controls are presented in Table 4. Although BMI did not differ between the two groups, patients showed a trend for increased waist circumference and exhibited higher abdominal fat level ($p = 0.008$). Regarding dietary intake, patients reported higher consumption of total and refined grains (both $p = 0.02$) and less daily alcohol intake ($p = 0.05$), although both groups reported low alcohol consumption. No other differences in food groups intakes were observed apart from nuts' consumption with patients reporting lower intakes than controls (3.0 ± 3.9 vs. 6.5 ± 10.9 servings/week, $p = 0.03$). Mean daily dietary fiber intake was also found to be similar between patients and matched controls (16.3 ± 6.9 vs. 17.3 ± 10.5 g/day, respectively, $p = 0.76$). Differences between the two groups were observed in several biochemical markers. Briefly, patients as expected had higher levels of ALT, AST and GGT (all $p < 0.001$), as well as higher levels of LDL, triglycerides, HOMA-IR, TNF-a (all $p \leq 0.001$) and

Table 4 Comparisons between patients with non-alcoholic fatty liver disease (NAFLD) and matched controls

	NAFLD ($N = 58$)	Controls ($N = 58$)	p[a]
Age (years)	44.5 ± 11.6	$44.6 \pm 12.$	0.96
Gender—males, *n* (%)	36 (62.1)	36 (62.1)	>0.99
Body Mass Index (kg/m^2)	28.7 ± 3.9	27.7 ± 3.6	0.15
Waist circumference (cm)			
Males	99 ± 7	97 ± 8	0.15
Females	101 ± 7	95 ± 7	0.07
Abdominal fat level (1–59)	15.0 ± 4.4	12.5 ± 5.1	0.008
Current smokers, *n* (%)	14 (24)	26 (45)	0.031
Number of steps per day	$7{,}076 \pm 3{,}178$	$7{,}371 \pm 3{,}401$	0.67
Sedentary activities (h/day)	2.8 ± 1.6	2.7 ± 1.4	0.66
Cereal grain intake			
Total grains (servings/week)	61.5 ± 31.9	48.8 ± 23.6	0.02
Refined grains (servings/week)	52.9 ± 31.7	40.9 ± 23.5	0.02
Refined grains (% of total grains)	82.1 ± 21.9	82.8 ± 19.8	0.83
Whole grains (servings/week)	8.7 ± 11.2	8.0 ± 9.4	0.70
Whole grains (g/day)	36.0 ± 47.0	35.8 ± 43.3	0.57
Whole grains (% of total grains)	17.9 ± 21.9	17.1 ± 19.8	0.83
Alcohol intake (g/day)	8.0 ± 11.5	13.4 ± 11.8	0.05

Data are presented as mean ± SD or frequencies

[a] Probability values as derived by Chi-squared test, two-sample *t* test or Mann–Whitney *U* test

Table 5 Logistic regression analysis models, exploring the association between consumption of cereal grains and the likelihood of the presence of NAFLD ($N = 58$ cases and 58 controls)

	Model 1[a]			Model 2[b]			Model 3[c]		
	OR	95 % CI	*p*	OR	95 % CI	*p*	OR	95 % CI	*p*
Total grains (servings/week)	1.024	1.006–1.042	0.01	1.022	1.003–1.041	0.02	1.024	1.004–1.044	0.02
Refined grains (servings/week)	1.022	1.004–1.039	0.02	1.019	1.001–1.037	0.04	1.021	1.001–1.042	0.04
Whole grains (g/day)	1.001	0.993–1.010	0.74	1.003	0.994–1.011	0.56	1.003	0.992–1.013	0.64

NAFLD non-alcoholic fatty liver disease, *OR* odds ratio, *CI* confidence interval, *HOMA-IR* homeostasis model of assessment of insulin resistance, *LDL* low-density lipoprotein, *TNF-α* tumor necrosis factor-α

[a] Model 1: adjusted for age, sex and daily energy intake

[b] Model 2: adjusted for age, sex, daily energy intake and abdominal fat level

[c] Model 3: adjusted for age, sex, daily energy intake, abdominal fat level, HOMA-IR, LDL, adiponectin and TNF-α levels

hsCRP ($p = 0.03$), whereas they had lower levels of HDL and adiponectin ($p < 0.001$ and 0.007, respectively).

The age-, sex- and energy intake-adjusted odds ratio of having NAFLD based on total grain consumption was 1.024 meaning that one serving increase in weekly consumption was associated with 2.4 % higher likelihood of having the disease (Table 5). Similar were the findings for refined grain consumption (OR 1.022), while there was no significant association between consumption of whole grains expressed as either g/day or servings/week and the likelihood of NAFLD. We also adjusted for other variables that varied between patients and controls, namely abdominal fat level (Model 2, Table 5) and HOMA-IR, LDL, adiponectin and TNF-α (Model 3, Table 5), but the odds ratios of having NAFLD based on total and refined grain consumption remained unchanged.

Discussion

In the present study, potential associations between consumption of cereal grains and development or severity of NAFLD were explored. According to our results, higher intakes of both total and refined grains were associated with higher likelihood of having NAFLD in the case–control analysis. Furthermore, higher intake of whole grains was associated with lower likelihood of having NASH; however, this association was weakened after adjusting for abdominal fat level or IL-6 levels.

So far there is little evidence regarding the association between consumption of grains and the development or severity of NAFLD. Results from a recent case–control study with a sample of 200 university faculty members and staff suggested that NAFLD patients consumed more candies and pastries and less coarse cereals compared to healthy controls [22]. Another cross-sectional study, aiming at comparing dietary habits of 18 patients with simple fatty liver and 28 with NASH, showed that NASH patients exhibited higher intake of sweets and lower intake of cereals [23]. In accordance with the evidence on other metabolic diseases, the aforementioned data propose that the absolute and/or relative consumption of grains with a different processing degree, namely refined and whole grains, may be more important for NAFLD development and progression than the total amount of grains consumed.

A high refined grain intake has been associated with higher risk of type 2 diabetes and higher cardiovascular risk. The suggested underline mechanisms include the enhanced insulin resistance due to their low fiber intake and sometimes due to their higher glycemic index, as well as an increase in very low-density lipoprotein hepatic synthesis and lipoprotein lipase activity, leading to decreased HDL and increased triglycerides, main components of the metabolic syndrome [24]. Furthermore, a diet rich in refined grains has been linked to higher inflammatory load (namely higher CRP and IL-18 levels and lower adiponectin levels) [25]. Although the pathogenesis of NAFLD is not entirely elucidated, overweight, increased visceral adiposity with production of adipocytokines and hormones, increased lipolysis and flow of free fatty acids to the liver have been proposed as starting events leading to the development of insulin resistance [26]. Therefore, given our results and the fact that refined grains were associated with higher likelihood of having NAFLD, one could speculate that refined grain intake can affect the mechanisms leading to the "first hit" of the liver, namely increased influx of free fatty acids and insulin resistance and hence the development of simple steatosis.

In addition, increased whole-grain consumption has been consistently linked to health benefits, including a reduced risk for prediabetes (impaired glucose tolerance and/or impaired fasting glucose) [27], type 2 diabetes [28] and metabolic syndrome [29], cardiovascular disease and weight gain [10], as well as to lower total body and abdominal fat mass and lower subcutaneous and visceral adipose tissue levels [30–32]. The beneficial role of whole

grains in health outcomes has been attributed to the synergistic effect of their nutrient and non-nutrient compounds, namely dietary fiber, minerals, vitamins, phenolic compounds, phytoestrogens, methyl donors and lipotropes, as well as plant sterols and stanols [33]. Although the potential underline mechanisms remain partly elucidated, whole grains increase the rate of gastric emptying and insulin sensitivity through increased anti-inflammatory effects and even changes in gut microbiota [28, 34]. Regarding NAFLD pathogenesis, after the development of simple steatosis, the liver becomes vulnerable to possible "secondary hits" which may be involved in the progression to NASH, namely oxidative stress, mitochondrial dysfunction, imbalance of production of adipocytokines and production of endotoxins and endogenous ethanol from the intestinal microflora [26, 35]. Therefore, whole grains could favorably affect the severity of NAFLD through several mechanisms, including their beneficial effect on intestinal microbiota, insulin resistance and subclinical inflammation. Evidence from both epidemiological and clinical studies suggests improved blood glucose control in patients with diabetes, as well as improved insulin sensitivity in non-diabetic individuals consuming high amounts of whole grains [10, 28], which was also true for subjects with NAFLD participating in the current study. Regarding inflammatory biomarkers, consumption of whole grains has been associated with decreased circulating concentrations of CRP [9] and IL-6 levels [36, 37] and increased adiponectin levels [38]. This beneficial effect of whole grains on inflammatory biomarkers could also explain the fact that the negative association between consumption of whole grains and the likelihood of NASH observed in the present study was weakened after adjusting for abdominal fat or for IL-6 levels, suggesting a possible mediating effect of abdominal fat and the cytokines it produces, on the likelihood of NASH development.

To our best knowledge, this is the first study focusing on cereal grain intake and its association with the development and severity of NAFLD. Among the limitations of our work, it should be mentioned that dietary assessment through a FFQ is susceptible to measurement errors, such as recall bias. However, correlations between total and whole-grain intake as assessed by the FFQ and the 24-h recalls applied in the study were explored and coefficients were 0.52 and 0.40 (both $p < 0.001$) for whole- and total-grain intake, respectively. Furthermore, the use of biomarkers such as plasma alkylresorcinols could have been useful for assessing the accuracy of the 24-h recalls in terms of whole-grain intake. A more detailed estimation of the whole-grain products consumed, based on the % of whole grains contained, could lead to more accurate estimation of the actual gram(s) of whole grains consumed and could allow even recommendations for the optimal whole-grain consumption. In addition, the cross-sectional nature of our study does not allow us to make a causal inference or elucidate underlying mechanisms and although we carefully adjusted for several confounders in our analyses, we cannot rule out the possibility of residual confounding. For example, whole-grain consumption could probably reflect a healthier lifestyle in general that is difficult to assess and correct for in statistical analyses. Finally, the sample size of patients with liver biopsies was rather small and not representing the more advanced stages of liver disease.

In conclusion, our results support the detrimental effect of refined grain consumption on the probability of having NAFLD and a beneficial effect of whole-grain intake on clinical factors involved in the progression and severity of NAFLD, such as abdominal obesity, insulin resistance and inflammatory biomarkers. These preliminary findings should be confirmed by future larger prospective epidemiological studies or clinical trials and could be incorporated in dietary guidelines for the prevention and treatment of NAFLD.

Acknowledgments We would like to thank Prof. Mary Yannakoulia for her valuable comments during the writing of the manuscript. This research was supported financially by a research grant from the Hellenic Foundation of Gastroenterology & Nutrition.

Conflict of interest All authors declare no conflicts of interest.

References

1. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ (2012) The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology 142:1592–1609. doi:10.1053/j.gastro.2012.04.001
2. Levene AP, Goldin RD (2012) The epidemiology, pathogenesis and histopathology of fatty liver disease. Histopathology 61:141–152. doi:10.1111/j.1365-2559.2011.04145.x
3. Tsochatzis E, Papatheodoridis GV, Archimandritis AJ (2006) The evolving role of leptin and adiponectin in chronic liver diseases. Am J Gastroenterol 101:2629–2640. doi:10.1111/j.1572-0241.2006.00848.x
4. Tsochatzis EA, Manolakopoulos S, Papatheodoridis GV, Archimandritis AJ (2009) Insulin resistance and metabolic syndrome in chronic liver diseases: old entities with new implications. Scand J Gastroenterol 44:6–14. doi:10.1080/00365520802273058
5. Vernon G, Baranova A, Younossi ZM (2011) Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther 34:274–285. doi:10.1111/j.1365-2036.2011.04724.x
6. Sullivan S (2010) Implications of diet on nonalcoholic fatty liver disease. Curr Opin Gastroenterol 26:160–164. doi:10.1097/MOG.0b013e3283358a58

7. Mouzaki M, Allard JP (2012) The role of nutrients in the development, progression, and treatment of nonalcoholic fatty liver disease. J Clin Gastroenterol 46:457–467. doi:10.1097/MCG.0b013e31824cf51e
8. Williams PG (2012) Evaluation of the evidence between consumption of refined grains and health outcomes. Nutr Rev 70:80–99. doi:10.1111/j.1753-4887.2011.00452.x
9. Lefevre M, Jonnalagadda S (2012) Effect of whole grains on markers of subclinical inflammation. Nutr Rev 70:387–396. doi:10.1111/j.1753-4887.2012.00487.x
10. Ye EQ, Chacko SA, Chou EL, Kugizaki M, Liu S (2012) Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. J Nutr 142:1304–1313. doi:10.3945/jn.111.155325
11. Ross AB, Godin JP, Minehira K, Kirwan JP (2013) Increasing whole grain intake as part of prevention and treatment of nonalcoholic Fatty liver disease. Int J Endocrinol 2013:585876. doi:10.1155/2013/585876
12. World Medical Association declaration of Helsinki (1997) Recommendations guiding physicians in biomedical research involving human subjects. JAMA, J Am Med Assoc 277:925–926
13. Goulet J, Nadeau G, Lapointe A, Lamarche B, Lemieux S (2004) Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. Nutr J 3:13. doi:10.1186/1475-2891-3-13
14. Trichopoulou A, Lagiou P (1999) Dietary guidelines for adults in Greece. Arch Hell Med 16:516–524
15. Nanos GD, Gerasopoulos DG (2001) Fruits, Vegetable, Legumes and Grains. In: Zampelas A, Stavrinos V, Wolinsky I, Matalas AL (eds) The mediterranean diet: constituents and health promotion. CRC Press LLC, Boca Raton
16. Manios Y, Moschonis G, Koutsikas K, Papoutsou S, Petraki I, Bellou E, Naoumi A, Kostea S, Tanagra S (2009) Changes in body composition following a dietary and lifestyle intervention trial: the postmenopausal health study. Maturitas 62:58–65. doi:10.1016/j.maturitas.2008.11.005
17. Browning LM, Mugridge O, Chatfield MD, Dixon AK, Aitken SW, Joubert I, Prentice AM, Jebb SA (2010) Validity of a new abdominal bioelectrical impedance device to measure abdominal and visceral fat: comparison with MRI. Obesity (Silver Spring) 18:2385–2391. doi:10.1038/oby.2010.71
18. Friedewald WT, Levy RIFD (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
19. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
20. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94:2467–2474. doi:10.1111/j.1572-0241.1999.01377.x
21. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ (2005) Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41:1313–1321. doi:10.1002/hep.20701
22. Shi L, Liu ZW, Li Y, Gong C, Zhang H, Song LJ, Huang CY, Li M (2012) The prevalence of nonalcoholic fatty liver disease and its association with lifestyle/dietary habits among university faculty and staff in Chengdu. Biomed Environ Sci 25:383–391. doi:10.3967/0895-3988.2012.04.002
23. Toshimitsu K, Matsuura B, Ohkubo I, Niiya T, Furukawa S, Hiasa Y, Kawamura M, Ebihara K, Onji M (2007) Dietary habits and nutrient intake in non-alcoholic steatohepatitis. Nutrition 23:46–52. doi:10.1016/j.nut.2006.09.004
24. Liu S (2002) Intake of refined carbohydrates and whole grain foods in relation to risk of type 2 diabetes mellitus and coronary heart disease. J Am Coll Nutr 21:298–306
25. Giugliano D, Ceriello A, Esposito K (2006) The effects of diet on inflammation: emphasis on the metabolic syndrome. J Am Coll Cardiol 48:677–685. doi:10.1016/j.jacc.2006.03.052
26. Krawczyk M, Bonfrate L, Portincasa P (2010) Nonalcoholic fatty liver disease. Best Pract Res Clin Gastroenterol 24:695–708. doi:10.1016/j.bpg.2010.08.005
27. Wirström T, Hilding A, Gu HF, Östenson C-G, Björklund A (2013) Consumption of whole grain reduces risk of deteriorating glucose tolerance, including progression to prediabetes. Am J Clin Nutr 97:179–187. doi:10.3945/ajcn.112.045583
28. Murtaugh MA, Jacobs DR Jr, Jacob B, Steffen LM, Marquart L (2003) Epidemiological support for the protection of whole grains against diabetes. Proc Nutr Soc 62:143–149
29. Sahyoun NR, Jacques PF, Zhang XL, Juan W, McKeown NM (2006) Whole-grain intake is inversely associated with the metabolic syndrome and mortality in older adults. Am J Clin Nutr 83:124–131
30. McKeown NM, Troy LM, Jacques PF, Hoffmann U, O'Donnell CJ, Fox CS (2010) Whole- and refined-grain intakes are differentially associated with abdominal visceral and subcutaneous adiposity in healthy adults: the Framingham Heart Study. Am J Clin Nutr 92:1165–1171. doi:10.3945/ajcn.2009.29106
31. Liu S, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G (2003) Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. Am J Clin Nutr 78:920–927
32. McKeown NM, Yoshida M, Shea MK, Jacques PF, Lichtenstein AH, Rogers G, Booth SL, Saltzman E (2009) Whole-grain intake and cereal fiber are associated with lower abdominal adiposity in older adults. J Nutr 139:1950–1955. doi:10.3945/jn.108.103762
33. Fardet A (2010) New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev 23:65–134. doi:10.1017/S0954422410000041
34. Salas-Salvado J, Martinez-Gonzalez MA, Bullo M, Ros E (2011) The role of diet in the prevention of type 2 diabetes. Nutr Metab Cardiovasc Dis 21(Suppl 2):B32–B48. doi:10.1016/j.numecd.2011.03.009
35. Tilg H, Moschen AR (2008) Insulin resistance, inflammation, and non-alcoholic fatty liver disease. Trends Endocrinol Metab 19:371–379. doi:10.1016/j.tem.2008.08.005
36. Lutsey PL, Jacobs DR Jr, Kori S, Mayer-Davis E, Shea S, Steffen LM, Szklo M, Tracy R (2007) Whole grain intake and its cross-sectional association with obesity, insulin resistance, inflammation, diabetes and subclinical CVD: the MESA Study. Br J Nutr 98:397–405. doi:10.1017/S0007114507700715
37. Nettleton JA, Steffen LM, Mayer-Davis EJ, Jenny NS, Jiang R, Herrington DM, Jacobs DR Jr (2006) Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr 83:1369–1379
38. Yannakoulia M, Yiannakouris N, Melistas L, Kontogianni MD, Malagaris I, Mantzoros CS (2008) A dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products and low consumption of refined cereals is positively associated with plasma adiponectin levels in healthy women. Metabolism 57:824–830. doi:10.1016/j.metabol.2008.01.027