Journal of Translational Medicine

RESEARCH

Open Access

Liver steatosis is positively associated with plasminogen activator inhibitor-1 in apparently healthy individuals with overweight and obesity: A FibroScan-Based Cross-Sectional study

Giuseppe Lisco[1*†], Davide Guido[2†], Nicole Cerabino[3], Martina Di Chito[3], Rosanna Donvito[3], Caterina Bonfiglio[2], Endrit Shahini[4], Marianna Zappimbulso[4], Cristiana Randazzo[5,6], Domenico Barletta[7], Dolores Stabile[8], Anna Ancona[8], Sergio Coletta[8], Pasqua Letizia Pesole[8], Gianluigi Giannelli[9] and Giovanni De Pergola[3]

Abstract

Background Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common cause of steatotic liver disease and has major implications on cardiovascular safety.

Study aims As the precise role linking MASLD to cardiovascular diseases is still unclear, the present study aims to investigate the association between liver steatosis and fibrosis and circulating plasminogen activator inhibitor-1 (PAI-1) levels.

Methods Eighty-two patients (41.6 ± 12.4 yrs, 34 men, 41%), naïve to medications, who attended the Nutrition Center for the Research and Care of Obesity and Metabolic Diseases at the National Institute of Gastroenterology "Saverio de Bellis" for weight management, were cross-sectionally evaluated. Demographic, anthropometric, clinic, and laboratory data were collected and analyzed. All patients underwent liver ultrasonographic assessment by FibroScan to diagnose liver steatosis (controlled attenuation parameter or CAP > 275 dBm) and fibrosis (liver stiffness > 8.2 kPa).

Results Sixty-one individuals (74.4%) had liver steatosis, and 17 (20.7%) had liver fibrosis. PAI-1 mean levels were 3,261 ± 1,270 pg/mL, mean body mass index (BMI) and waist circumference (WC) values were 36.6 ± 7.1 kg/m^2 and 114.1 ± 16.5 cm, respectively. Mild systolic and diastolic arterial pressure elevation and significantly high values of fasting plasma insulin (19.6 ± 12.6 IU/mL) and homeostatic model assessment of insulin resistance or HOMA-IR (4.8 ± 3.5) were also found. CAP values were correlated with several anthropometric, clinical, and laboratory

†Giuseppe Lisco and Davide Guido contributed equally to this work as first authors.

*Correspondence:
Giuseppe Lisco
giuseppe.lisco@uniba.it

Full list of author information is available at the end of the article

parameters of insulin resistance. We found a significant association between PAI-1 and CAP (β = 1.605; $p = 0.004$), and noteworthily, when PAI-1 increased by 100 units, the expected variation of CAP values was by + 1.6 units ($p = 0.004$). Notably, the association was independent of gender, age, and insulin resistance.

Discussion Circulating PAI-1 levels are correlated with liver steatosis and, to a lesser extent, fibrosis in apparently healthy patients with a BMI ≥ 25 kg/m^2. This is the first study to show these results in patients naïve to medications, using FibroScan assessment. The bidirectional relationship between circulating PAI-1 levels and CAP measurement highlights the relevance of our research from a diagnostic and pathophysiological-prognostic viewpoint. Longitudinal trials are needed to clarify the cause-effect association between MASLD and PAI-1 levels.

Keywords Obesity, Overweight, Liver steatosis, Liver fibrosis, Fibroscan, PAI-1

Introduction

Steatotic liver disease (SLD) encompasses a wide spectrum of disorders, ranging from metabolic dysfunction-associated steatotic liver disease (MASLD) to alcohol or predominantly alcohol-related liver disease (ALD) [1]. SLD phenotypes include uncomplicated hepatic steatosis, histologically defined as an intracellular accumulation of lipid droplets in at least 5% of the hepatic mass; steatohepatitis, characterized by marked hepatic steatosis, Ito's cells activation prompting intrahepatic inflammation, and fibrosis; progression to cirrhosis and hepatocellular carcinoma [2]. Owing to a drastic reduction in cases of chronic hepatitis C due to the development of direct antiviral agents [3], the relatively constant number of cases of chronic alcohol-related liver disease [4], and the spread of metabolic disorders associated with weight excess, SLD and particularly MASLD are now the most prevalent chronic liver diseases worldwide [5]. About one in four individuals of the general population can be affected by SLD, and the incidence increases among those with prediabetes and diabetes mellitus (35%), obesity (50%), and hypercholesterolemia (65%) [6].

When associated with metabolic disorders (MASLD), SLD is responsible for an increased risk of a wide range of non-communicable diseases, including cardiovascular diseases (CV) [7]. An intricate association of insulin signaling disruption, glucose, and lipid metabolism impairment, proinflammatory cytokines, vasoconstrictors, and prothrombotic factors drives the risk of cardiovascular events in MASLD [8]. However, the precise contribution of ectopic, either hepatic or extrahepatic, adipose tissue accumulation and related mechanisms participating in atherosclerosis is unclear. A pivotal study observed that patients with SLD had elevated circulating levels of pro-coagulative factors, including plasminogen-activator inhibitor 1 (PAI-1), independently of weight excess expressed as body mass index (BMI) [9]. Data from mechanistic studies confirmed that PAI-1 is a biomarker of metabolic syndrome, as it was involved in the regulation of lipid metabolism in the liver, systemic insulin resistance, and sarcopenia [10]. PAI-1 is the most important antifibrinolytic factor synthesized by many cells and tissues, such as endothelial cells, fibroblasts, smooth muscle cells, megakaryocytes, monocyte-macrophages, hepatocytes, and cardiomyocytes, to cite the most relevant sources [11]. Following synthesis, PAI-1 is stored in platelets in inactive form [12], or it can accumulate in the subendothelial space, participating in atherosclerotic plaque remodeling [13]. The remaining amount circulates in the bloodstream in the active form, at levels influenced by genetic factors, plasma lipid levels, insulin resistance, adipose tissue expansion, and inflammation [14, 15]. Data from an experimental mice model highlighted that plasma PAI-1 levels were more closely related to fat accumulation and PAI-1 expression in the liver than in adipose tissue, and were mediated by the tumor necrosis factor pathway activity [16]. Similar results were found by other authors, indicating that PAI-1 concentrations were higher in patients with SLD than those without and that the levels of systemic inflammation were strictly and independently related to the visceral adipose tissue expansion [17]. Patients with biopsy-confirmed metabolic dysfunction-associated steatohepatitis (MASH) show higher levels of PAI-1 compared to healthy controls, with a positive correlation between circulating PAI-1 levels and BMI [18]. Moreover, MASH was found to be an independent predictor of systemic inflammation and prothrombotic risk, apart from the BMI and visceral adipose tissue mass [19]. Improvement of MASH, achieved with physical exercise, attenuates inflammation, and significantly reduces PAI-1 levels [20].

Overall, data indicated that systemic inflammation originating at the adipose tissue site could stimulate the hepatic production of PAI-1. On the other hand, as observed for the expansion of the visceral adipose tissue, systemic inflammation is also driven by MASH, which is an independent contributor to the atherothrombotic risk also at endothelial sites, where proinflammatory cytokines may stimulate the synthesis of adhesion molecules to recruit leukocytes, and prothrombotic factors, including PAI-1.

However, compared to other metabolic parameters, the precise role in driving the elevation of PAI-1 levels played by MASLD and liver fibrosis is unclear, as is the

magnitude of this connection as a risk factor. The present study aims to investigate the relationship between PAI-1 circulating levels (a biomarker of the thrombotic risk) and features of liver steatosis and fibrosis assessed with FibroScan in individuals with SLD, overweight or obesity, naïve to medications, and without background chronic diseases, in the context of primary prevention of apparently healthy individuals.

Materials and methods

Study design and population

This cross-sectional study was conducted at the Center of Nutrition for the Research and Care of Obesity and Metabolic Diseases at the National Institute of Gastroenterology "Saverio de Bellis" Research Hospital sited at Castellana Grotte, Bari, Italy. The study is registered on ClinicalTrials.gov with the identifier code NCT05477212. The study protocol was formally approved by the Local Medical Ethics Committee with protocol number 170/CE De Bellis. All the procedures were conducted on human beings in accordance with the 1964 Helsinki Declaration and standards of Good Clinical Practice.

All participants provided written informed consent to participate before study entry. Patients were enrolled from February 2023 to July 2024. Inclusion criteria were age between 18 and 65 years, BMI ≥ 25 kg/m², naïve to medications. Exclusion criteria were established or newly diagnosed diabetes mellitus, CV, respiratory insufficiency, severe gastrointestinal diseases, chronic renal failure (i.e., estimated glomerular filtration rate < 60 mL/min/1.73 m^2), mental diseases, pregnancy and lactation, eating disorders, chronic hepatic diseases with other-than-metabolic etiology, alcohol intake exceeding 30 g/day for men and > 20 g/day for women, substance abuse, frailty, infectious diseases or other acute conditions affecting the levels of inflammation biomarkers, and rare metabolic disorders (e.g., porphyria or deficiencies in carnitine, carnitine–palmitoyl transferase, carnitine–acylcarnitine translocase, pyruvate carboxylase) or mitochondrial fatty acid oxidation disorders.

Cigarette smoking exposure and alcohol consumption were evaluated, and demographic information, anthropometric parameters, and fasting blood samples collected. All patients underwent liver ultrasonographic assessment with FibroScan within one week of enrollment.

Anthropometric parameters

Body weight and height were measured according to a standardized protocol, whereby patients observed overnight fasting, wore light clothing and were barefoot and with an empty bladder. Body mass index (BMI) was calculated according to the standard formula as weight (expressed in kg) divided by the square of height (expressed in m) [21]. Waist circumference (WC) was measured at the midpoint between the iliac crest line and the lower costal margin and expressed in cm. Systolic and diastolic arterial pressures were measured with patients seated at rest using an automated monitor (OMRON M6). Pressure values were measured and recorded three times and then the mean values were calculated.

Definition of associated chronic diseases or comorbidities

Chronic diseases, such as arterial hypertension, dyslipidemia, diabetes mellitus, and established CV diseases, were diagnosed in patients having diagnostic criteria or were already under pharmacological treatment.

Assessment of liver steatosis and fibrosis

FibroScan has proved to be an accurate, low-expensive, and non-invasive tool for estimating liver steatosis and fibrosis in at-risk individuals [22]. Although histological characterization of hepatic tissue is the best method for a precise grading of steatosis, fibrosis, and inflammation, ultrasonographic imaging with FibroScan is a painless alternative to liver biopsy, allowing investigation of the entire liver and is recommended by guidelines as the first-level examination for the diagnosis and staging of liver steatosis and fibrosis [23].

Vibration-controlled transient elastography (VCTE) with the controlled attenuation parameter (CAP) at a frequency of 3.5 MHz estimates the lipid content in the hepatic parenchyma. It was used to assess liver steatosis with the following rule-in cut-off: 248 dB/m for mild steatosis; 268 dB/m for moderate steatosis; 280 dB/m for severe steatosis [23]. Liver stiffness (VCTE-LSM) is be used to estimate extracellular volume fraction and fibrosis in the liver and was used to assess the presence of liver fibrosis with a rule-out cut-off of 8 kPa and a rule-in cut-off of 12 kPa for grade 3 fibrosis [23].

Laboratory tests

Blood samples were collected in the morning between 8:00 and 9:00 am. Samples were processed to extract sera and plasma for the measurement of fasting plasma glucose (FPG), fasting serum insulin, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate-amino transferase (AST), alanine-amino transferase (ALT), gamma glutamyl-transferase (γGT), uric acid, ferritin, creatinine, iron, high-sensitive c-reactive protein, PAI-1, basic thyroid panel test, and 25-hydroxy-vitamin D levels. All measurements were performed with the COBAS 8000 autoanalyzer (ROCHE 182 Diagnostic SPA, Monza, Italy).

Glycated hemoglobin (HbA1c) levels were estimated using the Capillarys 3 OCTA automated capillary electrophoresis system (Sebia Italia S.r.l., Bagno a Ripoli, Florence, Italy).

Insulin resistance was calculated with the homeostasis model assessment of insulin resistance (HOMA-IR) [24], a widely used index, applying the following formula: [FPG (mg/dL) x fasting serum insulin (μ IU/mL)] / 405.

Assessment of physical activity level

The level of physical activity was estimated with the International Physical Activity Questionnaire (IPAQ) [25].

Study outcomes

The primary study outcome was to estimate the frequency of liver steatosis and fibrosis, assessed by FibroScan with controlled attenuation parameter (CAP) and liver stiffness measurement (LSM), respectively, in our study population. The rule-in cut-off for the steatosis definition was CAP > 275 dB/m [26], and the rule-in cut-off for the fibrosis definition was LSM > 8kPa [27]. The primary study endpoint was the Pearson correlation coefficient (r) between CAP and PAI-1.

Variables of exposure and confounders

The exposure variable was PAI-1. Three confounding parameters– gender, age, and HOMA-IR - were considered to better investigate the association between PAI-1 and liver steatosis and fibrosis. In particular, the three factors were included in the analysis to adjust the association estimations between PAI-1 levels and Fibroscan markers (i.e., CAP and LSM).

Statistical analyses

Descriptive statistics were first calculated, with continuous variables reported as mean ± standard deviation (sd) and categorical variables as frequencies. Preliminary Pearson's correlation coefficient (r) was used to evaluate the raw association between FibroScan markers (i.e., CAP and LSM) and PAI-1 and other clinical parameters. Then, multiple linear regression models were applied to evaluate the associations, adjusting the results for gender, age, and HOMA-IR by regression coefficients (β). At the same time, simple linear models were also fitted to evaluate the β (expected) variation after the removal of the adjustment covariate set.

Firth's logistic regression models for small sample size [28] were also applied to assess associations between PAI-1 and binary outcomes of steatosis and fibrosis, computed by dichotomizing FibroScan measures as follows: CAP > 275 dB/m (yes/no), and LSM > 8 kPa (yes/no). These models provided the adjusted odds ratios (OR), accounting for covariates such as gender, age, and HOMA-IR. Notably, in the regression analysis, the PAI-1 regressor was divided by 100 units because of interpretation issues (due to its high variability) of the beta parameter.

Statistical significance was set at p-value < 0.05, calculating 95% confidence intervals (95%CI). Suggestive results ($0.05 < p < 0.10$) were also reported. All statistical analyses were performed using R software [29] and StataCorp 2023 Stata Statistical Software: Release 18 (College Station, TX, USA: StataCorp LLC). Missing data, which accounted for less than 5%, were imputed using the Random Forest algorithm through the missForest package in R [30].

The sample size was calculated with G*power 3.1 (correlation test) based on the primary study endpoint (correlation between PAI-1 levels and CAP values) after considering a mean r value of 0.55, an alfa error of 0.05, and a power (1– beta) value of 0.95. The sample size was 73 patients (lower critical r, -0.02; upper critical r, 0.4).

Results

Table 1 summarizes descriptive statistics. In total, 82 participants (41.6 ± 12.4 yrs, 34 men, 41%) were analyzed. Eighteen subjects (22%) were current smokers and 64 (78%) were past smokers or never smoked. Sixty-one individuals (74.4%) had liver steatosis, comprising 30 men (36.6%) and 31 women (37.8%). Seventeen participants (20.7%) had liver fibrosis, including 9 men (11%) and 8 women (9.8%). The mean CAP and LSM values were 303.5 ± 64.2 dB/m and 6.91 ± 4.1 kPa, respectively. PAI-1 mean levels were 3,261 ± 1,270 pg/mL.

According to anthropometric parameters, the study participants were overweight ($n = 12$, 4 men) or were diagnosed with obesity ($n = 70$, 30 men) with a mean BMI and WC of 36.6 ± 7.1 kg/m^2 and 114.1 ± 16.5 cm, respectively. None had an established diagnosis of chronic CV. Weight excess reflected other associated pathological features, including mild elevation of both systolic and diastolic arterial pressure (130.9 ± 12.1 and 82.6 ± 10.3 mmHg, respectively). None had type 2 diabetes (T2D). Baseline FPG were 97.6 ± 12.4 mg/dL, HbA_{1c} 5.5 ± 0.5%, and fasting plasma insulin was 19.6 ± 12.6 IU/mL. The mean HOMA-IR value was 4.8 ± 3.5, indicating a diagnosis of insulin resistance (n.v. <2.5). Apart from slightly lower-than-normal values of 25-hydroxy-Vitamin D and a modest elevation of total and LDL-C in a population with apparently low cardiovascular risk, all the other laboratory parameters were in normal range.

To visualize the joint distribution between PAI-1 levels and FibroScan measurements, a scatter plot with a regression line between them was also extracted (Fig. 1). Notably, the left-side plot shows the distribution between PAI-1 and CAP values, whereas the right-side plot shows the joint distribution with LSM.

Table 2 presents the results of correlation analysis between FibroScan measurements and circulating PAI-1 levels. A significant direct association was observed with CAP ($r = 0.32$, $p = 0.003$; 95%CI = 0.108; 0.500), whereas

Table 1 Background characteristics of the study population

	Overall (*n* = 82)
Ultrasonographic measures of liver steatosis and fibrosis	
FibroScan CAP (dB/m)	303.5 ± 64.2
FibroScan LSM (kPa)	6.91 ± 4.1
Steatosis* (yes/no; n and %)	61/21 (74.4%/25.6%)
Fibrosis* (yes/no; n and %)	17/65 (20.7%/79.3%)
Variable of exposure	
PAI-1 (pg/mL)	3261 ± 1270
Hallmarks of glucose control and insulin resistance	
FPG (mg/dL)	97.6 ± 12.4
HbA_{1c} (%)	5.5 ± 0.5
Fasting serum insulin (U/mL)	19.6 ± 12.6
HOMA-IR (score)	4.8 ± 3.5
Demographic and lifestyle characteristics	
Age (yrs)	41.6 ± 12.4
Sex (m/f; n and %)	34/48 (41%/59%)
Current smokers (yes/no; n and %)	18/64 (22%/78%)
IPAQ (score)	1819.9 ± 1604.2
Anthropometric and clinical parameters	
BMI (kg/m^2)	36.6 ± 7.1
Waist circumference (cm)	114.1 ± 16.5
Systolic blood pressure (mmHg)	130.9 ± 12.11
Diastolic blood pressure (mmHg)	82.59 ± 10.31
Laboratory tests	
Triglycerides (mg/dL)	122.21 ± 72.59
HDL cholesterol (mg/dL)	51.27 ± 14.63
LDL cholesterol (mg/dL)	137.9 ± 32.72
Total cholesterol (mg/dL)	203.7 ± 40.86
TSH (μmU/mL)	1.988 ± 1.34
FT3 (pg/mL)	3.367 ± 0.40
FT4 (ng/dL)	10.9 ± 1.93
25-hydroxi-vitamin D (ng/mL)	19.95 ± 6.59
Uric acid (mg/dL)	5.368 ± 1.22
Creatinine (mg/dL)	0.805 ± 0.14
AST (U/L)	24.13 ± 13.32
ALT (U/L)	35.72 ± 29.10
γGT (U/L)	29.15 ± 19.50
High-sensitive C-reactive protein (mg/dL)	1.131 ± 5.87
Ferritin (ng/mL)	152.72 ± 166.97

*Steatosis and fibrosis were diagnosed in the presence of CAP > 275 dB/m and LSM > 8 kPa, respectively

Descriptive statistics are shown as mean ± sd or frequencies when appropriate

Abbreviations: CAP, Controlled Attenuation Parameter; LSM, Liver Stiffness Measurement; PAI-1, Plasminogen Activator Inhibitor-1; FPG, Fasting Plasma Glucose; HbA_{1c}, Glycated hemoglobin; HOMA-IR, Homeostasis Model Assessment–Insulin Resistance; IPAQ, International Physical Activity Questionnaire; BMI, Body Mass Index; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; TSH, Thyroid-Stimulating Hormone; FT4, Free Tetraiodothyronine; FT3, Free Triiodothyronine; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; γGT, gamma Glutamyl-transferase

the direct association with LSM was only nearly reached ($r = 0.2$, $p = 0.065$; 95%CI = -0.013; 0.403). The Table 2 also shows the Pearson correlations with other clinical parameters. Notably, HDL-C levels were inversely correlated with liver steatosis (CAP; r = -0.23; $p = 0.035$) but not fibrosis (LSM; r = -0.12; $p = 0.283$). Moreover, we observed a strong direct correlation between CAP values and several anthropometric, clinical, and laboratory parameters of weight excess (BMI and WC), arterial pressure, insulin resistance, glucose control, and liver cytotoxicity (AST and ALT). Similar correlations were also reported for liver fibrosis. We found a direct and statistically significant correlation between circulating PAI-1 levels and fasting serum insulin levels ($r = 0.27$; $p = 0.012$) and HOMA-IR ($r = 0.22$; $p = 0.044$). Moreover, circulating PAI-1 levels were found to be directly correlated with WC ($r = 0.24$; $p = 0.031$), uric acid ($r = 0.25$; $p = 0.022$), GOT ($r = 0.29$; $p = 0.007$), GPT ($r = 0.34$; $p = 0.001$), and γGT ($r = 0.32$; $p = 0.003$) levels, and negatively correlated with HDL-C (r = -0.40; p = < 0.001) as well.

Table 3 shows the results of multiple regression analyses, including the associations between PAI-1 and FibroScan measurements, controlled by gender, age, and HOMA-IR. A significant association was observed for CAP (β = 0.011; $p = 0.026$) but not LSM (β = 0.033; $p = 0.312$). Notably, significant associations were also revealed by simple linear models: CAP (β = 1.605; $p = 0.004$); LSM (β = 0.065; $p = 0.066$). In other words, when PAI-1 increased by 100 units, the expected variation (beta) of CAP values was by + 1.6 units ($p = 0.004$) in the ordinary model and + 1.1 units ($p = 0.312$) in the multiple model. At the same time, the expected variation of the LSM was by + 0.065 units ($p = 0.066$) and + 0.033 units ($p = 0.312$), respectively, without statistical significance. Table 3 also shows Firth's logistic regression models. A significant association between PAI-1 and CAP values was observed in the ordinary model (odds ratio or OR = 1.050, $p = 0.032$). However, it is noteworthy that positive associations (OR > 1) were found in the multiple models for steatosis (CAP, ORs = 1.023; $p = 0.403$) and fibrosis (LSM, ORs = 1.019, $p = 0.458$), but without statistical relevance.

Discussion

Obesity is a common chronic metabolic disorder with a growing incidence and prevalence worldwide and earlier clinical presentation according to trends reported over the last three decades [31]. Consolidated evidence indicates that weight excess is associated with a high burden of chronic diseases and complications, in the context of the so-called metabolic syndrome, such as prediabetes and diabetes mellitus, arterial hypertension, dyslipidemia, CV, cancer, osteoarthritis, and gallbladder diseases

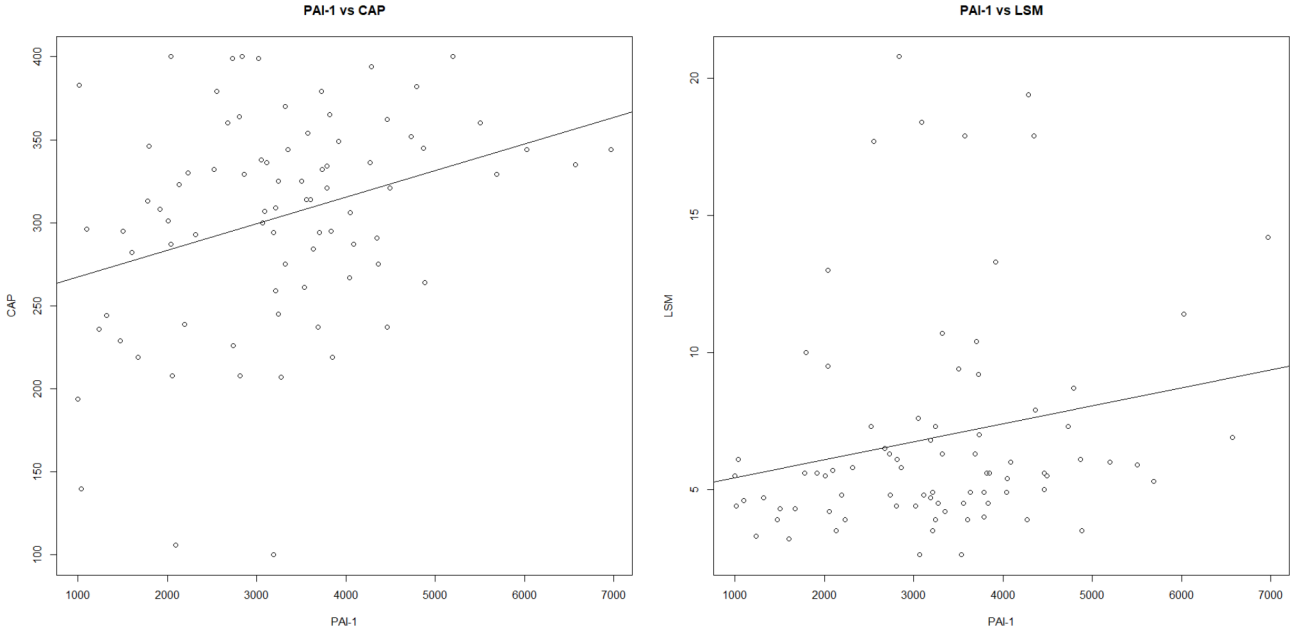

Fig. 1 Scatter plots of PAI-1 and ultrasonographic features of liver steatosis (CAP) and fibrosis (LSM) measurements and regression lines. On the left side, the scatter plot and regression line assessing the relationship between PAI-1 and CAP values. On the right side, plot and regression line assessing the relationship between PAI-1 and LSM values. Abbreviations: CAP, Controlled Attenuation Parameter; LSM, liver stiffness measurement; PAI, Plasminogen Activator Inhibitor-1

[32], significantly contributing to poor health quality [33] and healthcare costs [34].

The association between weight excess and CV is well-defined [35]. Most of the burden depends on potentially modifiable risk factors, already manifest or developing in later years, closely linked to obesity-related metabolic dysfunction [36]. On the other hand, obesity predisposes to atherosclerosis, pulmonary embolism, heart failure, and structural and non-structural cardiac disorders, including tachyarrhythmias and aortic valve stenosis, also well-known risk factors for CV and related mortality [37]. Moreover, obesity is associated with a variable degree of ectopic visceral fat accumulation and subsequent inflammation [38], which is responsible for a wide range of microvascular and macrovascular injuries inducing chronic low-grade inflammation, endothelial dysfunction, and thrombosis [39]. The results of a recently published study from a Danish nationwide registry indicated the presence of a 2-fold higher risk of cardiovascular and cerebrovascular events up to 30 years before the diagnosis of T2D, suggesting that predisposing factors to diabetes (with obesity dominating the pathohistological scenario) act several years before the onset of hyperglycemia in driving the cardiovascular risk [40]. This has been seen as a call to action for researchers and clinicians to counteract the cardiometabolic risk in the early stages of the disease.

More emphasis has been placed on MASLD during the last decade. MASLD is strictly linked to weight excess, particularly obesity, and drives a wide range of dysmetabolic changes, playing a crucial role in cardiovascular risk [41]. From a cardiovascular viewpoint, MASLD can also promote a prothrombotic state. The precise mechanisms driving this condition are unclear but may include the overproduction of coagulation factors, fibrinolysis inhibitors, and endothelial dysfunction as the earlier atherosclerosis precursor [42].

Our study investigated the role of PAI-1, a prothrombotic factor, as a possible determinant of the prothrombotic risk in relatively young, naïve to medication individuals with obesity (85%) individuals at an early stage of the disease with apparently low cardiovascular risk, naïve to medications, in the setting of primary prevention. Remarkably, we found liver steatosis in 3 of 4 participants, while different stages of liver fibrosis were present in 1 out of 4, in men as well as women. The study population was characterized by mildly elevated arterial pressure values and unequivocal laboratory and clinical signs of insulin resistance. The results are substantially in line with other data indicating a global prevalence of MASLD in 70% of examined cases and MASH in 33% [43]. Similar results were found in terms of baseline arterial pressure in a population with comparable characteristics [44].

PAI-1 levels correlated strongly and directly with both laboratory markers and ultrasonographic features of liver steatosis independent of age, gender, and metabolic variables, such as the BMI and HOMA-IR, related

Table 2 Pearson correlations of CAP and LSM with anthropometrical, clinical, and laboratory parameters

	CAP *r* (*p*-value)	LSM *r* (*p*-value)	PAI-1 *r* (*p*-value)
LSM	**0.36 (0.001)**	-	-
PAI-1	**0.32 (0.003)**	*0.20 (0.065)*	-
FPG	**0.22 (0.048)**	**0.23 (0.034)**	0.07 (0.540)
HbA_{1c}	**0.34 (0.002)**	**0.36 (0.001)**	*0.18 (0.096)*
Insulin	**0.48 (< 0.001)**	**0.47 (< 0.001)**	**0.27 (0.012)**
HOMA-IR	**0.48 (< 0.001)**	**0.49 (< 0.001)**	**0.22 (0.044)**
IPAQ	-0.16 (0.154)	-0.03 (0.762)	0.03 (0.767)
BMI	**0.50 (< 0.001)**	**0.65 (< 0.001)**	*0.19 (0.080)*
WC	**0.60 (< 0.001)**	**0.51 (< 0.001)**	**0.24 (0.031)**
Systolic blood pressure	**0.25 (0.024)**	*0.20 (0.073)*	0.05 (0.686)
Diastolic blood pressure	**0.38 (< 0.001)**	0.17 (0.135)	0.16 (0.145)
Triglycerides	**0.32 (0.003)**	0.11 (0.317)	0.17 (0.129)
HDL cholesterol	**-0.23 (0.035)**	-0.12 (0.283)	**-0.40 (< 0.001)**
LDL cholesterol	-0.02 (0.839)	*-0.22 (0.051)*	0.00 (0.968)
Total cholesterol	-0.11 (0.341)	-0.18 (0.108)	-0.11 (0.339)
TSH	*0.21 (0.057)*	-0.01 (0.953)	0.05 (0.669)
FT3	0.06 (0.611)	-0.04 (0.752)	0.18 (0.109)
FT4	**0.25 (0.022)**	0.10 (0.387)	0.03 (0.774)
Vitamin D	-0.06 (0.572)	-0.01 (0.962)	-0.17 (0.133)
Uric acid	**0.40 (< 0.001)**	0.18 (0.102)	**0.25 (0.022)**
Creatinine	*0.19 (0.094)*	0.03 (0.817)	-0.01 (0.903)
AST	**0.31 (0.004)**	**0.38 (< 0.001)**	**0.29 (0.007)**
ALT	**0.37 (< 0.001)**	**0.31 (0.004)**	**0.34 (0.001)**
γGT	**0.33 (0.002)**	**0.28 (0.010)**	**0.32 (0.003)**
Ferritin	**0.38 (< 0.001)**	*0.20 (0.073)*	-0.01 (0.893)

Abbreviations: CAP, Controlled Attenuation Parameter; LSM, Liver Stiffness Measurement; PAI-1, Plasminogen Activator Inhibitor-1; FPG, Fasting Plasma Glucose; HbA_{1c}, Glycated hemoglobin; HOMA-IR, Homeostasis Model Assessment–Insulin Resistance; IPAQ, International Physical Activity Questionnaire; BMI, Body Mass Index; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; TSH, Thyroid-Stimulating Hormone; FT4, Free Tetraiodothyronine; FT3, Free Triiodothyronine; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; γGT, gamma Glutamyl-transferase

r: Pearson correlation coefficient. In **bold**, significant results ($P < 0.05$), in *italics trends* ($0.05 < P < 0.10$)

to the presence of insulin resistance. When considering liver fibrosis, this association was less substantial (and not statistically significant). The leading determinant of this result was the relatively low number of liver fibrosis cases. Conflicting data have been published indicating, on one hand, a positive association between liver fibrosis and circulating PAI-1 levels, and experimentally, attenuation of liver fibrosis with PAI-1 inhibitors [45]. On the other hand, mechanistic studies reported that PAI-1 deficiency is associated with more extensive fibrosis in models of liver injury [46]. So, while early stages of MASLD are characterized by lipid accumulation into hepatocytes and strong inflammation and regeneration phenomena with an intense production of PAI-1 (explaining a direct association between PAI-1 levels and liver steatosis in our report), a fibrotic liver is a steady and metabolically less active tissue in which the association between PAI-1 levels (hepatic contribution) and fibrosis severity is lost, while PAI-1 plays a dual role, both protective (stimulating regeneration) and detrimental (hindering matrix degradation) [47].

According to our models, a 100 unit-increase in PAI-1 levels induces an expected elevation of CAP values by 1.6 units. In view of the bidirectional relationship between PAI-1 levels and CAP values, this could provide suitable diagnostic information for algorithmic tools.

Even despite specific estimation of the associations, an increasing trend of circulating PAI-1 levels towards the classes of hepatic steatosis severity has been observed also in younger individuals with overweight or obesity, posing real challenges in terms of cardiovascular risk at every age in apparently healthy individuals with overweight or obesity [48]. Although insulin resistance was associated with PAI-1 levels, confirming the pathophysiological link between insulin resistance, adipose tissue and systemic inflammation, and overproduction of pro-inflammatory cytokines to stimulate the hepatic and extra-hepatic synthesis and release of PAI-1 [49, 50], our study confirms, in line with another report, that insulin resistance-driven PAI-1 elevation in MASLD is independently mediated by liver steatosis per se instead of being direct consequence of insulin signaling pathway disruption and related consequences (i.e., proinflammatory cytokines and endothelial dysfunction) [51]. Thus, we can hypothesize that MASLD, a hepatic consequence of insulin resistance in metabolic syndrome, can stimulate intrahepatic or extrahepatic synthesis of PAI-1 regardless of systemic insulin resistance and related consequences [16]. In addition, mechanistic data indicate that PAI-1 expression is accentuated and adipose tissue inflammation is elevated in the visceral adipose tissue of mice after exposure to a high-fat diet. On the contrary, PAI-1 deficient mice were resistant to weight gain, visceral adipose tissue expansion, and inflammation, indicating a mechanistic role of PAI-1 in mediating weight gain and low-grade inflammation [52].

The study has some limitations and strengths. The limitations are the monocentric, cross-sectional nature of the research and related biases. Secondly, we considered only surrogate markers of the risks of thrombosis, liver steatosis, and fibrosis. Thirdly, given the nature of the study, the relationship between PAI-1 and liver steatosis could have diagnostic implications (i.e., algorithmic tools) rather than offering inferences on cardiovascular pathophysiology in primary prevention. Moreover, the study lacks data on serum levels of specific cytokines (e.g., interleukin 6 or tumor necrosis factor-alpha) to associate or correlate with PAI-1 levels and on baseline dietary

Table 3 – Results of the regression analysis on the associations between fibroscan measurements with PAI-1 levels

	β of PAI-1 (x100) on Fibroscan CAP	**β of PAI-1 (x100) on Fibroscan LSM**	**OR of PAI-1 (x100) on steatosis**	**OR of PAI-1 (x100) on fibrosis**
	Linear model	**Linear model**	**Firth's logistic models**	**Firth's logistic models**
Ordinary model	**β = 1.605**	*β = 0.065*	**OR = 1.050**	OR = 1.031
	P = 0.004	*P* = 0.066	*P* = 0.032	*P* = 0.144
	95%CI = 0.538; 2.671	*95%CI= -0.004; 0.135*	**95%CI = 1.004; 1.099**	95%CI = 0.989; 1.075
Multiple model*	**β = 1.088**	β = 0.033	OR = 1.023	OR = 1.019
	P = 0.026	*P* = 0.312	*P* = 0.403	*P* = 0.458
	95%CI = 0.135; 2.040	95%CI= -0.031; 0.097	95%CI = 0.969; 1.078	95%CI = 0.970; 1.069

Abbreviations: CAP, Controlled Attenuation Parameter; LSM, Liver Stiffness Measurement; PAI-1, Plasminogen Activator Inhibitor-1; β, linear regression coefficient, i.e., expected variation of the response variable in the model, per a 100-unit increase of PAI-1. OR: odds ratio. P: p-value

In the regression analysis, the PAI-1 regressor was considered divided by 100 units, because of interpretation issues of the β parameter

In **bold** significant results ($P < 0.05$), in *italics* ($0.05 < P < 0.10$) trends; 95%CI: 95% confidence intervals

*Adjusted for gender, age and HOMA

Results of the regression analysis on the associations between FibroScan measurements (outcomes) with PAI-1 levels: by column are presented the results returned by the models in terms of association measures (i.e., β or OR), by row the modelling nature

patterns associated with more extended visceral adipose tissue expansion and inflammation. The strengths are the robustness of the study protocol, consistency of procedures, and methodological approach to data collection, analysis, and interpretation. The use of ultrasonographic hallmarks, instead of algorithms and scores, of liver steatosis and fibrosis improved the quality of results. Moreover, the study aims and results are original, highlighting and quantifying the association between PAI-1 levels and measures of liver steatosis and, possibly, fibrosis. These may pave the way for future longitudinal and intervention studies addressing the association between MASLD and the atherothrombotic risk, with the mediation of PAI-1, for future diagnostic and therapeutic implications [53].

Conclusion

Circulating PAI-1 levels are correlated with liver steatosis and, to a lesser extent, fibrosis in apparently healthy individuals with overweight or obesity, independently of conventional extrahepatic risk factors. To our knowledge, this is the first study to show this association using FibroScan to diagnose liver fibrosis and steatosis in apparently healthy patients naïve to medications.

The bidirectional relationship between circulating PAI-1 levels and CAP measurement highlights the relevance of our research study from a diagnostic and pathophysiological-prognostic viewpoint. On one hand, we have generated a proof-of-concept for predicting diagnostic tools for hepatic steatosis in such an at-risk population according to circulating PAI-1 levels. On the other hand, we put the basis for a better understanding of the existing relationship between MASLD, insulin resistance, and PAI-1 elevation as a possible mediator of cardiovascular risk in apparently healthy individuals with obesity naïve to medications.

Longitudinal studies are needed to clarify the cause-effect association of this phenomenon and better investigate the source of PAI-1 overproduction (hepatic or endothelial) in patients with MASLD.

Abbreviations

SLD	Steatotic Liver Disease
MASLD	Metabolic Dysfunction-Associated Steatotic Liver Disease
ALD	Alcohol-Related Liver Disease
CV	Cardiovascular Diseases
PAI-1	Plasminogen-Activator Inhibitor 1
BMI	Body Mass Index
MASH	Dysfunction-Associated Steatohepatitis
WC	Waist Circumference
CAP	Controlled Attenuation Parameter
LSM	Liver Stiffness Measurement
FPG	Fasting Plasma Glucose
LDL-C	Low-Density Lipoprotein Cholesterol
HDL-C	High-Density Lipoprotein Cholesterol
AST	Aspartate-Amino transferase
ALT	Alanine-Amino transferase
γGT	Gamma Glutamyl-Transferase
HbA1c	Glycated hemoglobin
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
IPAQ	International Physical Activity Questionnaire

Acknowledgements

The authors thank all the participants and investigators for their efforts. We are also grateful to Mrs. Mary Pragnell for English editing.

Author contribution

Giuseppe Lisco: Writing– review & editing, Writing– original draft, Visualization, Methodology. Davide Guido: Writing– review & editing, Writing– original draft, Visualization, Software, Methodology, Formal analysis. Nicole Cerabino: Writing– original draft, Investigation, Data curation. Martina Di Chito: Writing– original draft, Investigation, Data curation. Caterina Bonfiglio: Software, Methodology, Formal analysis. Endrit Shahini: Validation, Methodology, Investigation. Marianna Zappimbulso: Validation, Methodology, Investigation. Domenico Barletta: Writing– review & editing. Sergio Coletta: Methodology, Data curation. Dolores Stabile: Data curation. Anna Ancona: Data curation. Pasqua Letizia Pesole: Methodology, Data curation. Gianluigi Giannelli: Writing– review & editing, Supervision, Resources, Funding acquisition. Giovanni De Pergola: Writing– review & editing, Writing– original draft, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Funding

The research was funded by the Italian Ministry of Health "Ricerca Corrente 2024–2026".

Declaration

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the National Institute of Gastroenterology and Research Hospital (DDG-CE-502/2005; DDG-CE-792/2014, on 20May 2005 and 14 February 2014, respectively).

Competing interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author details

[1]Interdisciplinary Department of Medicine, School of Medicine, University of Bari "Aldo Moro", Bari, Italy
[2]Unit of Data Science, National Institute of Gastroenterology "Saverio de Bellis", IRCCS Hospital, Castellana Grotte, Bari 70013, Italy
[3]Center of Nutrition for the Research and the Care of Obesity and Metabolic Diseases, National Institute of Gastroenterology IRCCS "Saverio de Bellis", Castellana Grotte, 70013 Bari, Italy
[4]Department of Gastroenterology, National Institute of Gastroenterology "Saverio de Bellis", IRCCS Hospital, Castellana Grotte, 70013 Bari, Italy
[5]Dipartimento di Promozione della Salute, Medicina Interna e Specialistica di Eccellenza (PROMISE), Materno-Infantile, University of Palermo, Palermo, Italy
[6]Unit of Clinical Nutrition, Obesity and Metabolic Diseases, AOU Policlinico "P. Giaccone", Palermo, Italy
[7]Laboratory of Clinical Pathology, National Institute of Gastroenterology "Saverio de Bellis", IRCCS Hospital, Castellana Grotte, 70013 Bari, Italy
[8]Core Facility Biobank, National Institute of Gastroenterology "Saverio de Bellis", IRCCS Hospital, Castellana Grotte, Bari 70013, Italy
[9]Scientific Direction, National Institute of Gastroenterology "Saverio de Bellis", IRCCS Hospital, Castellana Grotte, 70013 Bari, Italy

Received: 27 February 2025 / Accepted: 6 April 2025
Published online: 29 April 2025

References

1. Wong RJ. Epidemiology of metabolic dysfunction-associated steatotic liver disease (MASLD) and alcohol-related liver disease (ALD). Metab Target Organ Damage. 2024;4:35. https://doi.org/10.20517/mtod.2024.57.
2. Sharma N, Singh L, Sharma A, Kumar A, Mahajan D. NAFLD-associated hepatocellular carcinoma (HCC) - A compelling case for repositioning of existing mTORc1 inhibitors. Pharmacol Res. 2024;208:107375. https://doi.org/10.1016/j.phrs.2024.107375.
3. Manns MP, Maasoumy B. Breakthroughs in hepatitis C research: from discovery to cure. Nat Rev Gastroenterol Hepatol. 2022;19(8):533–50. https://doi.org/10.1038/s41575-022-00608-8.
4. Huang DQ, Mathurin P, Cortez-Pinto H, Loomba R. Global epidemiology of alcohol-associated cirrhosis and HCC: trends, projections and risk factors. Nat Rev Gastroenterol Hepatol. 2023;20(1):37–49. https://doi.org/10.1038/s41575-022-00688-6.
5. Miao L, Targher G, Byrne CD, Cao YY, Zheng MH. Current status and future trends of the global burden of MASLD. Trends Endocrinol Metab 2024, 35(8), 697–07. https://doi.org/10.1016/j.tem.2024.02.007
6. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73–84. https://doi.org/10.1002/hep.28431.
7. Quek J, Ng CH, Tang ASP, Chew N, Chan M, Khoo CM, Wei CP, Chin YH, Tay P, Lim G, Tan DJH, Lim WH, Chan KE, Teng M, Tan E, Tamaki N, Huang DQ, Siddiqui MS, Young DY, Noureddin M, Muthiah MD. Metabolic associated fatty liver disease increases the risk of systemic complications and mortality. A Meta-Analysis and systematic review of 12 620 736 individuals. Endocr Pract. 2022;28(7):667–72. https://doi.org/10.1016/j.eprac.2022.03.016.
8. Castillo-Núñez Y, Almeda-Valdes P, González-Gálvez G, Arechavaleta-Granell MDR. Metabolic dysfunction-associated steatotic liver disease and atherosclerosis. Curr Diab Rep. 2024;24(7):158–66. https://doi.org/10.1007/s11892-024-01542-6.
9. Tripodi A, Fracanzani AL, Primignani M, Chantarangkul V, Clerici M, Mannucci PM, Peyvandi F, Bertelli C, Valenti L, Fargion S. Procoagulant imbalance in patients with non-alcoholic fatty liver disease. J Hepatol. 2014;61(1):148–54. https://doi.org/10.1016/j.jhep.2014.03.013.
10. Kaji H. Adipose Tissue-Derived plasminogen activator Inhibitor-1 function and regulation. Compr Physiol. 2016;6(4):1873–96. https://doi.org/10.1002/cphy.c160004.
11. Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. N Engl J Med. 2000;342(24):1792–801. https://doi.org/10.1056/NEJM200006153422406.
12. Brogren H, Wallmark K, Deinum J, Karlsson L, Jern S. Platelets retain high levels of active plasminogen activator inhibitor 1. PLoS ONE. 2011;6(11):e26762. https://doi.org/10.1371/journal.pone.0026762.
13. Jovin IS, Willuweit A, Taborski U, Lehnhardt A, Schreiner K, Klövekorn WP, Müller-Berghaus G. Low-density lipoproteins induce the Polar secretion of PAI-1 by endothelial cells in culture. Am J Hematol. 2003;73(1):66–8. https://doi.org/10.1002/ajh.10314.
14. Huang J, Sabater-Lleal M, Asselbergs FW, Tregouet D, Shin SY, Ding J, Baumert J, Oudot-Mellakh T, Folkersen L, Johnson AD, Smith NL, Williams SM, Ikram MA, Kleber ME, Becker DM, Truong V, Mychaleckyj JC, Tang W, Yang Q, Sennblad B, Moore JH, Williams FM, Dehghan A, Silbernagel G, Schrijvers EM, Smith S, Karakas M, Tofler GH, Silveira A, Navis GJ, Lohman K, Chen MH, Peters A, Goel A, Hopewell JC, Chambers JC, Saleheen D, Lundmark P, Psaty BM, Strawbridge RJ, Boehm BO, Carter AM, Meisinger C, Peden JF, Bis JC, McKnight B, Öhrvik J, Taylor K, Franzosi MG, Seedorf U, Collins R, Franco-Cereceda A, Syvänen AC, Goodall AH, Yanek LR, Cushman M, Müller-Nurasyid M, Folsom AR, Basu S, Matijevic N, van Gilst WH, Kooner JS, Hofman A, Danesh J, Clarke R, Meigs JB, DIAGRAM Consortium, Kathiresan S, Reilly MP, CARDIoGRAM Consortium, Klopp N, Harris TB, Winkelmann BR, Grant PJ, Hillege HL, Watkins H; C4, D Consortium, Spector TD, Becker LC, Tracy RP, März W, Uitterlinden AG, Eriksson P, Cambien F; CARDIOGENICS Consortium;, Morange PE, Koenig W, Soranzo N., van der Harst P, Liu Y, O'Donnell CJ, Hamsten A. Genome-wide association study for circulating levels of PAI-1 provides novel insights into its regulation. Blood. 2012, 120(24), 4873-81. https://doi.org/10.1182/blood-2012-06-436188
15. Pannacciulli N, De Mitrio V, Marino R, Giorgino R, De Pergola G. Effect of glucose tolerance status on PAI-1 plasma levels in overweight and obese subjects. Obes Res. 2002;10(8):717–25. https://doi.org/10.1038/oby.2002.98.
16. Alessi MC, Bastelica D, Mavri A, Morange P, Berthet B, Grino M, Juhan-Vague I. Plasma PAI-1 levels are more strongly related to liver steatosis than to adipose tissue accumulation. Arterioscler Thromb Vasc Biol. 2003;23(7):1262–8. https://doi.org/10.1161/01.ATV.0000077401.36885.BB.
17. Targher G, Bertolini L, Scala L, Zoppini G, Zenari L, Falezza G. Non-alcoholic hepatic steatosis and its relation to increased plasma biomarkers of inflammation and endothelial dysfunction in non-diabetic men. Role of visceral adipose tissue. Diabet Med. 2005;22(10):1354–8. https://doi.org/10.1111/j.1464-5491.2005.01646.x.
18. Yener S, Akarsu M, Demir T, Akinci B, Sagol O, Bayraktar F, Ozcan MA, Tankurt E, Yesil S. Plasminogen activator inhibitor-1 and thrombin activatable fibrinolysis inhibitor levels in non-alcoholic steatohepatitis. J Endocrinol Invest. 2007;30(10):810–9. https://doi.org/10.1007/BF03349221.
19. Targher G, Bertolini L, Rodella S, Lippi G, Franchini M, Zoppini G, Muggeo M. Day C.P. NASH predicts plasma inflammatory biomarkers independently of visceral fat in men. Obes (Silver Spring). 2008;16(6):1394–9. https://doi.org/10.1038/oby.2008.64.
20. Stine JG, Schreibman IR, Faust AJ, Dahmus J, Stern B, Soriano C, Rivas G, Hummer B, Kimball SR, Geyer NR, Chinchilli VM, Loomba R, Schmitz K, Sciamanna C. NASHFit: A randomized controlled trial of an exercise training program to reduce clotting risk in patients with NASH. Hepatology. 2022;76(1):172–85. https://doi.org/10.1002/hep.32274.
21. Nuttall FQ. Body mass index: obesity, BMI, and health: A critical review. Nutr Today. 2015;50(3):117–28. https://doi.org/10.1097/NT.0000000000000092.
22. Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, Guha IN, Cobbold JF, Deeks JJ, Paradis V, Bedossa P, Newsome PN. Accuracy of fibroscan controlled Attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology. 2019;156(6):1717–30. https://doi.org/10.1053/j.gastro.2019.01.042.
23. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD). European association for the study of obesity (EASO). EASL-EASD-EASO clinical practice guidelines on the

management of metabolic dysfunction-associated steatotic liver disease (MASLD). J Hepatol. 2024;81(3):492–542. https://doi.org/10.1016/j.jhep.2024.04.031.
24. Wallace TM, Matthews DR. The assessment of insulin resistance in man. Diabet Med. 2002;19(7):527–34. https://doi.org/10.1046/j.1464-5491.2002.00745.x.
25. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381–95. https://doi.org/10.1249/01.MSS.0000078924.61453.FB.
26. European Association for the Study of the Liver; Clinical Practice Guideline Panel. Chair:; EASL governing board representative:; panel members:. EASL clinical practice guidelines on non-invasive tests for evaluation of liver disease severity and prognosis– 2021 update. J Hepatol. 2021;75(3):659–89. https://doi.org/10.1016/j.jhep.2021.05.025.
27. Expert Panel on Liver Stiffness Measurement. Clinical application of transient elastography in the diagnosis of liver fibrosis: an expert panel review and opinion. J Clin Transl Hepatol. 2014;2(2):110–6. https://doi.org/10.14218/JCTH.2014.00008.
28. Wang X. Firth logistic regression for rare variant association tests. Front Genet. 2014;5:187. https://doi.org/10.3389/fgene.2014.00187.
29. R Core Team. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/; 2024 [accessed on 26 September 2024].
30. Stekhoven DJ, Buehlmann P. MissForest - non-parametric missing value imputation for mixed-type data. Bioinformatics. 2021;28(1):112–18.
31. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in underweight and obesity from 1990 to 2022: a pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. Lancet. 2024;403(10431):1027–50. https://doi.org/10.1016/S0140-6736(23)02750-2.
32. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA. 1999;282(16):1523–9. https://doi.org/10.1001/jama.282.16.1523.
33. Hecker J, Freijer K, Hiligsmann M, Evers SMAA. Burden of disease study of overweight and obesity; the societal impact in terms of cost-of-illness and health-related quality of life. BMC Public Health. 2022;22(1):46. https://doi.org/10.1186/s12889-021-12449-2.
34. Pearson-Stuttard J, Banerji T, Capucci S, de Laguiche E, Faurby MD, Haase CL, Sommer Matthiessen K, Near AM, Tse J, Zhao X, Evans M. Real-world costs of obesity-related complications over eight years: a US retrospective cohort study in 28,500 individuals. Int J Obes (Lond). 2023;47(12):1239–46. https://doi.org/10.1038/s41366-023-01376-4.
35. Limpijankit T, Vathesatogkit P, Matchariyakul D, Wiriyatanakorn S, Siriyotha S, Thakkinstian A, Sritara P. Causal relationship of excess body weight on cardiovascular events through risk factors. Sci Rep. 2022;12(1):5269. https://doi.org/10.1038/s41598-022-08812-x.
36. Kivimäki M, Strandberg T, Pentti J, Nyberg ST, Frank P, Jokela M, Ervasti J, Suominen SB, Vahtera J, Sipilä PN, Lindbohm JV, Ferrie JE. Body-mass index and risk of obesity-related complex Multimorbidity: an observational multicohort study. Lancet Diabetes Endocrinol. 2022;10(4):253–63. https://doi.org/10.1016/S2213-8587(22)00033-X.
37. Koskinas KC, Van Craenenbroeck EM, Antoniades C, Blüher M, Gorter TM, Hanssen H, Marx N, McDonagh TA, Mingrone G, Rosengren A, Prescott EB. Obesity and cardiovascular disease: an ESC clinical consensus statement. Eur J Prev Cardiol. 2024;zwae279. https://doi.org/10.1093/eurjpc/zwae279.
38. Powell-Wiley TM, Poirier P, Burke LE, Després JP, Gordon-Larsen P, Lavie CJ, Lear SA, Ndumele CE, Neeland IJ, Sanders P, St-Onge MP, American Heart Association Council on Lifestyle and Cardiometabolic Health; Council on Cardiovascular and Stroke Nursing. Council on clinical cardiology; Council on epidemiology and prevention; and stroke Council. Obesity and cardiovascular disease: A scientific statement from the American heart association. Circulation. 2021;143(21):e984–1010. https://doi.org/10.1161/CIR.0000000000000973.
39. Perone F, Spadafora L, Pratesi A, Nicolaio G, Pala B, Franco G, Ruzzolini M, Ambrosetti M. Obesity and cardiovascular disease: risk assessment, physical activity, and management of complications. Int J Cardiol Cardiovasc Risk Prev. 2024;23:200331. https://doi.org/10.1016/j.ijcrp.2024.200331.
40. Gyldenkerne C, Kahlert J, Thrane PG, Olesen KKW, Mortensen MB, Sørensen HT, Thomsen RW, Maeng M. 2-Fold more cardiovascular disease events decades before type 2 diabetes diagnosis: A nationwide registry study. J Am Coll Cardiol. 2024;84(23):2251–59. https://doi.org/10.1016/j.jacc.2024.06.050.
41. Guido D, Cerabino N, Di Chito M, Di Stasi V, De Nucci S, Shahini E, Giannuzzi V, Cozzolongo R, Coletta S, Stabile D, Ancona A, Longo G, Fontana L, Giannelli G, De Pergola G. Association between liver steatosis, fibrosis, and the onset of type 2 diabetes in overweight individuals: A fibroscan-based study in Southern Italy. Diabetes Res Clin Pract. 2024;218:111911. https://doi.org/10.1016/j.diabres.2024.111911.
42. Swan D, Lisman T, Tripodi A, Thachil J. The prothrombotic tendency of metabolic-associated fatty liver disease. J Thromb Haemost. 2023;21(11):3045–55. https://doi.org/10.1016/j.jtha.2023.06.017.
43. Quek J, Chan KE, Wong ZY, Tan C, Tan B, Lim WH, Tan DJH, Tang ASP, Tay P, Yong XJ, Zeng JN, Chew RW, Nah NWS, Kulkarni B, Siddiqui A, Dan MS, Wong YY, Sanyal VW, Noureddin AJ, Muthiah M, Ng M. Global prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2023;8(1):20–30. https://doi.org/10.1016/S2468-1253(22)00317-X.
44. Ghosh AK, Vaughan DE. PAI-1 in tissue fibrosis. J Cell Physiol. 2012;227(2):493–507. https://doi.org/10.1002/jcp.22783.
45. Omori K, Hattori N, Senoo T, Takayama Y, Masuda T, Nakashima T, Iwamoto H, Fujitaka K, Hamada H, Kohno N. Inhibition of plasminogen activator Inhibitor-1 attenuates transforming growth Factor-β-Dependent epithelial mesenchymal transition and differentiation of fibroblasts to myofibroblasts. PLoS ONE. 2016;11(2):e0148969. https://doi.org/10.1371/journal.pone.0148969.
46. Beier JI, Arteel GE. Alcoholic liver disease and the potential role of plasminogen activator inhibitor-1 and fibrin metabolism. Exp Biol Med (Maywood). 2012;237(1):1–9. https://doi.org/10.1258/ebm.2011.011255.
47. Li P, Huang Y, Wong A. Behavioural strategies to reduce obesity among lower socio-economic adults living in high-income countries: a grades of recommendation, assessment, development and Evaluation-assessed systematic review and meta-analysis of randomised controlled trials. Br J Nutr. 2024;131(3):544–52. https://doi.org/10.1017/S0007114523001940.
48. Holzberg JR, Jin R, Le NA, Ziegler TR, Brunt EM, McClain CJ, Konomi JV, Arteel GE, Vos MB. Plasminogen activator Inhibitor-1 predicts quantity of hepatic steatosis independent of insulin resistance and body weight. J Pediatr Gastroenterol Nutr. 2016;62(6):819–23. https://doi.org/10.1097/MPG.0000000000001096.
49. Juhan-Vague I, Alessi MC, Mavri A, Morange PE. Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. J Thromb Haemost. 2003;1(7):1575–9. https://doi.org/10.1046/j.1538-7836.2003.00279.x.
50. Bastard JP, Piéroni L, Hainque B. Relationship between plasma plasminogen activator inhibitor 1 and insulin resistance. Diabetes Metab Res Rev. 2000;16(3):192–201.
51. Ardigò D, Franzini L, Valtueña S, Numeroso F, Piatti PM, Monti L, Reaven GM, Zavaroni I. The increase in plasma PAI-1 associated with insulin resistance May be mediated by the presence of hepatic steatosis. Atherosclerosis. 2010;208(1):240–5. https://doi.org/10.1016/j.atherosclerosis.2009.06.026.
52. Wang L, Chen L, Liu Z, Liu Y, Luo M, Chen N, Deng X, Luo Y, He J, Zhang L, Hill MA, Li R, Wu J. PAI-1 exacerbates white adipose tissue dysfunction and metabolic dysregulation in high fat Diet-Induced obesity. Front Pharmacol. 2018;9:1087. https://doi.org/10.3389/fphar.2018.01087.
53. Sillen M, Declerck PJ. Targeting PAI-1 in cardiovascular disease: structural insights into PAI-1 functionality and Inhibition. Front Cardiovasc Med. 2020;7:622473. https://doi.org/10.3389/fcvm.2020.622473.

Publisher's note