

SUPPLEMENTARY DATA

Criteria for excluding competing causes of hepatic steatosis. Criteria for excluding competing causes of fatty liver disease were as follows: exposure to occupational hepatotoxins or drugs known to be steatogenic or hepatotoxic; positive autoimmune or celiac disease markers; abnormal serum α_1 -antitrypsin, ceruloplasmin, transferrin saturation, or thyroid hormones. Mutations in the hemochromatosis genes HFE and TRF2 were detected in patients and controls using multiplex amplification reaction (Nuclear Laser Medicine, Milan, Italy). Liver iron concentration (LIC) and hepatic iron index (HII) were assessed from 2 mg dry weight tissue by atomic absorption spectroscopy.

Details on liver histology in biopsy-proven NAFLD patients. Hepatic steatosis was mild (involving 5-33% hepatocytes) in 32% of NAFLD patients, moderate (33-66% hepatocytes) in 39% NAFLD patients, and severe (>66% hepatocytes) in 29% patients. Lobular inflammation was absent in 37% patients, grade 1 in 20% patients, grade 2 in 23% patients, and grade 3 in 20% patients. Hepatocyte ballooning was absent in 42% patients, grade 1 in 35% patients and grade 2 in 23% patients.

Mean necro-inflammatory score (defined as the sum of hepatocellular ballooning and lobular inflammatory scores, ranging 0-5) was 2.3 ± 0.4 . Mean NAFLD activity score (NAS) in the overall NAFLD cohort was 3.9 ± 0.5 .

NASH was present in 19 (48%) of the 40 biopsy-proven NAFLD patients. Fibrosis was stage 0 in 54% patients, 1 in 15% patients, 2 in 18%, and stage 3 in 13% subjects; cirrhotic changes were absent.

Daily intake of main dietary constituents in NAFLD developers and non-NAFLD developers at baseline and at the end of follow-up is reported. No significant difference between baseline and end of follow-up in any nutrient intake was found in either NAFLD developers and non-NAFLD developers (P value not shown). Data are expressed as mean \pm SEM.

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Supplementary Table 1. Daily intake of main dietary constituents in NAFLD developers and non-NAFLD developers

	Baseline		P	End of follow-up		P
	Non-NAFLD developers (n=127)	NAFLD developers (n=48)		Non-NAFLD developers (n=127)	NAFLD developers (n=48)	
Total energy Intake (kcal)	2398±142	2478±146	0.202	2589±187	2599±179	0.721
Kcal/kg BW	31± 1	32± 1	0.571	33± 1	32± 1	0.831
Alcohol (g)	4.1±0.7	4.3±1.0	0.591	3.8±0.9	4.5± 0.9	0.169
Fat (% kcal)	34.8±0.9	35.0±1.2	0.994	36.7±1.0	33.7±1.0	0.557
CHO (% kcal)	49.8±1.1	49.6±1.3	0.957	49.6±1.1	50.0±1.4	0.561
Simple CHO (%tot CHO)	40.0±1.4	39.3±1.3	0.545	36.8±1.1	34.1±0.9	0.972
Fiber (g)	26.9±2.2	21.4±1.4	0.861	28.9±1.8	21.2±1.6	0.780
Protein (% kcal)	15.9±0.4	16.1±0.4	0.882	16.0±1.4	15.6± 0.5	0.357
Chol (mg)	374±22	418±25	0.158	420±18	429± 35	0.289
SFA (% total kcal)	11.6±0.4	11.9±0.5	0.568	12.1±0.4	11.7±0.6	0.991
MUFA (% total kcal)	16.9±0.6	16.3±0.7	0.481	16.2±0.5	16.3±0.7	0.210
PUFA (% total kcal)	4.4±0.2	4.8±0.2	0.641	4.4±0.3	4.1±0.1	0.747
SFA (% total fat)	33.2±0.8	33.8±0.9	0.467	34.8±0.7	34.9±0.9	0.993
MUFA (% total fat)	48.2±0.9	46.7±1.1	0.291	47.1±0.9	49.0±1.0	0.198
PUFA (% total fat)	12.8±0.5	13.6±0.6	0.546	12.6±0.06	12.2±0.5	0.745
(P:S ratio)	0.39±0.02	0.41±0.02	0.994	0.37±0.02	0.36±0.02	0.841
Vit. A (µg)	708±48	691±35	0.658	717±54	711±41	0.735
Vit. C (mg)	158±10	153±12	0.846	150±13	145±18	0.489
Vit. E (mg)	9.3±0.6	8.9±0.5	0.312	9.0±0.8	8.7±0.5	0.459

Abbreviations: BW: body weight; CHO: carbohydrates; Chol: cholesterol; Vit: vitamin

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Supplementary Table 2. Main clinical, biochemical and histological parameters of biopsy-proven NAFLD patients and controls grouped according to SREBF-2 SNP (n=80) Data are expressed as mean±SEM. Statistically significant P values are written in bold characters.

	Controls			NAFLD		
	CC(n=20)	CT/TT(n=20)	P	CC(n=20)	CT/TT(n=20)	P
Age (yr)	57±2	57±2	0.999	57±2	57±2	0.999
Sex (%M)	65	65	0.999	65	65	0.999
Smokers (%)	15	15	0.981	14	16	0.832
METS (h/week)	19.8±1.4	21.2±1.3	0.761	20.5±1.2	20.0±1.3	0.742
BMI (kg/m ²)	26.7±0.4	26.6±0.3	0.897	26.7±0.4	26.6±0.3	0.913
Waist (cm)	93±4	94±3	0.798	93±4	95±3	0.841
Sys BP (mmHg)	133±4	135±4	0.898	134±4	136±5	0.751
Dia BP (mmHg)	83±2	83±2	0.912	84±2	83±2	0.902
Glucose (mg/dL)	92±2	93±2	0.802	96±2	98±2	0.935
Insulin (μU/mL)	7.0±0.7	9.5±1.0	0.046	9.8±1.0	13.2±1.3	0.047
Total C (mg/dL)	179±7	191±8	0.266	187±6	197±7	0.299
LDL-C (mg/dL)	115±7	125±8	0.437	123±7	130±8	0.534
AST (IU/L)	16±2	20±2	0.165	36±3	61±4	0.001
ALT (IU/L)	18±2	21±2	0.411	66±3	103±5	0.001
MTP -493 G/T(%)						
GG	46	53	0.628	44	50	0.934
GT/TT	54	47	0.703	56	50	0.812
ApoE (%)						
2-3	12	14	0.810	13	14	0.901
3-3	68	67	0.880	68	66	0.899
3-4	20	19	0.902	19	20	0.947
CRP(mg/L)	1.5±0.1	2.0±0.2	0.033	2.2±0.2	3.4±0.3	0.004

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	Controls			NAFLD		
	CC(n=20)	CT/TT(n=20)	p	CC(n=20)	CT/TT(n=20)	p
E-selectin(ng/mL)	18.5±1.8	28.6±2.2	0.006	37.3±2.4†±	49.8±3.0	0.004
ICAM-1(ng/mL)	181.2±5.33	197.8±6.2	0.035	239.4±7.3*	279.1±9.1†	0.003
T2DM(%)	0	0	0.999	0.999	0.999	0.999
Obesity(%)	15	15	0.999	15	15	0.999
Met sy(%)	15	15	0.999	15	15	0.999
Abdominal obesity (%)	40	35	0.851	40	35	0.882
Hypertension (%)	54	58	0.340	53	59	0.361
High FPG(%)	15	25	0.693	15	25	0.701
High Tg(%)	15	20	0.976	15	20	0.991
Low HDL- C(%)	20	40	0.301	20	40	0.332
Histological steatosis (% hepatocytes)	-	-	-	18±4	41±6	0.004
Hepatocellular ballooning	-	-	-	0.4±0.2	1.4±0.4	0.032
Lobular inflammation	-	-	-	0.6±0.2	1.8±0.4	0.013
Necro-inflammatory score				1.1±0.5	3.6±0.6	0.005
NAS score	-	-	-	2.0±0.5	5.8±1.0	0.003
Fibrosis stage	-	-	-	0.5±0.2	1.6±0.4	0.021
Patients with NASH (%)	-	-	-	15%	80%	0.002

Data are expressed as mean±SEM. Statistically significant P values are written in bold characters.

Abbreviations: BP: blood pressure; C: cholesterol; CRP: C-reactive protein; Dia: diastolic; HOMA-IR: homeostasis model assessment of insulin resistance; ICAM: intercellular adhesion molecule; METS: Metabolic equivalent of activity (h/week); MTP: microsomal triglyceride transfer protein; SREBF: sterol regulatory element-binding factor; Sys: systolic; T2DM: type 2 diabetes mellitus; Tg: triglycerides;

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Met Sy: metabolic syndrome according to the joint statement of AHA, IDF and NHLBI, requires the presence of ≥ 3 of the following criteria:

-abdominal obesity: waist circumference ≥ 102 cm (males) and ≥ 88 cm (females)

-high triglycerides: ≥ 150 mg/dL (1.7 mmol/L) or on drug treatment for elevated triglycerides

-low HDL-C: < 40 mg/dL (1.0 mmol/L) (males) or < 50 mg/dL (1.3 mmol/L) (females) or on drug treatment for reduced HDL-C

-hypertension: systolic BP ≥ 130 and/or diastolic BP ≥ 85 mm Hg or on drug treatment

-high fasting plasma glucose (FPG): FPG ≥ 100 mg/dL (5.6 mmol/L) or on drug treatment for elevated glucose.