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Association between Plasminogen Activator Inhibitor-1 in Young Adulthood and Nonalcoholic Fatty Liver Disease in Midlife: CARDIA

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Abstract

Background: Prior studies have demonstrated a cross-sectional association between elevated plasminogen activator inhibitor-1 (PAI-1) levels and nonalcoholic fatty liver disease (NAFLD). However, there are no prospective longitudinal assessments of the association between PAI-1 and NAFLD. We aimed to describe the association between PAI-1 levels in early adulthood with NAFLD in midlife.

Methods: Among the 5115 participants in the Coronary Artery Risk Development In Young Adults (CARDIA) study, participants were randomly selected from a subset that was free of obesity, diabetes, and hypertension at the 1992–93 exam and attended the 2005–06 exam (n=996). A subset of participants (n=896) also had CT liver fat measured (2010–11). Participants with secondary causes of steatosis were excluded (n=87). NAFLD was defined as liver attenuation ≤51 Hounsfield units. Logistic regression models assessed the association between PAI-1 and NAFLD.

Results: Of 809 participants, 53% were female, 37% black with a mean age of 32 years. Median PAI-1 level at 1st assessment (1992–93) was 23.4 ng/mL among participants with NAFLD vs 11.9 ng/mL among those without NAFLD (p<0.0001). Median PAI-1 level at 2nd assessment (2005–06) was 55.6 ng/mL among participants with NAFLD vs. 19.5 ng/mL among those without NAFLD (p<0.0001). Higher PAI-1 levels were independently associated with NAFLD (1st assessment

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Author Contributions:

SSK, LBV, and AH inspired study concept/design. SSK, PTC, LBV interpreted results and formed analysis plan. LAC performed statistical analysis. SSK, DLJ, DEV assisted with data collection, funding and supervision of study. PTC wrote the manuscript. All authors provided critical review and edits of the manuscript.

adjusted OR [AOR] 2.16 per 1 standard deviation higher log(PAI-1) level (95% confidence interval [CI] 1.63–2.85); 2nd assessment AOR 2.71 (95% CI 2.03–3.61)).

Conclusions: Plasma PAI-1 levels in young adulthood were independently associated with NAFLD in midlife. Further studies may indicate whether PAI-1 plays a role in NAFLD pathophysiology.

Lay Summary:

Levels of plasminogen activator inhibitor-1 (PAI-1) in young adulthood were independently associated with the presence of nonalcoholic fatty liver disease (NAFLD) in mid-life. In addition, the change in PAI-1 over a 13 year period was also independtly associated with the presence of NAFLD in mid-life. These findings further support the hypothesis that PAI-1 is playing an important role in the mechanisms that predisopose to NAFLD.

Keywords

steatosis; obesity; metabolic syndrome; computed tomography (CT); hepatic

Amid an increasing burden of risk factors such as obesity and insulin resistance, the worldwide prevalence of nonalcoholic fatty liver disease (NAFLD) is at an all time high and continuing to increase.[1,2] Currently, NAFLD prevalence globally and in the US is estimated to be approximately 25%.[1,3,4] While NAFLD's hepatic complications such as cirrhosis[5] and hepatocellular carcinoma[6] are well known, it has also been independently associated with many extrahepatic complications including cardiovascular disease[7], chronic kidney disease[8] and diabetes[9,10]. However, the underlying molecular mechanisms predisposing individuals to development of NAFLD are not well understood. Given the sizeable and rapidly mounting public health implications of this disease, it remains critical to further elucidate the pathophysiology of NAFLD to advance preventive and therapeutic strategies.

Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor that inhibits tissue plasminogen activator. Through this mechanism, PAI-1 has been shown to play a role in thrombosis[11]. In recent years, PAI-1 has been identified to have a broader biologic relevance and has been linked with the development of cardiometabolic diseases (e.g. obesity[12], the metabolic syndrome[13,14], and cardiovascular disease[15,16]) and organ fibrosis[15]. Obesity and insulin resistance contribute to underlying risk of NAFLD and data from *cross-sectional* studies have demonstrated associations between elevated plasma levels of PAI-1 and presence and severity of NAFLD in humans.[17–19] Preclinical data supporting the biologic plausibility of PAI-1 playing a causal role in the development of NAFLD include findings in a murine model exposed to a high fructose diet in which PAI-1 deficient mice were protected against the development of hepatic steatosis.[20] Furthermore, administration of a small molecule inhibitor of PAI-1 attenuated high-fat diet induced hepatic steatosis in two separate murine studies,[21,22] showing the potential to prevent NAFLD development with PAI-1 targeted therapies.

However, there are no prospective longitudinal assessments of PAI-1 in a young, non-obese (at baseline) human cohort and associations with NAFLD in midlife, and it is unkown

whether associatsion may differ by race. We hypothesize that higher levels of PAI-1 and greater change in PAI-1 will be associated with prevalent NAFLD in middle age.

MATERIALS AND METHODS

Study Population

Coronary Artery Risk Development in Young Adults (CARDIA) is a longitudinal cohort study of 5115 biracial men and women from four geographically diverse United States cities (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA). The study design has been described previously.[23] Participants aged 18–30 were initially recruited in 1985–1986 (CARDIA Exam Year 0) and have undergone subsequent follow-up in-person examinations 2, 5, 7, 10, 15, 20, 25, and 30 years after enrollment. Retention rates among surviving participants at each in-person examination were 91%, 86%, 81%, 79%, 74%, 72%, 72%, and 71%, respectively. Contact is maintained with participants via telephone, mail, or email every 6 months, with annual interim medical history ascertainment. Over the last 5 years, >90% of the surviving cohort members have been directly contacted, and follow up for vital status is virtually complete through related contacts and intermittent National Death Index searches. Written informed consent was obtained from each participant at each follow-up examination and the study was approved annually by institutional review boards from each field center (University of Alabama at Birmingham, Birmingham, Alabama; Northwestern University, Chicago, Illinois; University of Minnesota, Minneapolis, Minnesota; and Kaiser Permanente, Oakland, California).

Among the 5115 men and women in the CARDIA study, participants were eligible for the PAI-1 ancillary study if they were free of obesity (defined as body mass index (BMI) ≥30kg/m^2), diabetes, and hypertension at baseline for this study in 1992–93 (CARDIA exam year 7), attended follow-up exams in 2005–06 (CARDIA exam year 20) and 2010–11 (CARDIA exam year 25); and were not pregnant at these visits and had a sufficient number of stored EDTA samples (eligible n=1730). Of those eligible for the PAI-1 ancillary study, 996 participants were randomly selected using the SAS procedure PROC SURVEYSELECT and specifying simple random sampling for participation. Among these 996 participants, 985 had PAI-1 measured in both 1992–93 (first assessment) and 2005–06 (second assessment). A subset of participants (n= 896) also had computed tomography (CT) liver fat data measured in 2010–11. In order to define the NAFLD-eligible population, we excluded participants with self reported history of cirrhosis or viral hepatitis (n=9) but did not have available data on serologies or antibody status. We also excluded participants for secondary causes of hepatic steatosis including excessive alcohol use (defined as ≥14 drinks/wk in women and ≥ 21 drinks/wk in men)[24] (n=56), human immunodeficiency virus (n=10), and medications known to cause hepatic steatosis (corticosteroids, amiodarone, methotrexate, tamoxifen, valproic acid) (n=12). The remaining 809 participants comprised the NAFLD-eligible study cohort (Figure 1).

Measurements

PAI-1 measurement: Plasma PAI-1 levels were measured using the Molecular Innovations Plasminogen Activator Inhibitor-1 ELISA assay from plasma samples that were

collected and stored in 1992–1993 (first PAI-1 assessment) and 2005–06 (second PAI-1 assessment); 10% of duplicates were randomly sampled within and across plates with an intra-assay coefficient of variation of 3.7%.

CT examination: Non-contrast abdominal CT was performed in 2010–11 using GE (GE 750HD 64 and GE LightSpeed VCT 64 at Birmingham and Oakland Centers, respectively; GE Healthcare, Waukesha, WI, USA) or Siemens (Sensation 64 at Chicago and Minneapolis Centers; Siemens Medical Solutions, Erlangen, Germany) as previously described.[25] Quality control and image analysis were performed at a central reading center (Wake Forest University Health Sciences, Winston-Salem, NC). Prior studies have shown non-contrast CT liver attenutation (LA) values ≤51 Hounsfield Units (HU) correlate with biopsy confimed steatosis (of any severity) and ≤40 HU with moderate-to-severe steatosis (>30%).[26–28] Therefore, we assessed NAFLD via CT using both LA ≤40 HU and LA ≤51 HU after exclusions of competing causes of hepatic steatosis such as detailed above. LA was calculated by averaging 9 measurements taken on 3 CT slices of the right hepatic lobe. The intraclass correlation coefficient between different readers on a random sample of 156 participants was 0.975 for LA, indicating high reproducibility of CT measured LA.[25]

Covariates: Participant demographics, medical history, current medications and tobacco use were obtained via participant surveys per CARDIA protocols that have been described previously.[23] Participants self-reported race as black or white. Participant ethnicity was not evaluated in the CARDIA study. Medication use was self-reported and verified by review of medications brought to study visits. Blood pressure measurements were obtained at each visit. Hypertension was defined as antihypertensive medications use and/or systolic pressure ≥140 mmHg or diastolic pressure ≥90 mmHg. Certified technicians performed anthropometric measurements at each visit; BMI was calculated as weight (kg)/height $(m)^2$. Plasma triglycerides, high-density lipoprotein cholesterol (HDL-C), total cholesterol[29,30] and serum glucose were measured at first assessment (1992–93) and second assessment (2005–2006). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedwald equation. Diabetes was defined as fasting glucose≥126 mg/dl, 2 h oral glucose tolerance test≥200 mg/dl, hemoglobin HbA1c≥6.5% or use of diabetes medications.

Statistical analysis

Participant characteristics at first and second assessments were compared using the paired t-test. Logistic regression models assessed the longitudinal association between PAI-1 levels (at first and second assessment, measured continuously and by tertiles) and NAFLD at follow-up (2010–11) adjusted for age, sex, race, CARDIA center, education, BMI, hypertension, total cholesterol, diabetes and current smoking at the corresponding PAI-1 assessment. Effect modification of each association by sex, race, and menopause status was assessed. A Chi-Squared test for linear trend was used to assess for a linear trend to the association between tertiles of PAI-1 and NAFLD. Statistical significance was defined as a two-tailed p-value <0.05.

RESULTS

Among the 809 participants that met inclusion criteria, 53% were female, 63% Caucasian with a mean age of 32 years at first PAI-1 assessment and 45 years at second PAI-1 assessment. Average BMI at first assessment was 24.1 kg/m^2, compared to 27.1 kg/m^2 at second assessment. No participants had diabetes at time of first PAI-1 assessment (by study design) compared to 3.6% at second PAI-1 assessment. No participants had hypertension at time of first PAI-1 assessment (by study design) compared to 11.4% at second PAI-1 assessment Current smoking prevalence decreased from 20% at first assessment to 13% at second assessment. Age, BMI, total cholesterol, HDL-C, LDL-C, triglycerides and systolic blood pressure are summarized by tertile of PAI-1 at first PAI-1 assessment (Table 1). No significant interactions by race or sex were observed. A significant interaction by menopausal status was not observed in the models evaluating PAI-1 at the first or second assessment; while an interaction by menopausal status was observed, this did not meaningfully change the direction or magnitude of the associations.

PAI-1 assocation with any severity NAFLD (defined as CT LA≤51 HU in the absence of secondary causes of hepatic steatosis):

Using the CT LA≤51 HU definition of NAFLD (in the absence of secondary causes of hepatic steatosis), NAFLD prevalence was 17.1% (n=138) at the 2010–2011 CARDIA exam. The median PAI-1 level at first assessment was 23.4 (IQR 13.9, 36.5) vs. 11.9 (IQR 7.2, 19.8) ng/mL among those with and without NAFLD at follow-up, respectively ($p<0.0001$). Median PAI-1 level at second assessment was 55.6 (IQR 32.4, 87.1) among participants with NAFLD vs. 19.5 (IQR 12.5, 37.2) ng/mL among those without NAFLD ($p<0.0001$). The median change in PAI-1 was 28.7 (IQR 5.7, 62.4) among those with NAFLD vs 6.9 (IQR 0.5, 19.2) ng/mL among those without NAFLD ($p<0.0001$) (Table 2).

Higher PAI-1 levels (at both first and second assessment) were independently associated with prevalent NAFLD at follow-up after adjusting for relevant covariates (First PAI-1 assessment adjusted OR [AOR] per 1 standard deviation (SD) higher log(PAI-1) (1 SD= 0.84) 2.16, (95% confidence interval [CI] 1.63–2.85); Second PAI-1 assessment AOR per 1 SD (1 SD= 0.85) was 2.71 (95% CI 2.03–3.61). The change in PAI-1 from first to second assessment had a somewhat weaker, but still highly significant independent association with prevalent NAFLD AOR per 1 SD (1 SD= 37.2) 1.77 (95% CI 1.41–2.22) (Table 3).

Odds of NAFLD at follow-up were significantly higher among participants in higher tertiles of PAI-1 levels measured at both first and second assessments, even after adjustment for demographics and risk factors. The AOR for NAFLD by first PAI-1 assessment was 1.82 (95% CI 0.95–3.51) for tertile 2 and 3.79 (95% CI 1.99–7.22) for tertile 3 (trend test $p<0.0001$). The AOR for NAFLD by PAI-1 levels obtained at second assessment was 3.20 (95% CI 1.34–7.64) for tertile 2 and 8.55 (95% CI 3.65–20.02) for tertile 3 (trend test $p<0.0001$) (Figure 2). The odds of NAFLD by change in PAI-1 (from first to second assessment) was not statisticially significant for tertile 2 (AOR 0.66, 95% CI 0.35–1.25) of change in PAI-1 but was statistically significant for tertile 3 (AOR 2.36, 95% CI 1.37–4.06) (trend test $p<0.0001$) (Figure 3). Spline analyses support a linear association across the

spectrum of log-transformed PAI-1 exposure with prevalent NAFLD (Supplemental Figure 1).

PAI-1 assocation with moderate-severe NAFLD (defined as CT LA≤40 HU in the absence of secondary causes of hepatic steatosis):

Using the CT LA ≤40 HU definition of NAFLD (in the absence of secondary causes of hepatic steatosis), prevalence was 7.2% (n=58) at the year 25 CARDIA exam in 2010–11. The median PAI-1 level at first PAI-1 assessment was 29.5 (IQR 14.5, 43.3) vs. 12.5 (IQR 7.6, 21.4) ng/mL among those with and without moderate-severe NAFLD at follow up, respectively ($p<0.0001$). Median PAI-1 level at second PAI-1 assessment was 63.6 (IQR 45.6, 97.5) among participants with moderate-severe NAFLD vs. 21.9 (IQR 13.1, 42.2) ng/mL among those without NAFLD ($p<0.0001$). The median change in PAI-1 was 32.7 (IQR 10.1, 68.0) among those with moderate-severe NAFLD vs 7.9 (IQR 0.6, 21.6) among those without ($p<0.0001$) (Supplemental Table 1).

Higher PAI-1 levels (at both first and second PAI-1 assessments) were independently associated with prevalent moderate-severe NAFLD at follow-up after adjusting for relevant covariates (First PAI-1 assessment AOR 2.45 [95% CI 1.65, 3.10; Second PAI-1 assessment AOR 3.42 [95% CI 2.23, 5.26). The change in PAI-1 from first assessment to second assessment had a somewhat weaker independent association with prevalent moderate-severe NAFLD (AOR 1.73 [95% CI: 1.34, 2.23). (Supplemental Table 2).

Relative to the lowest tertile, odds of moderate-severe NAFLD at follow-up were significantly higher among participants in higher tertiles of PAI-1 levels measured at both first and second PAI-1 assessments, even after adjustment for demographics and risk factors. The AOR for moderate-severe NAFLD by PAI-1 levels at first PAI-1 assessment was not statistically significant for tertile 2 (AOR 1.84, 95% CI 0.62–5.40) but was statistically significant for tertile 3 (AOR 4.81, 95% CI 1.70–13.65) (trend test $p=0.0003$). The AOR for moderate-severe NAFLD by PAI-1 levels at second PAI-1 assessment was 6.59 (95% CI 0.82–53.10) for tertile 2 and 21.28 (95% CI 2.75–164.36) for tertile 3 (trend test $p<0.0001$) (Figure 4). The odds of moderate-severe NAFLD by change in PAI-1 (from first to second assessment) was not statisticially significant for tertile 2 (AOR 0.81, 95% CI 0.29–2.32) but was statistically significant for tertile 3 (AOR 3.19, 95% CI 1.38–7.37) (trend test $p=0.0006$) (Figure 5).

DISCUSSION

In this large prospective study of a young and healthy (without obesity or diabetes at baseline) cohort, we found that higher plasma levels of PAI-1 in young adulthood were associated with prevalent NAFLD in midlife. This association was independent of demographic and metabolic covariates measured in younger adulthood. Higher tertiles of PAI-1 in younger adulthood and midlife were also independently associated with prevalent NAFLD in midlife. Additionally, change in PAI-1 over an average 13 years from first PAI-1 assessment to second assessment had an independent association with prevalent NAFLD in midlife.

PAI-1 has consistently been associated with many disease states that are also associated with NAFLD, including obesity, insulin resistance and cardiovascular disease. Upregulated by selected cytokines in adipose tissue,[31] plasma and adipose PAI-1 levels are elevated in individuals with obesity.[12] PAI-1 levels are also higher in individuals with insulin resistance prior to the development of diabetes and improvement in insulin resistence via weight loss lowers plasma PAI-1 levels.[32,33] However, evidence is emerging that PAI-1 may be playing a larger and more independent role in NAFLD than simply being upregulated in disease states commonly linked with NAFLD. For example, PAI-1 levels have a higher correlation with liver fat content than visceral fat content.[34] In addition, prior work in murine models showing that PAI-1 deficient mice and wild type mice given a small molecular inhibitor of PAI-1 were protected against diet induced steatosis formation[20–22] further supports the hypothesis that PAI-1 may be playing an important role in NAFLD pathophysiology.

Our study builds upon the prior cross-sectional work describing the relationship between NAFLD and PAI-1 in humans that has consistently shown higher plasma PAI-1 levels are associated with concurrent NAFLD. One study in which NAFLD cases were confirmed with biopsy found that the association between higher plasma levels of PAI-1 and NAFLD was attenuated after controlling for age, sex and insulin resistance.[35] However, several other studies have found the cross-sectional association between PAI-1 and NAFLD to be independent of other metabolic covariates. A recent study of patients with histologic or imaging confirmed NAFLD evaluated 32 different biomarkers, and only plasma PAI-1 activity was independently associated with NAFLD.[17] In addition, two other studies including patients with biopsy proven nonalcoholic steatohepatitis[36] and NAFLD[37] found a positive graded relationship between plasma PAI-1 levels and severity of steatosis and fibrosis. However, all of these studies were cross-sectional in nature and involved relatively small cohorts (n=85–648). The current study is the first longitudinal assessment of PAI-1 levels involving a large human cohort of young and healthy individuals at baseline without prevalent obesity or diabetes. Findings from our study further supports the hypothesis that PAI-1 may play an important role in NAFLD pathophysiology.

Our study has several strengths. CARDIA involves a large well-characterized biracial cohort that has been followed longitudinally for many years with excellent participant retention. NAFLD diagnosis was also made using two well defined CT liver attenuation cutoffs (in the absence of secondary causes of hepatic steatosis) and similar assocations with PAI-1 were found using both imaging definitions of NAFLD. The LA ≤40 HU cutoff is more specific for hepatic steatosis and has been found to identify moderate-severe steatosis, while LA ≤51 HU is more sensitive but less specific, and identifies any severity hepatic steatosis. The Multi-Ethic Study of Atherosclerosis documented a NAFLD prevalence of 17.3% with LA ≤51 HU and 6.3% LA ≤40 HU.[26] Our findings largely support and confirm these prevalence estimates by finding a prevalence of 17.1% and 7.2%, respectively.

This study also has several limitations. We can not infer temporal nor causal relationships as hepatic steatosis was only assessed at one time point. However, a prior study of lean United States adults (average age 40 years and BMI 22 kg/m^2, excluded if BMI >25 kg/m^2) found a NAFLD prevalence of only 7.4% and multivariate analysis showed that Hispanic ethnicity, diabetes, and hypertension were associated with prevalent NAFLD in lean individuals.[38]

Given the participants in the present study had a similar average BMI (24 kg/m^2 vs 22 kg/m^2), were on average 8 years younger (32 years vs 40 years), and were free of both diabetes and hypertension, we would expect the NAFLD prevalence at time of first PAI-1 assessment (CARDIA Exam Year 7, 1992–93) to be less than the lean NAFLD prevalence of 7.4% observed by Younossi et al. It is highly plausible that a more significant proportion of CARDIA participants had prevalent NAFLD at the time of second PAI-1 assessment, however the direction of association between PAI-1 and prevalent NAFLD in mid-life was consistent independent of time of PAI-1 assessment further strengthening the potential role of PAI-1 in the pathogenesis of NAFLD. It is also important to acknowledge new data that suggests a possible bi-directional relationship between NAFLD and diabetes/metabolic syndrome where perhaps NAFLD may be associated with increased risk of incident diabetes and metabolic syndrome[39,40] but similar to our study, all data are observational and thus causality cannot be inferred. An additional limitation includes CT being a relatively insensitive method of detecting hepatic steatosis compared to hepatic triglyceride content measured by proton magnetic resonance imaging (MRI),[9,27] which may have led to falsely low estimates of prevalence in this cohort. In fact, abdominal ultrasound (US), not CT or MRI, is the consensus first-line option for identifying hepatic steatosis given its low cost, wide availability and lack of radiation exposure.[41] Furthermore, US based semi-quantitative indices, such as the US-Fatty Liver Index (US-FLI), have been shown to correlate with metabolic and histological features of NAFLD.[42,43] However, the sensitivity of US can be limited by morbid obesity (BMI > 40 kg/m^2) and US can miss steatosis content of less than <20%.[41] While several novel non-invasive modalities for assessment of steatosis and fibrosis, such as measurement of liver stiffness with vibration-controlled transient elastography (VCTE), have shown promising results and may revolutionize our approach to studying NAFLD epidemiology[44], iver biopsy is still the gold standard for diagnosing and classifying the severity of NAFLD[24]. However, performing liver biopsy is not feasible in epidemiologic studies given the risks associated with the procedure. In addition, despite recent improvements in biomarkers and biomarker panels in the ability to predict likelihood of steatosis and subsequent inflammation/fibrosis,[45,46] NAFLD cannot currently be diagnosed via a laboratory test. Therefore, imaging documentation of steatosis in the presence of risk factors and after exclusion of other secondary causes of hepatic steatosis confirms the diagnosis. As a result, the NAFLD definition applied in this study is similar to how NAFLD is currently diagnosed in clinical practice (and sensitivity analysis was performed with an additional threshold value). Third, certain data that might be helpful in characterizing an individual's severity of liver disease are not available in CARDIA, such as liver chemistries and the previously mentioned noninvasive liver fibrosis scores (e.g., NAFLD fibrosis score, Fibrosis-4 index). Future prospective studies with serial liver imaging (ideally with the most sensitive modality of proton MRI) and/or confirmation with liver biopsy are needed to strengthen the findings of PAI-1 and NAFLD to support future development of PAI-1 targeted therapies. An additional limitation is that serologies or antibody status was not available to confirm viral hepatitis diagnoses, but medical history updates were obtained via annual questionnaires and all hospitalization records were obtained and adjudicated for events by trained physicians.

In conclusion, PAI-1 levels in healthy participants in young adulthood were independently associated with prevalent NAFLD in midlife. Taken together with animal studies suggesting a causal role for PAI-1 in NAFLD, it is plausible that PAI-1 may play an important role in human NAFLD pathophysiology. Further work is needed in order to further investigate the true role of PAI-1 in NAFLD pathophysiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

The authors take responsibility for decision to submit the manuscript for publication. Dr. Khan had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The authors thank the participants of the CARDIA study for their long-term commitment and important contributions to the study.

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Abbreviations:

NAFLD	Non alcoholic fatty liver disease
PAI-1	plasminogen activator inhibitor-1
CARDIA	Coronary Artery Risk Development in Young Adults
BMI	body mass index
CT	computed tomography
LA	liver attenuation
HU	Hounsfield units
HTN	hypertension
HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
SD	standard deviation
CI	confidence interval
AOR	adjusted odds ratio

MRI	magnetic resonance imaging
US	ultrasound
US-FLI	ultrasound fatty liver index
VCTE	vibration-controlled transient elastography
HIV	human immunodeficiency virus
SBP	systolic blood pressure

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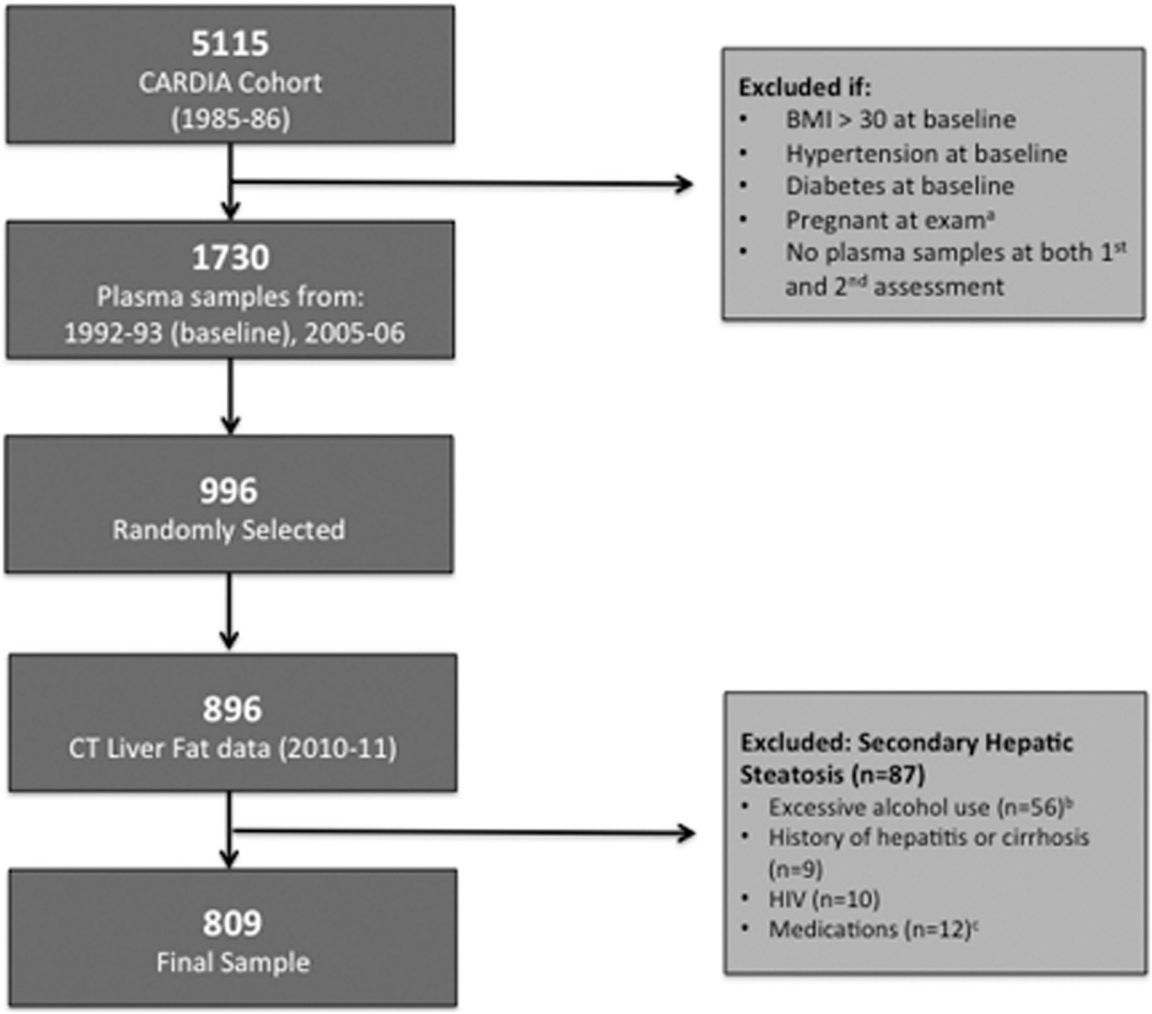

Figure 1.

Cohort selection. [a]Participants pregnant at any exam (1992–1993, 2005–2006, 2010–2011) were excluded. [b]Excessive alcohol use was defined as ≥14 drinks/wk in women and ≥21 drinks/wk in men. [c]Medications = corticosteroids, amiodarone, methotrexate, tamoxifen, valproic acid. BMI, body mass index; CT, computed tomography; HIV, human immunodeficiency virus

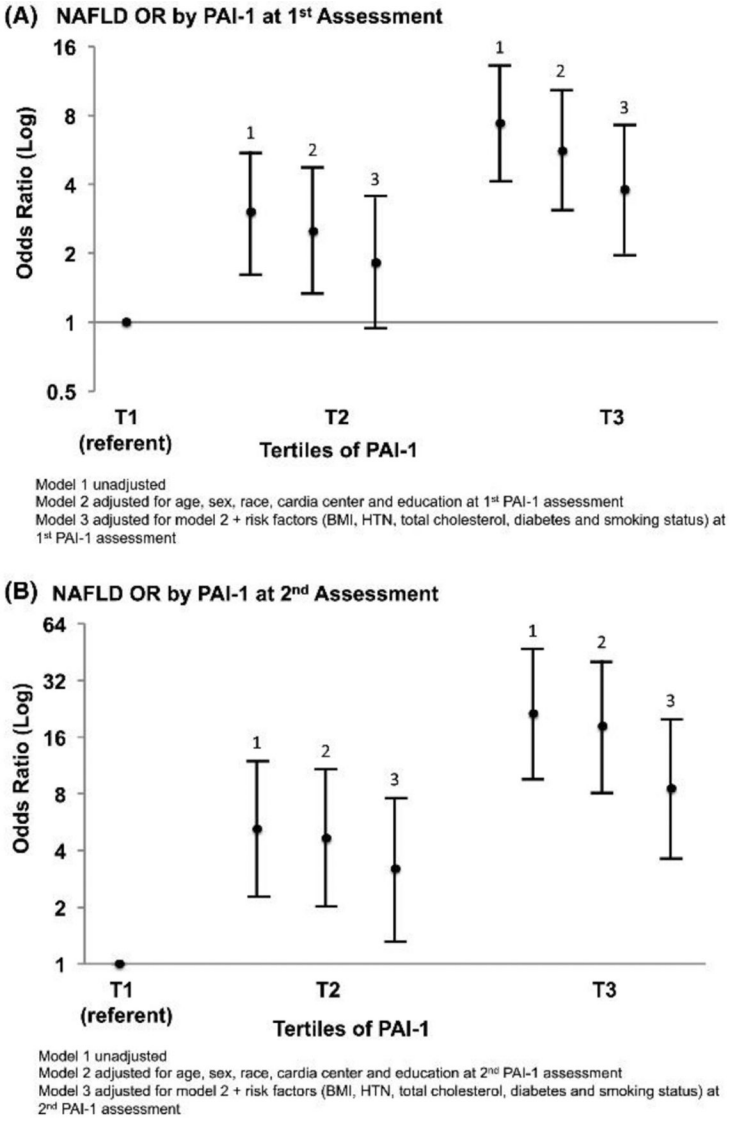

Figure 2.

Association of PAI-1 by tertiles measured at 1st PAI-1 assessment (1992–1993) (A) and 2nd PAI-1 assessment (2005–2006) (B) with NAFLD (any severity, defined as CT LA ≤51 HU) at follow-up (2010–2011). PAI-1 tertile ranges (ng/mL): (A) T1: 0.34–9.38; T2: 9.40–18.65; T3: 18.67–178.83. (B) T1: 2.11–16.38; T2: 16.39–36.61; T3: 36.67–400.00 diabetes defined as fasting glucose ≥126 mg/dL, 2-hour glucose tolerance test ≥200 mg/dL, haemoglobin HbA1c ≥6.5% or use of diabetic medications. Hypertension defined as antihypertensive medication use and/or systolic pressure ≥140 mm Hg or diastolic pressure ≥90 mm Hg. BMI, body mass index; HTN, hypertension

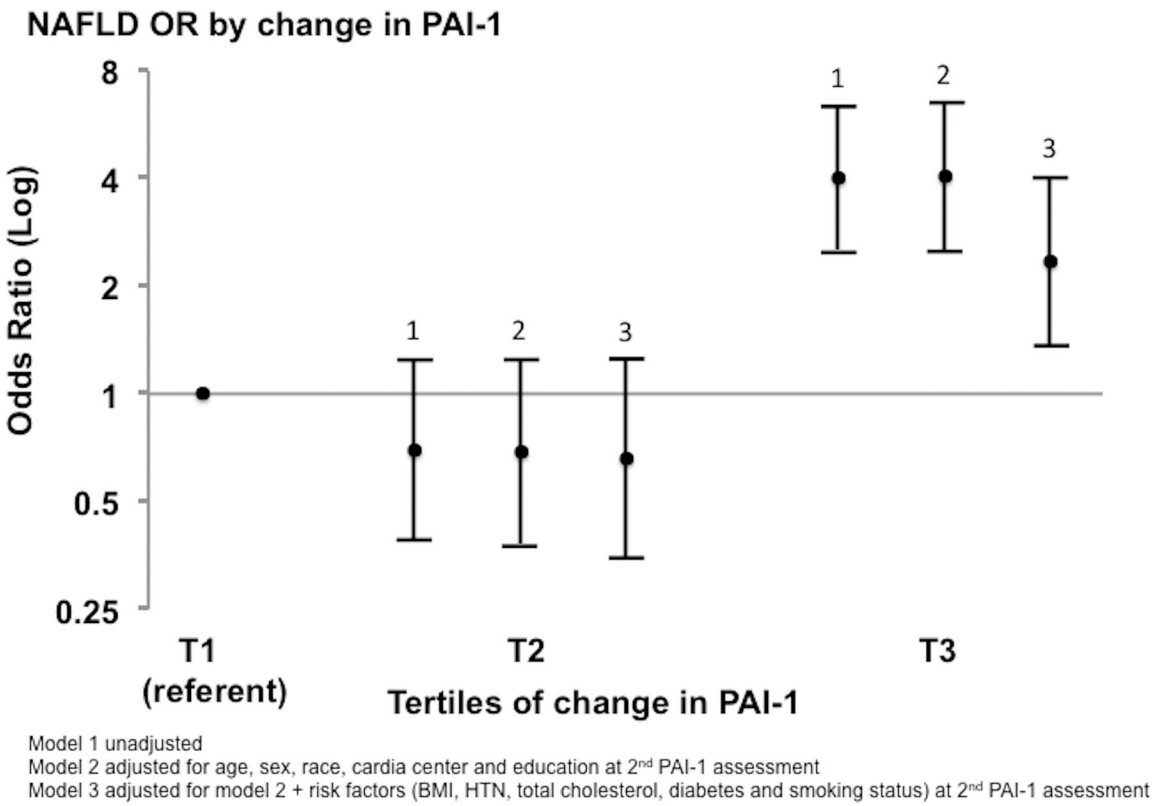

Figure 3.

Association of change in PAI-1 (from 1st PAI-1 assessment [1992–1993] to 2nd PAI-1 assessment [2005–2006]) by tertiles with NAFLD (any severity, defined as CT LA ≤51 HU) at follow-up (2010–2011). Change in PAI-1 tertile ranges: T1: −115.83–3.39; T2: 3.44–17.14; T3: 17.21–358.52 diabetes defined as fasting glucose ≥126 mg/dL, 2-hour glucose tolerance test ≥200 mg/dL, haemoglobin HbA1c ≥6.5% or use of diabetic medications. Hypertension defined as antihypertensive medication use and/or systolic pressure ≥140 mm Hg or diastolic pressure ≥90 mm Hg. BMI, body mass index; HTN, hypertension

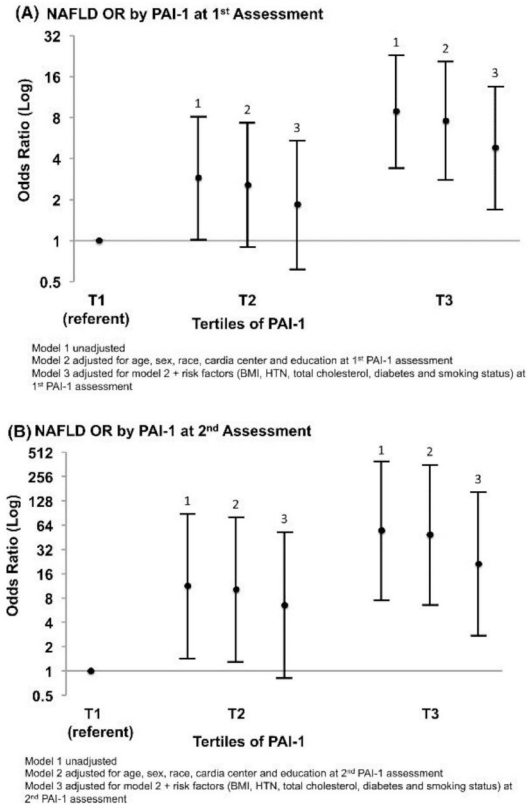

Figure 4.

Association of PAI-1 by tertiles measured at 1st PAI-1 assessment (1992–1993) (A) and 2nd PAI-1 assessment (2005–2006) (B) with NAFLD (moderate-severe severity, defined as CT LA ≤40 HU) at follow-up (2010–2011). PAI-1 tertile ranges (ng/mL): (A) T1: 0.34–9.38; T2: 9.40–18.65; T3: 18.67–178.83. (B) T1: 2.11–16.38; T2: 16.39–36.61; T3: 36.67–400.00 diabetes defined as fasting glucose ≥126 mg/dL, 2-hour glucose tolerance test ≥200 mg/dL, haemoglobin HbA1c ≥6.5% or use of diabetic medications. Hypertension defined as antihypertensive medication use and/or systolic pressure ≥140 mm Hg or diastolic pressure ≥90 mm Hg. BMI, body mass index; HTN, hypertension

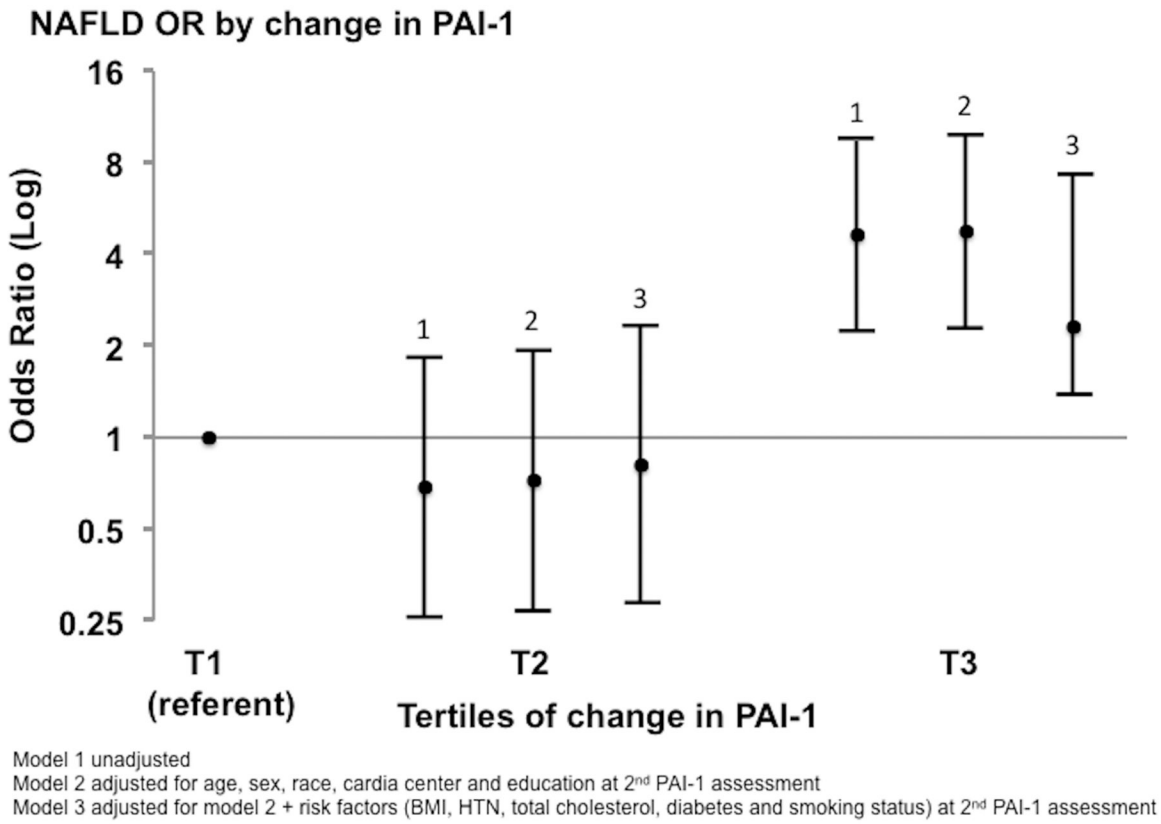

Figure 5.

Association of change in PAI-1 (from 1st PAI-1 assessment [1992–1993] to 2nd PAI-1 assessment [2005–2006]) by tertiles with NAFLD (moderate-severe severity, defined as CT LA ≤40 HU) at follow-up (2010–2011). Change in PAI-1 tertile ranges: T1: −115.83–3.39; T2: 3.44–17.14; T3: 17.21–358.52 diabetes defined as fasting glucose ≥126 mg/dL, 2-hour glucose tolerance test ≥200 mg/dL, haemoglobin HbA1c ≥6.5% or use of diabetic medications. Hypertension defined as antihypertensive medications use and/or systolic pressure ≥140 mm Hg or diastolic pressure ≥90 mm Hg. BMI, body mass index; HTN, hypertension

Table 1:

Cohort characteristics by Tertile of PAI-1 (n=809)

	PAI-1 in 1992–1993, ng/mL		
	1st Tertile	**2nd Tertile**	**3rd Tertile**
Age, years	31.7 (3.6)	32.3 (3.6)	32.7 (3.5)
Female, n (%)	205 (76.2)	135 (50)	91 (33.7)
Caucasian, n (%)	157 (58.4)	180 (66.7)	176 (65.10
Education (Grade completed)	15.3 (2.4)	14.9 (2.4)	14.8 (2.6)
BMI, kg/m^2	22.7 (2.6)	24.4 (2.8)	25.3 (2.6)
Total Cholesterol, mg/dL	171 (34)	173 (32)	180 (33)
HDL-C, mg/dL	58 (13)	53 (12)	49 (15)
LDL-C, mg/dL	100 (31)	106 (30)	111 (32)
Triglycerides, mg/dL	60 (26)	70 (35)	99 (58)
Average SBP	103 (9)	105 (9)	109 (10)

Data expressed as mean (SD) or n (%)

Abbreviations: PAI-1, plasminogen activator inhibitor-1; SD, standard deviation; BMI, body mass index; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; SBP, systolic blood pressure.

Table 2:

PAI-1 levels by NAFLD* status

	NAFLD 2010–11 (n=138)	No NAFLD 2010–11 (n=671)	P value+
1st Assessment PAI-1 level (1992–93) (ng/mL)	23.4 (13.9, 36.5)	11.9 (7.2, 19.8)	<0.0001
2nd Assessment PAI-1 level (2005–06) (ng/mL)	55.6 (32.4, 87.1)	19.5 (12.5, 37.2)	<0.0001
Change in PAI-1	28.7 (5.7, 62.4)	6.9 (0.5, 19.2)	<0.0001

Data expressed as median (IQR)

Abbrevations: PAI-1, plasminogen activator inhibitor 1; NAFLD, non-alcoholic fatty liver disease; IQR, interquartile range.

*NAFLD defined as LA≤51 HU on CT in the absence of secondary causes of steatosis

+kruskal-Wallis test

Table 3:

Association of PAI-1 (1992–93, 2005–06) with prevalent NAFLD* (2010–11)

	1st PAI-1 Assessment 1992–93	2nd PAI-1 Assessment 2005–06	Change in PAI-1
Unadjusted OR	2.29 (1.86, 2.81)	3.51 (2.76, 4.47)	1.87 (1.54, 2.27)
Model 1 AOR	2.06 (1.65, 2.58)	3.35 (2.62, 4.28)	1.87 (1.54, 2.28)
Model 2 AOR	1.75 (1.37, 2.23)	2.52 (1.92, 3.32)	1.66[a] (1.33, 2.06)
Model 3 AOR	2.16 (1.63, 2.85)	2.71 (2.03, 3.61)	1.77[a] (1.41, 2.22)

Model 1 adjusted for age, sex, race, cardia center, education, with age and education at the corresponding PAI-1 assessment

Model 2 adjusted for model 1 + risk factors (body mass index, hypertension, total cholesterol, diabetes and smoking status) at corresponding PAI-1 exam

Model 3 adjusted for model 2 + BMI at NAFLD assessment (2010–11)

[a]Additionally adjusted for PAI-1 from 1992–93

*NAFLD defined as LA≤51 HU on CT in the absence of secondary causes of steatosis

AOR reported per 1 SD higher log (PAI-1): 1st PAI-1 assessment SD 0.84, 2nd PAI-1 assessment SD 0.85, Change in PAI-1 SD 37.2

Abbreviations: PAI-1, plasminogen activator inhibitor 1; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; AOR, adjusted odds ratio

Diabetes defined as fasting glucose≥126 mg/dl, 2 h glucose tolerance test≥200 mg/dl, hemoglobin HbA1c≥6.5% or use of diabetic medications. Hypertension defined as antihypertensive medications use and/or systolic pressure ≥140 mmHg or diastolic pressure ≥90 mmHg.

ORs reported are for a 1 SD higher log(PAI-1) for 1st and 2nd assessments and 1 SD higher Change in PAI-1