Serum paraoxonase 1 activity is paradoxically maintained in nonalcoholic fatty liver disease despite low HDL cholesterol[S]

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Abstract **Nonalcoholic fatty liver disease (NAFLD) is characterized by low HDL cholesterol, but the activity of the HDL-associated antioxidative enzyme paraoxonase-1 (PON-1) remains unclear. To determine the association of PON-1 with suspected NAFLD, we measured serum enzyme activity in 7,622 participants of the Prevention of Renal and Vascular End-Stage Disease cohort. A fatty liver index (FLI) ⩾60, a proxy of NAFLD, was present in 2,083 participants (27.3%) and coincided with increased prevalence of T2D, metabolic syndrome (MetS), (central) obesity, elevated triglycerides, and low HDL cholesterol (all $P < 0.001$). In men and women combined, serum PON-1 activity did not vary according to elevated FLI ($P = 0.98$), whereas in men with elevated FLI PON-1 activity was increased ($P = 0.016$). In multivariable linear regression analyses (adjusted for age, sex, T2D, MetS, alcohol use, and smoking), PON-1 activity was unexpectedly associated with elevated FLI ($\beta = 0.083$; $P < 0.001$). In a sensitivity analysis ($n = 5{,}126$) that excluded subjects with positive cardiovascular history, impaired estimated glomerular filtration rate, elevated urinary albumin excretion, and drug use, PON-1 activity was also independently associated with elevated FLI ($\beta = 0.045$; $P = 0.017$). These results indicate that PON-1 is paradoxically maintained and may even be increased in NAFLD despite inverse associations with metabolic disorders and low HDL cholesterol.**—van den Berg, E. H., E. G. Gruppen, R. W. James, S. J. L. Bakker, and R. P. F. Dullaart. **Serum paraoxonase 1 activity is paradoxically maintained in nonalcoholic fatty liver disease despite low HDL cholesterol.** ***J. Lipid Res.*** **2019.** 60:**168–175.**

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Nonalcoholic fatty liver disease (NAFLD) is emerging as the most common cause of chronic liver disease in the developed world, and its increasing prevalence parallels the obesity epidemic (1–3). The spectrum of NAFLD comprises simple hepatic steatosis, nonalcoholic steatohepatitis, fibrosis, and eventually cirrhosis (1). NAFLD is commonly seen as the liver manifestation of metabolic syndrome (MetS) (4) but in itself may represent a risk marker for the development of MetS and T2D (5). Elevations in apoB-containing lipoproteins are frequently found in NAFLD together with decreased levels of HDL cholesterol (6–8), which probably at least in part explains why patients with NAFLD may also be predisposed to atherosclerotic CVD (9, 10).

Paraoxonase-1 (PON-1) is a calcium-dependent esterase enzyme that can hydrolyze lipid peroxides (11, 12). PON-1 has important antioxidative and anti-inflammatory properties (11–15) that likely contribute to its alleged protection against atherosclerotic vascular damage (16, 17). PON-1 is primarily synthesized in the liver (18–20) and secreted in the circulation (11). In the liver, PON-1 is believed to counteract oxidative stress-mediated hepatocyte injury (21–23). In serum, PON-1 is primarily bound to HDL and to some extent to VLDLs and protects LDLs from oxidative modification (11, 12, 24, 25). The transfer of circulating HDL-associated PON-1 to cell membranes may also contribute to the oxidative damage protective properties of the enzyme (26).

Several cross-sectional small-scale reports have shown that serum PON-1 activity is decreased in the context of chronic liver disease, showing a reduced ability of HDL to retard LDL oxidation and an inverse association in regulating

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; hsCRP, high-sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; FLI, fatty liver index; GGT, γ-glutamyltransferase; HSI, hepatic steatosis index; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; PON-1, paraoxonase-1; PREVEND, Prevention of Renal and Vascular End-Stage Disease; UAE, urinary albumin excretion.

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oxidative stress, fibrosis, and cell apoptosis (21, 27). Furthermore, it has been suggested that PON-1 measurement could improve the assessment of impaired liver function (28). Surprisingly limited data are available with respect to possible alterations in serum PON-1 in the context of NAFLD despite extensive evidence that shows that serum PON-1 is decreased in MetS and T2D, particularly in relation to low HDL cholesterol (11, 13, 29, 30). In rat and sheep models, diet-induced (e.g., a high-fat diet) hepatic steatosis may reduce serum PON-1 activity (23, 31). So far, only a few studies have addressed the impact of NAFLD on serum PON-1 regulation in humans. In a Turkish study, serum PON-1 activity toward paraoxon was decreased in 49 individuals with NAFLD confirmed by ultrasound compared with 25 control subjects (32). Likewise, serum PON-1 activity toward paraoxon was impaired in 50 Egyptian subjects with NAFLD compared with 20 healthy subjects (33). In contrast, a report from Iran demonstrated that serum PON-1 activity, measured as its arylesterase activity, was elevated in 83 patients with NAFLD compared with 138 healthy subjects (34).

In the absence of large-scale studies among Caucasian subjects that aim to investigate the impact of NAFLD on serum PON-1, we initiated this study to determine the extent to which serum PON-1 activity is associated with NAFLD. To this end we carried out a cross-sectional analysis among 7,622 men and women participating in the Prevention of Renal and Vascular End-Stage Disease (PREVEND) cohort, which comprises a large and well-characterized population from the north of the Netherlands.

MATERIALS AND METHODS

Study population

The study was approved by the Medical Ethics Committee of the University Medical Center Groningen and was performed in accordance with Declaration of Helsinki guidelines. The study included participants of the PREVEND cohort study (35, 36). Pregnant women and diabetic subjects using insulin were not allowed to participate. All participants with a urinary albumin concentration ⩾10 mg/l were invited to our clinic together with randomly selected subjects with a urinary albumin concentration <10 mg/l. The initial study population comprised 8,592 subjects who completed the total screening program. All participants gave written informed consent. For this study, we excluded subjects for which data on serum PON-1 activity and liver function tests were not available, leaving a study population of 7,622 participants.

Measurements and definitions

BMI was calculated as weight (kilograms) divided by height (meters) squared. Waist circumference was measured as the smallest girth between the rib cage and iliac crest. The waist-hip ratio was determined as the waist circumference divided by the largest girth between the waist and thigh. Blood pressure was measured using an automatic device. T2D was defined as a fasting glucose ⩾7.0 mmol/l, a random glucose ⩾11.1 mmol/l, self-report of a physician diagnosis, or the use of glucose-lowering drugs. Alcohol consumption was defined as ⩾10 g/day, with one alcoholic drink being assumed to contain 10 g alcohol. Smoking was categorized as either current or never/former. Urinary albumin excretion (UAE) was measured as described in two 24-h urine collections, and the results were averaged for analysis. The estimated glomerular filtration rate (eGFR) was calculated by applying the combined creatinine cystatin C-based chronic kidney disease epidemiology collaboration equation (37). Information on medication use was combined with information from a pharmacy-dispensing registry that has complete information on drug usage of >95% of subjects in the PREVEND study. Venous blood samples were drawn after an overnight fast after the participants had rested for 15 min.

For the diagnosis of NAFLD, the algorithm of the fatty liver index (FLI) was used in subjects from the PREVEND cohort study (10). The FLI was calculated as $[e^{(0.953 \times \log_e(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log_e(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745)} / [1 + e^{(0.953 \times \log_e(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log_e(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745)}] \times 100$, where GGT is γ-glutamyltransferase (38). The optimal cutoff value for the FLI has been documented to be 60 with an accuracy of 84%, sensitivity of 61%, and specificity of 86% for detecting NAFLD as determined by ultrasonography (38). The FLI is currently considered as one of the best-validated steatosis scores (39) and was validated in a Caucasian population from Italy that conforms to our Western European population. Alternatively, we used the hepatic steatosis index (HSI) (40). The HSI (evaluated in an Asian population in the Republic of Korea) was estimated as follows: HSI = 8 × alanine aminotransferase (ALT)-aspartate aminotransferase (AST) ratio + BMI (+2, if diabetes; +2, if female). The cutoff value of the HSI for detecting NAFLD is 36 (40). MetS was defined according to the revised National Cholesterol Education Program Adult Treatment Panel III criteria (41). Three or more of the following criteria were required to categorize subjects with MetS: waist circumference >102 cm for men and >88 cm for women; plasma triglycerides ⩾1.7 mmol/l; HDL cholesterol <1.0 mmol/l for men and <1.3 mmol/l for women; hypertension (blood pressure ⩾130/85 mm Hg or the use of antihypertensive medication); and hyperglycemia (fasting glucose ⩾5.6 mmol/l or the use of glucose-lowering drugs).

Laboratory methods

Heparinized plasma and serum samples were obtained by centrifugation at 1,400 *g* for 15 min at 4°C. Plasma and serum samples were stored at −80°C until analysis. Glucose was measured directly after blood collection. Plasma total cholesterol, triglycerides, and HDL cholesterol were measured as described previously (35, 36). Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. LDL cholesterol was calculated by the Friedewald formula if triglycerides were <4.5 mmol/l. Serum ALT and AST were measured using the standardized kinetic method with pyridoxal phosphate activation (Roche Modular P; Roche Diagnostics, Mannheim, Germany). Serum GGT was assayed by an enzymatic colorimetric method (Roche Modular P). ALT, AST, and GGT were standardized according to International Federation of Clinical Chemistry guidelines (42–44). High-sensitivity C-reactive protein (hsCRP) was assayed by nephelometry. Serum creatinine was measured by an enzymatic method on a Roche modular analyzer. Serum cystatin C was measured by a cystatin C immunoassay (Gentian AS, Moss, Norway) on a Roche modular analyzer. Urinary albumin was measured by nephelometry (Dade Behring Diagnostics, Marburg, Germany). Serum PON-1 enzymatic activity was measured as its arylesterase activity, i.e., as the rate of hydrolysis of phenyl acetate into phenol, as described previously (13, 17). The interassay coefficient of variation was 8%. Arylesterase activity, as measured with this assay, is positively correlated with PON-1 enzymatic activity toward paraoxon as well as with PON-1 mass (45).

Statistical analysis

SPSS version 23.0 (IBM, Armonk, NY) was used for data analysis. Results are expressed as mean ± SDs or medians (interquartile

ranges) unless otherwise stated. Between-group differences in variables were determined by unpaired *t*-tests or by Chi-square tests where appropriate. Triglycerides, transaminases, GGT, hsCRP, UAE, and PON-1 activity values were $\log_e$-transformed for analysis to achieve approximately normal distributions. Multivariable linear regression analyses were carried out to disclose the independent associations of PON-1 activity with an elevated FLI and HSI when taking account of clinical covariates and laboratory parameters. $P < 0.05$ was considered significant.

RESULTS

Clinical and laboratory characteristics according to elevated FLI and HSI

The study population consisted of 7,622 subjects, of which 2,083 (27.3%) were categorized with an FLI ⩾60. **Table 1** shows the clinical characteristics and laboratory data of the participants according to the FLI categorization. Subjects with an FLI ⩾60 were older and more likely to be men (men: 68.2%; women: 42.8%), were more likely to be classified with MetS and T2D, and had a positive cardiovascular history more frequently. Subjects with an elevated FLI also used antihypertensive, glucose-lowering, and lipid-lowering drugs more frequently. BMI, waist circumference, waist-hip ratio, systolic and diastolic blood pressure, plasma glucose, hsCRP, transaminases and GGT, UAE, total cholesterol, non-HDL cholesterol, LDL cholesterol, and triglycerides were higher in subjects with an elevated FLI, but eGFR and HDL cholesterol were lower in subjects with an elevated FLI (Table 1). Alcohol consumption ⩾10 g/day was recorded in subjects with an elevated FLI more frequently. Cigarette smoking was not different between subjects with and without an elevated FLI. Remarkably, serum PON-1 activity was not different between subjects with and without an elevated FLI. In a sex-stratified analysis, PON-1 activity was significantly higher in men with an FLI ⩾60 compared with men with an FLI <60 (supplemental Table S1). Of all women included, 41.9% were postmenopausal, and 23.5% were using oral contraceptives. PON-1 was significantly higher in women using oral contraceptives (59.61 vs. 52.63 U/L; $P < 0.001$) and varied according to menopausal status (premenopausal: 54.69 U/l; postmenopausal: 53.42 U/l; $P = 0.021$).

In subjects with an HSI >36 versus subjects with an HSI ⩽36, essentially similar differences in glucose, lipid levels, hsCRP, and UAE were also found (**Table 2**). PON-1 activity was also not decreased in subjects with an elevated HSI, also when subdivided into men and women separately (supplemental Table S2).

Subjects with an FLI ⩾60 and HSI >36 showed 78.5% overlap. In this overlap group serum PON-1 activity was not different between subjects with and without suspected NAFLD (supplemental Table S3).

Independent positive association of PON-1 activity with elevated FLI and HSI

Multivariable linear regression analyses were subsequently performed to establish any association of PON-1 activity with an elevated FLI (**Table 3**). Remarkably, in age- and sex-adjusted analyses, PON-1 activity was positively

TABLE 1. Clinical and laboratory characteristics of serum paraoxonase-1 activity in 5,539 subjects with an FLI <60 and 2,083 subjects with an FLI ⩾60

	FLI <60	FLI ⩾60	*P*
Age (years)	47.8 ± 12.5	54.8 ± 11.5	<0.001
Sex [men/women (*n*)]	2,375/3,164	1,422/661	<0.001
T2D (*n*)	92	177	<0.001
MetS (*n*)	476	1,310	<0.001
History of CVD (*n*)	398	344	<0.001
Current smokers (*n*)	1,873	695	0.77
Alcohol intake ⩾10 g/day (*n*)	1,301	616	<0.001
Antihypertensive medication (*n*)	576	600	<0.001
Glucose-lowering drugs (*n*)	59	74	<0.001
Lipid-lowering drugs (*n*)	247	236	<0.001
Systolic blood pressure (mm Hg)	125 ± 19	140 ± 19	<0.001
Diastolic blood pressure (mm Hg)	72 ± 9	79 ± 9	<0.001
BMI (kg/m^2)	24.4 ± 2.9	30.5 ± 4.1	<0.001
Waist circumference (cm)	83 ± 10	103 ± 9	<0.001
Waist-hip ratio	0.85 ± 0.08	0.96 ± 0.07	<0.001
Glucose (mmol/l)	4.65 ± 0.82	5.42 ± 1.63	<0.001
hsCRP (mg/l)	0.97 (0.44–2.33)	2.38 (1.20–4.91)	<0.001
ALT (U/l)	18 (14-24)	28 (20–39)	<0.001
AST (U/l)	23 (20–27)	27 (23–32)	<0.001
GGT (U/l)	20 (14–28)	43 (29–65)	<0.001
eGFR (ml/min/1.73 m^2)	97.2 ± 16.5	89.2 ± 17.8	<0.001
UAE (mg/24 h)	8.4 (6.0–14.0)	14.0 (8.0–33.1)	<0.001
Total cholesterol (mmol/l)	5.46 ± 1.08	6.10 ± 1.11	<0.001
Non-HDL cholesterol (mmol/l)	4.04 ± 1.13	5.01 ± 1.13	<0.001
LDL cholesterol (mmol/l)	3.54 ± 1.02	4.05 ± 1.01	<0.001
HDL cholesterol (mmol/l)	1.41 ± 0.40	1.09 ± 0.30	<0.001
Triglycerides (mmol/l)	1.00 (0.76–1.33)	1.90 (1.38–2.65)	<0.001
PON-1 activity (U/l)	53.1 (43.2–65.2)	53.3 (43.7–64.7)	0.98

Data are means ± SDs or medians (interquartile ranges) unless otherwise stated. LDL cholesterol was calculated by the Friedewald formula in 5,509 subjects with an FLI <60 and in 1,973 subjects with an FLI ⩾60.

TABLE 2. Clinical and laboratory characteristics of serum paraoxonase-1 activity in 5,117 subjects with an HSI ⩽36 and 2,505 subjects with an HSI >36

	HSI ⩽36	HSI >36	*P*
Age (years)	48.4 ± 12.8	52.4 ± 11.9	<0.001
Sex [men/women (*n*)]	2,499/2,618	1,298/1,207	0.016
T2D (*n*)	70	199	<0.001
MetS (*n*)	531	1,255	<0.001
History of CVD (*n*)	428	314	<0.001
Current smokers (*n*)	1,847	721	<0.001
Alcohol intake ⩾10 g/day (*n*)	1,318	599	0.093
Antihypertensive medication (*n*)	572	604	<0.001
Glucose-lowering drugs (*n*)	42	91	<0.001
Lipid-lowering drugs (*n*)	268	215	<0.001
Systolic blood pressure (mm Hg)	125 ± 19	137 ± 20	<0.001
Diastolic blood pressure (mm Hg)	72 ± 10	77 ± 9	<0.001
BMI (kg/m^2)	24.0 ± 2.5	30.3 ± 3.9	<0.001
Waist circumference (cm)	83 ± 10	99 ± 11	<0.001
Waist-hip ratio	0.86 ± 0.09	0.93 ± 0.09	<0.001
Glucose (mmol/l)	4.64 ± 0.80	5.30 ± 1.57	<0.001
hsCRP (mg/l)	0.97 (0.43–2.35)	2.07 (1.00–4.41)	<0.001
ALT (U/l)	18 (14–23)	28 (20–39)	<0.001
AST (U/l)	23 (20–27)	25 (22–31)	<0.001
GGT (U/l)	20 (14–30)	33 (23–53)	<0.001
eGFR (ml/min/1.73 m^2)	96.3 ± 17.0	92.3 ± 17.4	<0.001
UAE (mg/24 h)	8.7 (6.1–15.2)	11.3 (7.0–24.1)	<0.001
Total cholesterol (mmol/l)	5.49 ± 1.11	5.92 ± 1.11	<0.001
Non-HDL cholesterol (mmol/l)	4.09 ± 1.18	4.75 ± 1.15	<0.001
LDL cholesterol (mmol/l)	3.54 ± 1.04	3.95 ± 1.02	<0.001
HDL cholesterol (mmol/l)	1.41 ± 0.41	1.17 ± 0.34	<0.001
Triglycerides (mmol/l)	1.03 (0.77–1.43	1.51 (1.08–2.20)	<0.001
PON-1 activity (U/l)	53.1 (43.1–65.2)	53.5 (43.7–64.9)	0.74

Data are means ± SDs or medians (interquartile ranges) unless otherwise stated. LDL cholesterol was calculated by the Friedewald formula in 565 subjects with an HSI ⩽36 and in 2,417 subjects with an HSI >36.

associated with an elevated FLI (Table 3; model 1). This positive association was also demonstrated after additionally adjusting for the presence of T2D, MetS, alcohol consumption, and current smoking (Table 3; model 2), as well as after further adjusting for individual MetS components (Table 3; model 3). The association of PON-1 activity with an elevated FLI also remained present after additionally adjusting for eGFR, UAE, a positive cardiovascular history, and the use of antihypertensive medication, glucose-lowering drugs, and lipid-lowering drugs (Table 3; model 2: β = 0.081, $P < 0.001$; model 3: β = 0.055, $P < 0.001$). In an alternative analysis, with an elevated HSI instead of an elevated FLI, a similar positive association of PON-1 activity with an elevated HSI was found (**Table 4**). This association also remained present in a fully adjusted analysis (Table 4; model 2: β = 0.037, $P < 0.001$; model 3: β = 0.036, $P = 0.01$). In these analyses, there were positive associations of alcohol consumption and inverse associations with smoking, as well as inverse associations of PON-1 activity with low HDL cholesterol and elevated triglycerides. When adjusted for

TABLE 3. Multivariable regression analysis demonstrating the positive association of serum paraoxonase-1 activity with an elevated FLI (⩾60) after adjusting for clinical and laboratory covariates in 7,622 subjects

	Model 1[a]		Model 2[b]		Model 3[c]	
	β	*P*	β	*P*	β	*P*
Age	−0.106	<0.001	−0.097	<0.001	−0.124	<0.001
Sex (men vs. women)	−0.074	<0.001	−0.094	<0.001	−0.100	<0.001
FLI ⩾60 vs. <60	0.043	<0.001	0.083	<0.001	0.064	<0.001
T2D (yes/no)			−0.032	0.007		
MetS (yes/no)			−0.066	<0.001		
Alcoholic intake ⩾10 g/day			0.088	<0.001	0.069	<0.001
Current smoking (yes/no)			−0.078	<0.001	−0.059	<0.001
Elevated blood pressure					0.023	0.083
Enlarged waist					−0.006	0.67
Elevated glucose					−0.037	0.002
Low HDL cholesterol					−0.183	<0.001
Elevated triglycerides					0.088	<0.001

[a]Adjusted for age and sex.
[b]Adjusted for age, sex, T2D, MetS, alcohol consumption, and current smoking.
[c]Adjusted for age, sex, waist circumference, and individual MetS criteria (elevated blood pressure, enlarged waist, elevated glucose, low HDL cholesterol).

TABLE 4. Multivariable regression analysis demonstrating the positive association of serum paraoxonase-1 activity with an elevated HSI (>36) after adjusting for clinical and laboratory covariates in 7,622 subjects

	Model 1[a]		Model 2[b]		Model 3[c]	
	β	*P*	β	*P*	β	*P*
Age	−0.009	<0.001	−0.091	<0.001	−0.122	<0.001
Sex (men vs. women)	−0.066	<0.001	−0.079	<0.001	−0.089	<0.001
HSI ⩾36 vs. <36	0.020	0.075	0.038	0.003	0.038	0.007
T2D (yes/no)			−0.034	0.005		
MetS (yes/no)			−0.038	0.005		
Alcoholic intake ⩾10 g/day			0.091	<0.001	0.072	<0.001
Current smoking (yes/no)			−0.075	<0.001	−0.057	<0.001
Elevated blood pressure					0.025	0.058
Enlarged waist					0.005	0.73
Elevated glucose					−0.036	0.003
Low HDL cholesterol					−0.183	<0.001
Elevated triglycerides					0.102	0.003

[a]Adjusted for age and sex.
[b]Adjusted for age, sex, T2D, MetS, alcohol consumption, and current smoking.
[c]Adjusted for age, sex, waist circumference, and individual MetS criteria (elevated blood pressure, enlarged waist, elevated glucose, low HDL cholesterol).

oral contraceptives and menopausal status in a multivariable regression analysis, a positive association of PON-1 activity with an elevated FLI (data not shown) remained.

A sensitivity analysis of 5,126 subjects, which was performed after excluding subjects with a positive cardiovascular history, eGFR <60 ml/min/1.73 m^2, and UAE >30 mg/24 h and those using antihypertensive, glucose-lowering, and lipid-lowering drugs, also demonstrated an independent positive association of PON-1 activity with an elevated FLI independent of the presence of T2D, MetS, individual MetS components, alcohol consumption, and smoking (**Table 5**).

DISCUSSION

This cross-sectional study was carried out in a large, predominantly Caucasian population. We used an elevated FLI (38), and in alternative analyses an elevated HSI (40), as a proxy of NAFLD, thereby following recent international guidelines that recommend the use of biomarker-derived algorithms to categorize subjects with probable NAFLD in large-scale studies (39). Unexpectedly, in a univariate analysis, serum PON-1 activity was neither decreased in subjects with an elevated FLI nor in those with an elevated HSI (Tables 1, 2) despite increased prevalence of T2D and MetS as well as low levels of HDL cholesterol (6–8, 46). In multivariable linear regression analyses, serum PON-1 activity was positively and independently associated with an elevated FLI (Table 3) in addition to anticipated inverse associations of PON-1 activity with T2D, MetS, current smoking, and alcohol consumption ⩾10 g/day (8, 17, 47, 48). Analyses with an elevated HSI instead of an elevated FLI (Table 4) confirmed these findings, whereas a sensitivity analysis (Table 5), in which subjects with a cardiovascular history, impaired renal function, and elevated UAE and those using medication were excluded, also demonstrated an independent positive association of serum

TABLE 5. Multivariable regression analysis demonstrating the positive association of serum paraoxonase-1 activity with an elevated FLI (⩾60) after adjusting for clinical and laboratory covariates in 5,126 subjects, excluding subjects with a positive cardiovascular history, impaired estimated glomerular filtration rate, and elevated UAE and those using antihypertensive, glucose-lowering, and lipid-lowering drugs

	Model 1[a]		Model 2[b]		Model 3[c]	
	β	*P*	β	*P*	β	*P*
Age	−0.062	<0.001	−0064	<0.001	−0.085	<0.001
Sex (men vs. women)	−0.078	<0.001	−0.093	<0.001	−0.106	<0.001
FLI ⩾60 vs. <60	0.031	0.030	0.050	0.003	0.045	0.017
T2D (yes/no)			−0.012	0.38		
MetS (yes/no)			−0.032	0.057		
Alcoholic intake ⩾10 g/day			0.074	<0.001	0.051	<0.001
Current smoking (yes/no)			−0.067	<0.001	−0.046	0.001
Elevated blood pressure					0.037	0.013
Enlarged waist					−0.020	0.23
Elevated glucose					−0.001	0.97
Low HDL cholesterol					−0.185	<0.001
Elevated triglycerides					0.076	<0.001

[a]Adjusted for age and sex.
[b]Adjusted for age, sex, T2D, MetS, alcohol consumption, and current smoking.
[c]Adjusted for age, sex, waist circumference, and individual MetS criteria (elevated blood pressure, enlarged waist, elevated glucose, low HDL cholesterol).

PON-1 activity with an elevated FLI. Taken together, this large-scale study thus paradoxically shows that an elevated FLI per se, as a proxy of NALFD, is not associated with impaired serum PON-1 activity but instead may relate to higher circulating PON-1 activity.

In two previous studies demonstrating reduced serum PON-1 activity in the context of NAFLD, paraoxon was used as a substrate (32, 33). In a third report, serum PON-1 arylesterase activity was elevated, whereas PON-1 paraoxonase activity was not different in patients with NAFLD (34). In the current study, we assayed PON-1 enzymatic activity as its arylesterase activity with phenyl acetate as a substrate. PON-1 arylesterase activity has an approximately normal distribution after logarithmic transformation and has been previously reported to be less variable between subjects compared with its activity toward paraoxon (13, 17, 45). Therefore, we preferred to measure serum PON-1 arylesterase activity, considering that this assay method would be more sensitive compared with paraoxonase activity in testing PON-1 differences between subjects with and without an elevated FLI, as well as in multivariable linear analysis. Thus, it is possible that the difference in the substrate used to assay PON-1 activity could in part explain the discrepancy with earlier reports (32–34). Another consideration is the number of study participants, which was more than 30-fold higher in this study that included PREVEND study participants compared with earlier reports (32–34). Furthermore, it cannot be excluded that differences in ethnical background, i.e., Caucasian subjects versus subjects of Turkish or Egyptian descent, could to some extent be responsible for the apparent discrepancies.

The robust relationship of circulating PON-1 with HDL cholesterol and the concentration of HDL particles is well established (11, 13, 24). Accordingly, in multivariable linear regression analyses serum PON-1 activity was strongly associated with HDL cholesterol independent of alcohol consumption, smoking, and other MetS components. Notably, serum PON-1 activity was also positively associated with high triglycerides (Tables 3–5). Circulating PON-1 is to some extent bound to VLDL (25), and it is known that enhanced VLDL secretion is a feature of NAFLD (49), which could explain why serum PON-1 activity may be increased in the context of NAFLD. PON-1 release out of Chinese ovary cells and hepatocytes is stimulated by VLDL in vitro, thereby affecting PON-1 secretion and metabolism (25). PON-1 secreted by this pathway is active and retains its antioxidative functionality (25). Obviously, the precise mechanisms responsible for the association of higher circulating PON-1 with NAFLD remains to be established. Moreover, while this report essentially rules out reduced serum PON-1 activity as a feature of NAFLD, we cannot exclude a role of disturbed intracellular PON-1 activity in the pathogenesis of NAFLD, as shown in rodent models of hepatic steatosis (22, 23).

Several other methodological aspects of this study need to be considered. First, we performed a cross-sectional analysis. For this reason neither cause-and-effect relationships can be established with certainty nor can the possibility of reversed causation be excluded. Second, an elevated FLI was chosen to categorize subjects with suspected NAFLD. The FLI is considered to have sufficient accuracy for NAFLD assessment, and its use is in line with international recommendations to apply steatosis scores to characterize NAFLD in larger-sized cohorts (38, 39). Moreover, the positive association of serum PON-1 activity with suspected NAFLD was confirmed by using the HSI as an alternative proxy for NAFLD. Performing liver ultrasound was not feasible in the setup of the large-scale PREVEND cohort study. Third, we could not differentiate between simple hepatic steatosis and hepatic fibrosis or determine their association with circulating PON-1. Fourth, it is possible that the PREVEND cohort contains a high percentage of individuals with microalbuminuria. For this reason, we adjusted for eGFR and UAE in multivariable linear regression analyses and carried out a sensitivity analysis that excluded subjects with impaired eGFR and elevated UAE. This analysis yielded a similar positive and independent association of serum PON-1 activity with suspected NAFLD. In conclusion, we suggest that the circulating activity of the antioxidative enzyme PON-1 is paradoxically maintained and may even be increased in NAFLD despite inverse associations of PON-1 activity with T2D, MetS, and low HDL cholesterol.

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