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Cancer-related gene expression is associated with disease severity and modifiable lifestyle factors in non-alcoholic fatty liver disease

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ABSTRACT

Objective: The aim of this study was to determine whether hepatic gene expression related to hepatocellular carcinoma (HCC) is associated with disease severity and modifiable lifestyle factors in non-alcoholic fatty liver disease (NAFLD).

Methods: In a cross-sectional study, the associations between hepatic gene expression and liver histology, insulin resistance, anthropometrics, diet, and physical activity were assessed in patients with non-alcoholic steatohepatitis (NASH; n = 19) or simple steatosis (SS; n = 20). In a group of patients with NASH, we then conducted a 1-y, single-arm, pilot study using ω-3 polyunsaturated fatty acid (PUFA) supplementation to determine whether changes in hepatic PUFA content would have a modulating effect on hepatic gene expression and would affect liver histology.

Results: In the cross-sectional study, histological features of disease severity correlated with *AKR1B10, ANXA2, PEG10, SPP1, STMN2, MT1A,* and *MT1B* in NASH and with *EEF1A2, PEG10,* and *SPP1* in SS. In addition, *PEG10, SPP1, ANXA2,* and *STMN2* expression correlated positively with insulin resistance in NASH. *SPP1* and *UBD* correlated strongly with body mass index in SS. Associations between *ENPP2, AKR1B10, SPP1, UBD*, and waist circumference depended on sex and diagnosis. Several genes correlated with protein, fat, or carbohydrate intake. *PEG10* correlated positively with physical activity in NASH and inversely with plasma vitamin C in both groups. Despite increased erythrocyte and hepatic ω-3 PUFA, supplementation did not alter hepatic gene expression and liver histology.

Conclusions: HCC-related gene expression was associated with liver histology, body mass index, waist circumference, diet, and physical activity but was not affected by ω-3 PUFA supplementation.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) includes hepatic simple steatosis (SS) and non-alcoholic steatohepatitis (NASH). NAFLD, particularly NASH, poses an increased risk for hepatocellular carcinoma (HCC) [1,2]. Altered expression patterns for genes related to pathways such as nutrient and xenobiotic metabolism, apoptosis, liver regeneration, and fibrosis have been observed in patients with NAFLD, NASH, or both diseases, compared with healthy controls and between SS and NASH [3,4]. Dysregulation of these pathways may contribute to disease progression and HCC development

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[2,5,6]. Upregulation of genes associated with cancer progression and liver proliferation, particularly *AKR1B10,* was also found in patients with NASH compared with those with SS and controls [6,7].

The mechanisms underlying the progression from SS to NASH and HCC are still unclear, but metabolic disorders, particularly obesity and diabetes mellitus are risk factors for both NAFLD and HCC, which could explain the strong link between both diseases [8,9]. Because poor diet, a lack of physical activity, and resulting obesity and insulin resistance (IR) are highly prevalent in both SS and NASH, lifestyle interventions aiming at weight loss and increased physical activity are important approaches for the prevention of NAFLD-associated HCC [8]. In addition to general overnutrition, specific dietary intakes, such as low ω-3 polyunsaturated fatty acids (PUFAs) may predispose to NAFLD [10] or HCC [11], and some randomized controlled trials [12–15] but not all [16,17] have shown benefits of ω-3 PUFA supplementation on liver histology. Beneficial effects may be related to gene-modulating properties of ω-3 PUFA that can regulate transcription factors like *SREBP-1, PPAR,* or *NF–κB,* thus affecting lipid and carbohydrate metabolism and inflammatory pathways [18,19]. However, to our knowledge, none of the ω-3 PUFA supplementation trials examined the effect on hepatic gene expression.

The aim of the present study was to investigate the relationship between HCC-related hepatic gene expression and obesity, dietary intake, and physical activity as lifestyle-related modifiable risk factors in both SS and NASH. In addition, we conducted a 1-y, single-arm, pilot study in a subgroup of patients with NASH, using ω-3 PUFA supplementation as surrogate to a dietary intervention, to determine whether increasing hepatic PUFA content would alter hepatic gene expression and improve liver histology.

Methods and materials

Design

This was a cross-sectional study and a single-arm open-label pilot study with ω-3 PUFA supplementation.

Participants

For the cross-sectional study, adults with biopsy-proven NASH or SS were enrolled at a tertiary care center between March 2007 and November 2011, as described previously [7]. Patients with NASH were enrolled in the supplementation trial between October 2009 and May 2014. Inclusion criteria were as follows:

- age 18 to 65 y;
- body mass index (BMI) $\leq$40 kg/m^2;
- alcohol consumption <20g/d;
- non-smokers;
- be on a stable drug regimen if hyperlipidemia or diabetes is present.

Exclusion criteria were:

- other liver diseases;
- anticipated need for liver transplantation within 1 y or complications of liver disease;
- concurrent medical illnesses;
- any reasons contraindicating a liver biopsy;
- chronic gastrointestinal diseases, previous gastrointestinal surgery modifying the anatomy, diabetes requiring insulin;
- use of medications known to precipitate steatohepatitis or regular intake of non-steroidal anti-inflammatory drugs, antioxidant vitamin or ω-3/fish oil supplements, prebiotics, probiotics, antibiotics, or laxatives;
- ursodeoxycholic acid or any experimental drug in the 6 mo before study entry;
- pregnancy or lactating state.

Both studies followed the guidelines of the 1975 Declaration of Helsinki and its revisions and were approved by the local research ethics board and registered at www.clinicaltrials.gov. All participants provided informed written consent.

Intervention

Patients with NASH received ω-3 PUFA supplementation from fish oil (2 g/d, containing 740–840 mg eicosapentaenoic acid [EPA] and 400–440 mg docosahexaenoic acid [DHA] quantified as ethyl ester; Ocean Nutrition Canada Ltd., Dartmouth, NS, Canada) for 1 y. This supplementation was within the range used in previous studies, where doses of ω-3 PUFA and the EPA–DHA balance varied widely, and effects on liver steatosis were observed by ultrasound within 6 to 12 mo [20–22].

Patients were asked to maintain their habitual diet and physical activity.

Sample and data collection

For the cross-sectional study, participants provided a liver biopsy, a fasting blood sample, a 7-d food record, and an activity log. Anthropometric and clinical data were collected as described previously [7].

Patients in the intervention trial had the same baseline assessments described in the cross-sectional study. Those were repeated at the end of the 1-y intervention. Liver transaminases were measured at 6 mo for safety. Hepatic PUFA (% of total lipids and in lipid subfractions) were assessed at the same time points. Compliance was assessed by PUFA measurement in erythrocytes at baseline, 3, 6, 9, and 12 mo and pill counts. At each visit, patients were asked about side effects and changes in their medication or health status.

Liver histology

Samples were stained with hematoxylin and eosin for morphologic evaluation and Prussian Blue stain to rule out iron loading. Slides were reviewed by a single pathologist. SS or NASH were diagnosed [23], and the NAFLD activity score (NAS) was assessed [24].

Hepatic gene expression

RNA was extracted from liver biopsies (*mir*Vana miRNA Isolation kit, Life Technologies Corp., Carlsbad, CA, USA) and gene expression levels were measured (Whole Genome Gene DASL HT Assay with the Human HT-12 V4 BeadChip, Illumina Inc., San Diego, CA, USA). The data analysis and global gene expression profiles were described previously [7]. For the intervention trial, baseline and 12-mo data were compared.

Gene expression was analyzed by the Princess Margaret Genomics Centre, Toronto, Canada (www.pmgenomics.ca). The data are publicly available in the Gene Expression Omnibus repository (https://www.ncbi.nlm.nih.gov/geo/, accession # GSE89632 and GSE96971).

Hepatic PUFAs

Total lipids were extracted from 50 μL of liver homogenate and directly saponified and transmethylated to form fatty acid methyl esters (FAME) [7]. For lipid class analyses, lipids were extracted from 200 μL of liver homogenate and subjected to thin layer chromatography [25]. Individual bands were visualized using ultraviolet light, scraped from the plate, and methylated using boron trifluoride. The fatty acid methyl esters from total lipid and lipid classes were then separated using gas chromatography [7], identifying 63 peaks between C12:0 and C22:6ω3. The relative amount of single fatty acids was calculated as mole percent (mol%) of total lipids. The ratio of ω-6 to ω-3 was calculated as arachidonic acid (AA)/(EPA + DHA).

Nutritional assessment and blood biochemistry

BMI (weight [kg] / height [m]^2) and waist circumference (WC) were measured. Diet was assessed by 7-d food records, using Food Portion Visual (Nutrition Consulting Enterprises, Framingham, MA, USA) for portion size estimation and Food Processor SQL (ESHA Research, Salem, OR, USA) for nutrient analysis. Participants documented the duration and intensity of physical activity [26] for 1 wk. Plasma and serum were collected after 8 h of fasting to measure transaminases, glucose, and insulin [7]. Homeostasis model of assessment for insulin resistance (HOMA-IR) was calculated [27]. Plasma vitamin C, an important water-soluble antioxidant reflecting intakes of fruits and vegetables, was measured colorimetrically [28] from samples that were stabilized with 1:1 (v/v) 10% meta-phosphoric acid and stored at −80°C until analysis.

Statistical analysis

For the cross-sectional part, differentially expressed genes were identified by one-way analysis of variance and Tukey's honestly significant difference post hoc test. For the ω-3 PUFA supplementation study, hepatic gene expression before and after supplementation was compared using paired *t* test. Benjamini–Hochberg false-discovery rate of $q < 0.05$ was applied to control for multiple comparisons; significantly differentially expressed genes were subsequently filtered for at least twofold up- or downregulation. Details on the analysis were published previously [7]. The results are expressed as fold-changes between the participant groups.

Clinical and nutritional data are presented as median (Q1; Q3), or percent of cases. In the cross-sectional study, continuous variables were compared by Mann–Whitney test. Fisher's exact and χ^2 tests were applied for categorical variables. Spearman correlations were calculated. A principal component (PC) analysis was performed to summarize variation in expression levels of multiple genes and to determine whether certain genes have more effect on variation. For the fish oil supplementation study, before and after data were compared using Friedman test and Wilcoxon-signed rank test for continuous and McNemar test for categorical data.

Analyses were performed with SAS version 9.4 (SAS Institute Inc, Cary, NC, USA) and SPSS version 24 (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered significant.

Results

Cross-sectional study

Clinical characteristics, diet, and physical activity

Clinical characteristics of the participants included in the analysis are presented in Table 1. (A participant flowchart is presented in Supplementary Fig. 1). SS and NASH showed typical differences in BMI, laboratory measurements, and liver histology. Demography, WC, and plasma glucose were similar (Table 1). Dietary intake and estimated energy requirements were similar in SS and NASH. However, physical activity was higher and plasma vitamin C was lower in NASH (Supplementary Table 1).

Hepatic gene expression

The whole-genome analysis identified 22 differentially expressed genes between SS and NASH [7], of which 11 were related to HCC (Fig. 1; Supplementary Table 2).

SS and NASH have different prognosis, thus we assessed each separately for associations between gene expression and various parameters. Histological features were significantly correlated with *EEF1A2, PEG10*, and *SPP1* expression in the SS group and with *AKR1B10, ANXA2, PEG10, SPP1, MT1A*, and *MT1B* in NASH (Table 2; Supplementary Table 3 for a full list of correlations).

Of the nutrition and metabolic factors, HOMA-IR was positively correlated with *ANXA2* ($\rho = 0.640$), *PEG10* ($\rho = 0.587$), *SPP1* ($\rho = 0.547$), and *STMN2* ($\rho = 0.640$) in NASH (all $P < 0.05$), but not in SS. BMI was strongly correlated with *SPP1* ($\rho = 0.772$, $P < 0.001$) and *UBD* ($\rho = 0.667$, $P < 0.01$) only in SS. Because WC and its health implications are sex-dependent and men and women have different cutoff points for central obesity (>102 and >88 cm, respectively, are associated with a substantially increased risk for metabolic complications [29]), the associations between WC and gene expression were examined separately for both sexes. The correlation analysis yielded distinct results for men and women. *ENPP2* and WC were positively correlated in men with NASH but negatively in women with both SS and NASH (Fig. 2). In addition, *AKR1B10* ($\rho = 0.66$, $P = 0.015$), *SPP1* ($\rho = 0.83$, $P = 0.001$), and *UBD* ($\rho = 0.81$, $P = 0.001$) showed moderate to strong correlations with WC only in men with SS.

Hepatic gene expression did not correlate with absolute or adjusted energy intake in SS or NASH (data not shown). However, several HCC-related genes correlated negatively with energy intake from fat, carbohydrates, or protein (Table 3). Plasma vitamin C showed a highly significant inverse relationship with *PEG10* in both groups, whereas physical activity correlated with gene expression only in patients with NASH (for a full list of correlations see Supplementary Table 4).

Physical activity (units/d) correlated with *MT1A* ($\rho = -0.653$, $P < 0.05$) and *PEG10* ($\rho = 0.684$, $P < 0.01$), mild activity (min/d) with *MT1A* ($\rho = -0.609$, $P < 0.05$), and moderate to strenuous activity (min/d) with *ENPP2* ($\rho = -0.559$, $P < 0.05$) and *MT1B* ($\rho = 0.634$, $P < 0.05$).

In the PC analysis, all genes contributed similarly to PC1, which explained 55.2% of the variance in this data set. In contrast, PC2 (12.2%) was determined particularly by *PEG10, MT1A*, and *MT1B* (Fig. 3A, B). The component plot (Fig. 3C) distinguishes NASH from SS, although not perfectly.

ω-3 PUFA supplementation study

Eleven patients with NASH received ω-3 PUFA supplementation for 55.1 (54; 62) wk (patient flow in Supplementary Fig. 2, demography in Supplementary Table 5). Compliance assessed by pill

Table 1
Clinical data of participants in the cross-sectional study

Parameter	n	SS	n	NASH	*P*-value
Age (y)	20	45.5 (35.5; 51.8)	19	44.0 (34.0; 52.0)	NS
Men,% (n/n)	20	70.0 (14/20)	19	47.4% (9/19)	NS
BMI, kg/m^2	19	28.6 (25.2; 30.1)	18	31.9 (29.4; 33.5)	0.026
Waist circumference, cm					
Women	6	94.5 (89.7; 98.9)	8	103.8 (93.3; 111.4)	NS
Men	13	101.5 (95.0; 110.3)	8	107.7 (97.1; 110.5)	NS
Steatosis, % of hepatocytes	20	35.0 (10.0; 50.0)	19	40.0 (25.0; 70.0)	NS
Fibrosis stage 0/1/2/3/4, %	20	85/15/0/0/0	19	21.1/26.3/10.5/21.1/21.1	0.001
Fibrosis stage 0/1/2/3/4, n		17/3/0/0/0		4/5/2/4/4	
NAS (0–8)	19	2.00 (1.00; 2.00)	19	5.00 (4.00; 6.00)	<0.0001
AST (U/L)	19	30.0 (26.0; 33.0)	19	52.0 (36.0; 83.0)	<0.0001
ALT (U/L)	19	52.0 (37.0; 64.0)	19	81.0 (46.0; 118.0)	0.009
Glucose (mmol/L)	17	5.30 (4.75; 6.50)	17	5.40 (4.95; 6.65)	NS
Insulin (pmol/L)	17	61.0 (31.0;90.0)	14	130 (70.3; 203.3)	0.013
HOMA-IR	16	2.83 (1.72; 3.86)	14	5.62 (2.48; 13.89)	0.038

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; HOMA-IR, homeostasis model of assessment for insulin resistance; NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis; NS, not statistically significant; SS, simple steatosis.
Modified from [7]. Values given are median (Q1; Q3) or % of valid cases. *P*-value based on Mann–Whitney U test for continuous and Fisher's exact test for categorical variables. $P < 0.05$ considered significant.

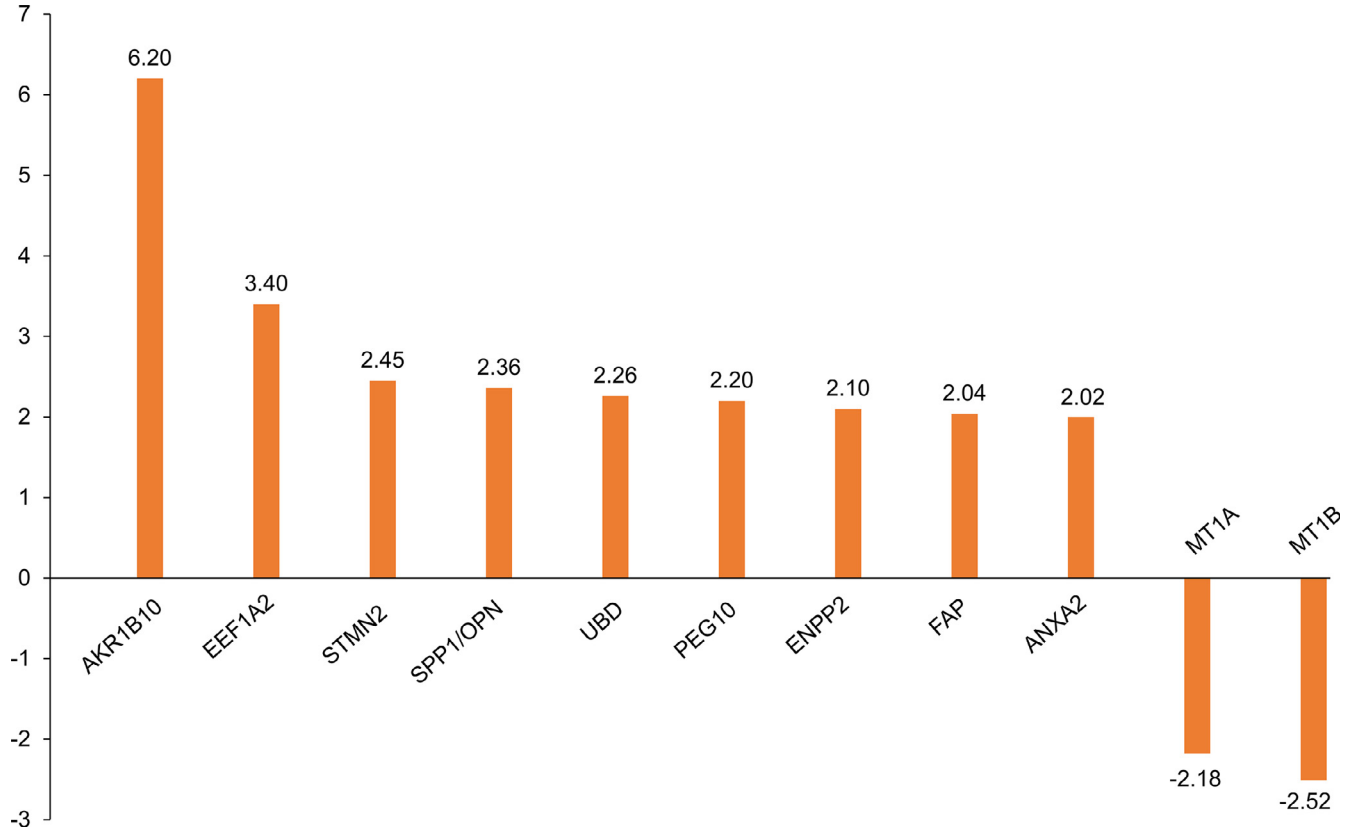


Fig. 1. Hepatocellular carcinoma-related genes that were differentially expressed between patients with NASH and SS. NASH, non-alcoholic steatohepatitis; SS, simple steatosis.

counts was 87.6% (76.8%; 99.4%), which was confirmed by a significant increase of ω-3 PUFA and a reduction of AA and the ω-6/ω-3 ratio in erythrocytes (Supplementary Fig. 3). While on supplementation, changes in hepatic fatty acid content were significant with DHA being enriched in total lipids, phospholipids, and triacylglycerides, whereas EPA decreased in diacylglycerols. Changes of DHA in erythrocytes correlated strongly with changes in hepatic total lipids ($\rho = 0.782$; $P = 0.008$) and phosphatidylcholine ($\rho = 0.721$, $P = 0.019$). Changes of erythrocyte EPA were reflected in hepatic phosphatidylcholine ($\rho = 0.903$, $P = 0.0003$; Supplementary Table 6).

BMI, WC, liver histology (Supplementary Table 5), plasma transaminases, blood lipids, or parameters of blood sugar control (Supplementary Table 7) remained unchanged.

Regarding gene expression, an unsupervised clustering showed no clear grouping of the baseline and 12-mo biopsies or of the paired samples from each patient (Supplementary Fig. 4). None of the 21 562 probes included in the analysis changed significantly according to the paired *t* test (data not shown).

Table 2
Spearman correlations* between hepatic gene expression and histology in the cross-sectional study

Parameter	SS (n = 20)		NASH (n = 19)	
	Genes	ρ	Genes	ρ
Steatosis %				
	EEF1A2	0.558[a]	*SPP1*	0.575[a]
	PEG10	0.546[a]		
	SPP1	0.452[a]		
Lobular Inflammation				
		N/A†	*PEG10*	0.595[b]
Fibrosis				
	PEG10	−0.474[a]	*AKR1B10*	0.468[a]
			MT1A	−0.530[a]
			MT1B	−0.550[a]
			STMN2	0.672[b]
NAS				
	PEG10	0.488[a]	*ANXA2*	0.483[a]
	SPP1	0.463[a]	*SPP1*	0.670[b]

Superscript letters a and b show the level of significance: [a]$P < 0.05$; [b]$P < 0.01$.
NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis; SS, simple steatosis.
*Only statistically significant correlations are presented. For a full list see Supplementary Table 3.
†Lobular inflammation is not present in patients with SS.

Discussion

Eleven genes implicated in HCC pathogenesis were differentially expressed between NASH and SS, and in each group, some of these genes correlated with liver histology and lifestyle-related risk factors such as obesity, diet, and physical activity. However, ω-3 PUFA supplementation, a surrogate intervention for dietary manipulation, did not affect hepatic gene expression or histology.

The expression of *AKR1B10*, a proposed biomarker for NASH and progression to HCC [6], was significantly higher in NASH than in SS and correlated with fibrosis in NASH. The only association between *AKR1B10* and nutritional status was a positive correlation with WC, exclusively observed in men with SS. Therefore, the present results do not suggest a major role of lifestyle factors for the overexpression of *AKR1B10*.

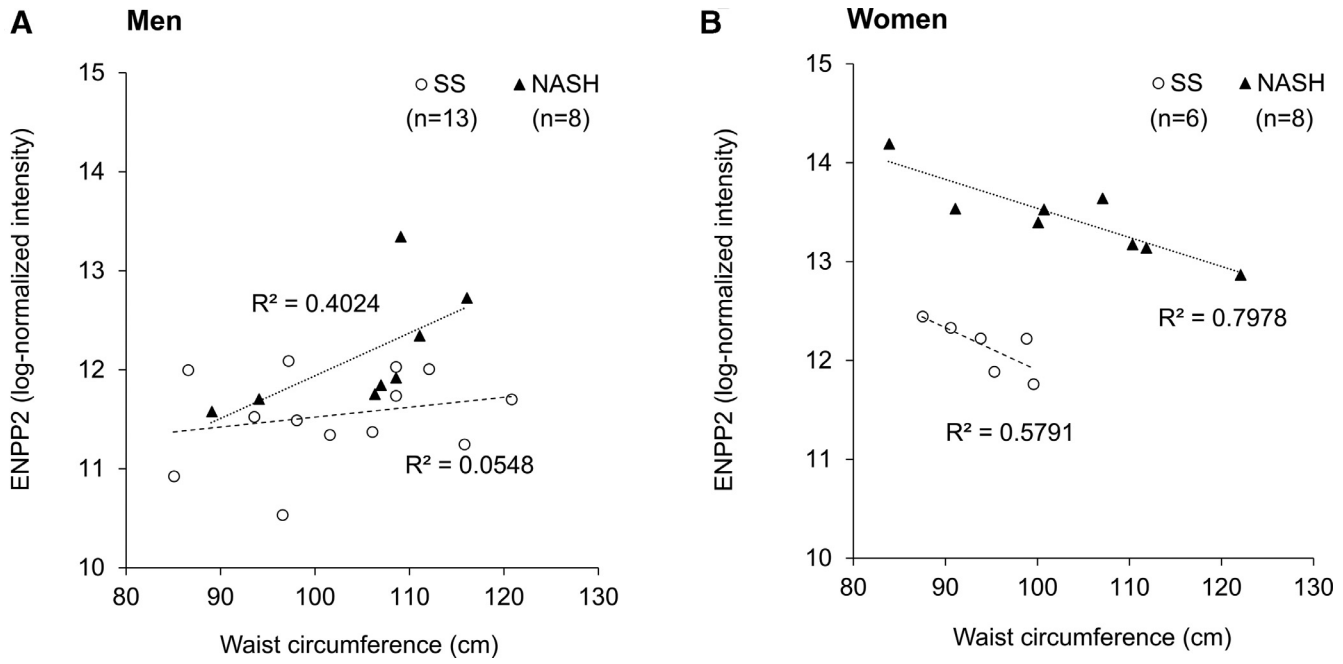


Fig. 2. Correlation between hepatic *ENPP2* gene expression and waist circumference (A) in men and (B) in women. Men–SS: ρ = 0.22, *P* = 0.478, men–NASH: ρ = 0.93, *P* = 0.0009; women–SS: ρ = −0.94, *P* = 0.005, women–NASH: ρ = −0.83, *P* = 0.010. NASH, non-alcoholic steatohepatitis; SS, simple steatosis.

Table 3
Spearman correlations* between hepatic gene expression and lifestyle-related parameters in the cross-sectional study including patients with SS and NASH

Parameter	SS (n = 16)		NASH (n = 15)	
	Genes	ρ	Genes	ρ
Energy intake from fat (%)				
	MT1B	0.612[a]	*MT1A*	0.525[a]
Energy intake from carbohydrates (%)				
	ENPP2	−0.597[a]		
	SPP1	0.556[a]		
Energy intake from protein (%)				
	MT1B	−0.556[a]	*AKR1B10*	−0.614[a]
	PEG10	−0.532[a]	*EEF1A2*	−0.718[b]
			SPP1	−0.643[b]
			STMN2	−0.600[a]

NASH, non-alcoholic steatohepatitis; SS, simple steatosis.
Superscript letters a and b show the level of significance: [a] $P < 0.05$, [b] $P < 0.01$.
*Only statistically significant correlations are presented. For a full list see Supplementary Table 4.

PEG10, shown to be upregulated in HCC [30,31], was also overexpressed in NASH compared with SS and correlated with liver histology. *PEG10* was positively correlated with HOMA-IR and physical activity in NASH and negatively with vitamin C in both SS and NASH. Oxidative stress and physical activity may alter epigenetic regulation, particularly DNA methylation [32,33], which in turn could affect the expression of *PEG10*, an imprinted gene. Altered DNA methylation has been shown in NAFLD [34,35]. In the PC analysis, *PEG10* together with *MT1A* and *MT1B* were the main contributors to PC2, which provides further evidence for a potential connection between *PEG10* expression and oxidative stress. *MT1A* and *MT1B* encode for metal binding and radical scavenging metallothioneins, which can protect from liver injury [36,37]. Both genes were downregulated in NASH, which may contribute to hepatic oxidative stress and inflammation. *MT1A* correlated negatively with total and mild physical activity in NASH, whereas *MT1B* correlated positively with moderate to very strenuous activity in this group. As part of the antioxidant defense, metallothioneins are expected to be upregulated by exercise, as shown in skeletal muscle and plasma [38]. The discrepancy for *MT1A* may suggest that mild exercise is not sufficient to induce *MT1A*. Alternatively, the metallothionein response of NASH patients might be impaired, like in patients with type 2 diabetes [38], particularly because 31.6% of the present NASH patients had diabetes.

SPP1 (osteopontin) may contribute to inflammation, fibrosis, and HCC in NASH [39,40]. Higher plasma *SPP1* was reported in NASH than in healthy controls, and higher plasma *SPP1* and hepatic *SPP1* expression was reported in NASH with advanced fibrosis compared with those with no or milder fibrosis [40]. Positive correlations between hepatic *SPP1* expression and steatosis, alanine aminotransferase, and HOMA-IR were observed in morbid obesity [41]. The present findings match these observations.

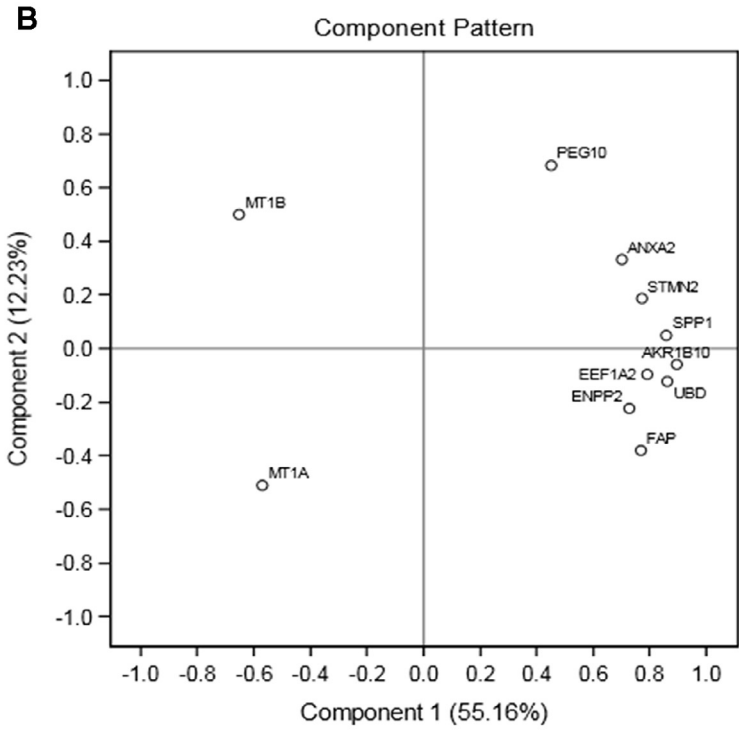
Consistent with previous reports, *ENPP2* (autotaxin) was overexpressed in NASH but not in SS [42,43]. However, the present results did not correspond to findings in obese women, where serum *ENPP2* correlated with presence and severity of steatosis [44]. This discrepancy may be due to dissimilarities between the study populations or may indicate that hepatic mRNA expression differs from serum *ENPP2*. Interestingly, the association between *ENPP2* expression and WC showed striking sex differences. Serum *ENPP2* was reported to be higher in women than in men [45]. We observed the same for hepatic *ENPP2* expression, particularly in women with NASH. Others previously described a positive correlation between serum *ENPP2* and BMI and WC [45]. Thus, there is evidence of a relationship between *ENPP2* and measures of obesity, but this may differ across the BMI spectrum and between sexes.

Patients with high protein intake had a more favorable gene expression profile, particularly in NASH, where protein intake was negatively correlated with *AKR1B10*, a biomarker for HCC development [6]. This contrasts with an epidemiologic study, where protein intake was associated with increased risk for cirrhosis and HCC [46]. However, the source of protein may play an important

A

Eigenvectors

	Prin1	Prin2
AKR1B10	0.363	-0.050
UBD	0.350	-0.108
SPP1	0.349	0.042
EEF1A2	0.322	-0.085
STMN2	0.313	0.162
FAP	0.312	-0.328
ENPP2	0.295	-0.194
ANXA2	0.285	0.285
PEG10	0.184	0.587
MT1A	-0.231	-0.441
MT1B	-0.264	0.430



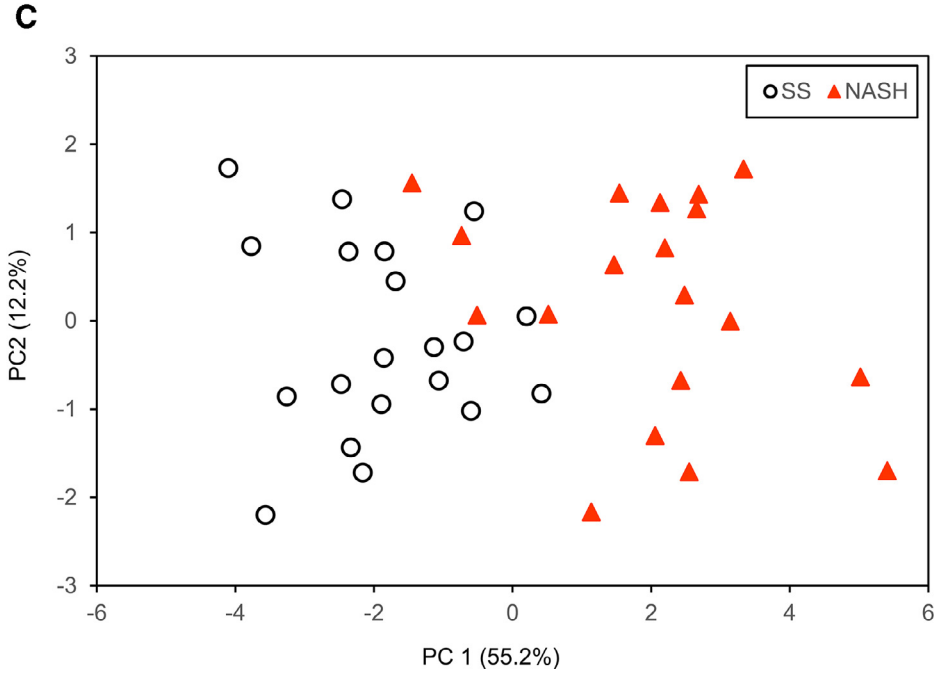


Fig. 3. Principal component analysis including 11 cancer-related genes that were differentially expressed between patients with non-alcoholic steatohepatitis and those with simple steatosis. (A) Eigenvectors for the first two PCs extracted. The 11 genes contribute similarly to PC1, except for a slightly lower value for *PEG10*. PC2 is most strongly determined by *PEG10*, *MT1A*, and *MT1B*. (B) Component pattern depicting the contribution of each gene to the two PCs. (C) This plot shows the separation of patients with NASH from those with SS by PC1 and PC2. NASH, non-alcoholic steatohepatitis; PC, principal component; SS, simple steatosis.

role. Dairy may increase the risk for chronic liver disease and HCC [47], whereas the consumption of fish or white meat may be protective [48–50]. The effect of red meat is controversial [48–50]. We also found correlations between gene expressions and fat or carbohydrate intake, so overall, relationships between genes, HCC risk, and diet may be worth exploring further.

No change was detected in hepatic gene expression or histology despite increases in liver ω-3 PUFA after supplementation. The lack of effect on gene expression was unexpected because gene-modulating effects of ω-3 PUFA are well documented [18,19]. The lack of effect could be due to the dose of ω-3 PUFA not being high enough as newer trials showing improvement in liver histology used

higher ω-3 PUFA concentrations [12,14]. The EPA/DHA balance also may have influenced the results as higher DHA enrichment has been associated with improvement of steatosis [14] and lobular inflammation [15].

There were limitations to this study. The explanatory power is restricted by the design, which was either cross-sectional or a single-arm intervention. In addition, the sample size was small for both studies because of the invasiveness of the liver biopsy, which made patient recruitment challenging, thus preventing more complex multivariate analyses and possibly limiting the reliability of the results. Patients with NASH had higher BMI than those with SS, which also may have influenced hepatic gene expression. However, because obesity is a risk factor for NASH, finding patients matching for BMI for both groups would be very challenging. We accounted for this by examining correlations between genes and BMI in both groups and showed moderate to strong, highly significant correlations between BMI and *SPP1* and *UBD* only in SS. Another limitation was that associations between gene expression and lifestyle factors were not corrected for multiple comparisons because this was an exploratory study. However, the results point to several potential associations between HCC-related gene expression and modifiable lifestyle factors that deserve closer investigation.

Conclusion

The expression of hepatic genes related to HCC was associated with liver histology and several modifiable lifestyle factors. However, ω-3 PUFA supplementation, as a way of changing hepatic fatty acid composition, was not successful in modulating gene expression or in improving histology.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2018.12.001.

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