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# Non-Alcoholic Fatty Liver Disease, Liver Fibrosis, and Regional Amyloid-β and Tau Pathology in Middle-Aged Adults: The Framingham Study

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## Abstract

**Background:** Liver steatosis and fibrosis are emerging as risk factors for multiple extrahepatic health conditions; however, their relationship with Alzheimer's disease pathology is unclear.

**Objective:** To examine whether non-alcoholic fatty liver disease (NAFLD) and FIB-4, a non-invasive index of advanced fibrosis, are associated with brain amyloid-β (Aβ) and tau pathology.

**Methods:** The study sample included Framingham Study participants from the Offspring and Third generation cohorts who attended exams 9 (2011–2014) and 2 (2008–2011), respectively. Participants underwent $^{11}$C-Pittsburgh Compound-B amyloid and $^{18}$F-Flortaucipir tau positron emission tomography (PET) imaging and abdomen computed tomography, or had information on all components of the FIB-4 index. Linear regression models were used to assess the relationship

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SUPPLEMENTARY MATERIAL

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of NAFLD and FIB-4 with regional tau and Aβ, adjusting for potential confounders and multiple comparisons.

**Results:** Of the subsample with NAFLD information (N = 169; mean age 52 ± 9 y; 57% males), 57 (34%) had NAFLD. Of the subsample with information on liver fibrosis (N = 177; mean age 50 ± 10 y; 51% males), 34 (19%) had advanced fibrosis (FIB-4 > 1.3). Prevalent NAFLD was not associated with Aβ or tau PET. However, FIB-4 index was significantly associated with increased rhinal tau (β = 1.03 ± 0.33, $p = 0.002$). Among individuals with prevalent NAFLD, FIB-4 was related to inferior temporal, parahippocampal gyrus, entorhinal and rhinal tau (β = 2.01 ± 0.47, $p < 0.001$; β = 1.60 ± 0.53, $p = 0.007$, and β = 1.59 ± 0.47, $p = 0.003$ and β = 1.60 ± 0.42, $p = 0.001$, respectively) and to Aβ deposition overall and in the inferior temporal and parahippocampal regions (β = 1.93 ± 0.47, $p < 0.001$; β = 1.59 ± 0.38, $p < 0.001$, and β = 1.52 ± 0.54, $p = 0.008$, respectively).

**Conclusion:** This study suggests a possible association between liver fibrosis and early Alzheimer's disease pathology, independently of cardio-metabolic risk factors.

### Keywords

Alzheimer's disease; amyloid-β; liver fibrosis; non-alcoholic fatty liver disease; positron emission tomography

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver condition, affecting approximately 25% of the population in the developed world [1]. Metabolic dysregulation, including type 2 diabetes, insulin resistance, and hyperlipidemia are closely associated with NAFLD in a bidirectional manner [2]. Thus, NAFLD prevalence may reach 68% in individuals with type 2 diabetes [3] and up to 80% in those with morbid obesity [4]. Evidence suggests that NAFLD may be directly related to multiple extra hepatic health conditions [5], which include measures of vascular dysfunction [6–8], and cardiovascular morbidity and mortality [9, 10]. Furthermore, an increasing amount of evidence suggests that NAFLD is independently associated with measures of brain health. Indeed, NAFLD has been previously related to smaller brain volume in the Framingham Study's Offspring cohort [11] as well as in other populations [12]. In addition, individuals with NAFLD demonstrated poorer cognitive function [13] and reduced brain activity [14]. In contrast, other studies have failed to identify a link of NAFLD with cognitive function [15] and incident dementia [16].

The natural history of NAFLD is diverse and can include various stages of liver fibrosis [17]. Histologically, NAFLD encompasses a broad range of pathologies ranging from simple steatosis, with no or minimal inflammation, to nonalcoholic steatohepatitis that is characterized by necroinflammation and an increased progression of fibrosis [18]. Similarly to NAFLD, liver fibrosis is often clinically silent, and is present in up to 9% of individuals without known liver disease [19]. It is increasingly recognized that liver fibrosis, rather than the existence of hepatic steatosis per se, is a strong prognostic factor for long-term complications including liver-related outcomes, cardiovascular mortality [20] and stroke

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[21]. Recent evidence also stresses the possible important role of liver fibrosis in cognitive function and dementia risk [22, 23].

Despite the growing support for the implications of NAFLD and liver fibrosis to brain health, the connection with Alzheimer's disease (AD) is unknown. *In vivo* quantification of amyloid-β (Aβ) and tau deposition are valid biomarkers for prodromal AD and can emerge decades prior to AD clinical diagnosis [24]. Thus, in the current study, we utilized data from PET imaging to examine the association of NAFLD and liver fibrosis with regional Aβ and tau deposition. We hypothesized that NAFLD and liver fibrosis will be associated with increased Aβ and tau PET retention in brain regions in which AD pathology initially emerges, and that these associations will be independent of cardio-metabolic measures.

## METHODS

### Study sample

The study sample is based on participants from the Offspring [25] and third generation [26] of the Framingham Heart Study (FHS). Figure 1 presents a flow chart of the study sample. We included a total of 5,841 participants, of them 2,430 Offspring and 3,411 third generation, who attended exams 9 (2011–2014) and 2 (2008–2011), respectively. Of these, 4,991 participated in the multi-detector CT 2 sub-study for evaluation of ectopic fat, including liver fat, between September 2008 and December 2011 or had information on all components of the liver fibrosis score (FIB-4). We excluded 803 participants with excessive alcohol consumption defined as self-report of >14 alcoholic drinks/week for men and >7 alcoholic drinks/week for women because we were interested in non-alcoholic fatty liver disease. Thus, 4,188 were eligible for inclusion, among them PET imaging was obtained for a representative sample (with respect to vascular risk) of 230 eligible participants. Of them, 169 (73%) had information on NAFLD, 177 (77%) had information on all components of FIB-4, and 116 (50%) had information on both NAFLD and all components of FIB-4. Eligibility for the PET imaging sub-study included absence of significant neurological conditions including clinical stroke, dementia, and multiple sclerosis. As previously described, FHS participants undergo routine cognitive screening and comprehensive monitoring for continual surveillance of dementia [27], which was exclusionary for participation in the PET imaging sub-study and therefore also in the current study. Data were obtained under a protocol approved by the institutional review board of the Boston University Medical Center, and written informed consent was obtained from all participants.

### Assessment of fatty liver

Multi-detector CT was performed using 8-slice MDCT technology (LightSpeed Ultra, General Electric, Milwaukee, WI, USA). A calibration phantom (Image Analysis, Lexington, KY, USA) with a water equivalent compound (CT-Water, Light Speed Ultra, General Electric, Milwaukee, WI, USA) and calcium hydroxyapatite at 0, 75, and 150 mg/cm3 was placed under each participant [28]. Three areas from the liver and one from an external phantom were measured, and the average of the liver measures were then calculated and used to create liver/phantom ratios. NAFLD was defined as having a liver/phantom ratio

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≤0.33, consistent with prior FHS publications [29]. Additional details on multi-detector CT scan protocol and measurement of fatty liver can be found elsewhere [29].

### Assessment of liver fibrosis

The FIB-4 index was calculated using the following formula: age(years) x AST[U/L]/ (platelets [$10^9$/L] x (ALT[U/L])$^{1/2}$) [30]. The components of the FIB-4 index derive from readily available blood tests that are routinely measured [31]. FIB-4 index have been shown high validity compared to liver biopsy [31]. In addition, FIB-4 have also been demonstrated in a general population to predict cardiovascular disease [32, 33] and increased overall and liver disease-specific mortality [34]. FIB-4 score is categorized into three categories (low, inconclusive, and advanced), according to NAFLD recommended cut-off values of ≤1.3 to rule out advanced fibrosis, >1.3 and <2.67 as intermediate, and ≥2.67 to suggest advanced fibrosis [35].

### Assessment of Aβ and tau pathology

Consented participants underwent Aβ and tau PET imaging using $^{11}$C-Pittsburgh Compound B (PiB) and $^{18}$F-Flortaucipir (FTP), respectively. PET data were acquired using either a Siemens/CTI ECAT HR+ scanner (3D mode; 63 image planes; 15.2 cm axial field of view; 5.6 mm transaxial resolution; 2.4 mm slice interval) or a Discovery MI (GE Healthcare) PET/CT scanner. For the latter, the full width half maximum spatial resolutions measured at the center of the axial field of view (radial position = 1 cm) were 4.3 mm and 5.1 mm in transverse and axial directions respectively. 10-min transmission scans were collected at the beginning of each HR+ scan for attenuation correction, and low-dose CT acquisitions were performed before each Discovery MI scan for the same purpose. After injection of 8.5–15 mCi of PiB, 60 min of dynamic data were acquired in 3D acquisition mode. These data were reconstructed in 39 frames (8 × 15s, 4 × 60s, and 27 × 120 s). FTP was prepared with a mean radiochemical yield of 14 ± 3% and specific activity of 216 ± 60 GBq/mol (5837 ± 1621 mCi/mol) at the end of synthesis (60 min) and validated for human use [36]. After a 10.0 ± 1.0 mCi bolus injection, images were acquired from 80 to 100 minutes in 4 × 5 min frames.

All PET data was co-registered to the corresponding T1 images for each participant using SPM12. FreeSurfer v6.0 was used to derive 215 regions of interest (ROIs) [37]. Images were inspected for adequate count statistics, and head motion between frames, if any, were compensated in the software. PET data were evaluated without partial volume correction given the relatively young age of the sample with minimal atrophy. PiB retention was expressed as the distribution volume ratio (DVR) using the cerebellar cortex as a reference. A PiB summary measure, frontal, lateral, and retrosplenial cortices (FLR), was derived from the mean of superior frontal, inferior frontal, rostral middle frontal, rostral anterior cingulate, medial orbitofrontal, inferior and middle temporal, inferior parietal, and precuneus regions [38]. Tau measurement parameters were expressed as the standardized uptake value ratio (SUVr) and included assessment of uptake at pre-defined ROIs in the entorhinal cortex, inferior temporal lobe and parahippocampal gyrus (compared to cerebellar cortex) [39]. A rhinal region that overlaps the entorhinal region was included because it has been shown to more accurately assess the earliest stage of temporal lobe tauopathy [40].

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## Covariate assessment

Covariates were chosen based on prior knowledge on their associations both with liver traits and AD risk. All covariates were assessed at the second examination cycle (May 2008 to March 2011) for the Third Generation and the ninth examination cycle (April 2011- March 2014) for the Offspring Cohort participants. Serum ALT, AST, platelets, HDL and total cholesterol levels were obtained from fasting morning samples using an automated Roche method (Roche cobas 501). The HDL to total cholesterol ratio rather than the inclusion of each measure separately was used as a covariate to avoid collinearity in the regression models and due to the high correlation of the ratio with cardiometabolic risk [41]. Alcohol use and smoking status were assessed using physician-administrated questionnaires. Participants were considered current smokers if they had smoked at least one cigarette per day in the year preceding the FHS examination. Using standard protocols, trained technicians measured blood pressure, height, and weight in all participants as has been previously reported [42]. Body mass index (BMI) was defined as weight (kg)/height$^2$ ($m^2$). The physical activity index (PAI) is a composite score of self-reported total physical activity, constructed for each participant by weighting each hour in a typical day based on their activity level [43]. Diabetes was defined as a fasting plasma glucose ≥126 mg/dL or treatment with a hypoglycemic agent or insulin. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or on treatment with an antihypertensive agent. Volume of visceral adipose tissue was assessed using a 8-slice supine multidetector CT as previously described [44]. Serum C-reactive protein was measured using high-sensitivity assay. Cardiovascular disease was considered as present (yes versus no) if a person had at least one of the following conditions: cardiovascular death, fatal or nonfatal myocardial infarction, stroke, angina pectoris, unstable angina (prolonged ischemic episode with documented reversible ST-segment changes), transient ischemic attack, heart failure and intermittent claudication.

## Statistical analysis

Statistical analysis was performed using SAS version 9.4 between February 25, 2021 and April 1, 2021. Descriptive statistics were calculated in the total sample and stratified by prevalent NAFLD, with values presented as mean and SD, median and interquartile range (IQR) or frequency and percent for continuous, skewed continuous, and categorical variables, respectively. *p*-values comparing characteristics between participants with and without prevalent NAFLD are presented from a *t*-test, non-parametric Wilcoxon rank sum test, or Chi-square test. Linear regression models were constructed to estimate beta coefficients and SE for the association between prevalent NAFLD (exposure) and amyloid and tau PET (outcome). Similarly, we assessed liver fibrosis as both a continuous, log-transformed measure, and as a dichotomous predictor in linear regression models. A FIB-4 cutoff of 1.3 was chosen because it is an established threshold for which values below it indicate the lack of advanced fibrosis in NAFLD patients who are under the age of 65 years [45, 46]. First, the associations between liver fibrosis and Aβ and tau deposition were tested in the total sample. All models were adjusted for age, sex, time between exposure and PET, and camera (Model 1). An additional model (Model 2) also adjusted for BMI, alcohol consumption, smoking, cardiovascular disease, C-reactive protein, total to HDL cholesterol ratio, diabetes, and hypertension. Second, we examined the association between fibrosis

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and PET in a subsample of individuals with prevalent NAFLD. Due to the small sample size of this analysis, only covariates included in model 1 were adjusted for. In all models, FLR and inferior temporal amyloid were log transformed due to skewness. As sensitivity analyses, we 1) reran the association between fibrosis and PET but excluded subjects with missing information on prevalent NAFLD; 2) reran the association of NAFLD and fibrosis with PET while additionally adjusting for visceral adipose tissue; and 3) examined the relationships between liver fibrosis and Aβ deposition in additional brain regions (i.e., caudal anterior cingulate, isthmus cingulate, medial orbitofrontal, posterior cingulate and rostral anterior cingulate). The selection of these regions was based on recent evidence suggesting that Aβ pathology appears in these regions at early disease stages [47], and all were log transformed due to skewness. A $p$-value below 0.05 was considered statistically significant. We also applied correction for multiple testing using the Benjamini-Hochberg False Discovery Rate (FDR) procedure [48], which controls for the expected proportion of falsely rejected hypotheses. We indicated those considered statistically significant using the FDR method (FDR $p$-value < 0.05) in the table footnotes.

## RESULTS

The characteristics of the total study sample (N = 230) and of those who were not included in our analyses are presented in Supplementary Table 1, and the characteristics of subgroups with NAFLD, fibrosis and both conditions are presented in Supplementary Table 2. The age of the participants in the study sample who had information on NAFLD or fibrosis ($n$ = 230) was 50 ± 10 years, and 112 (49%) were women. The prevalence of NAFLD was 34% (57 out of 169). Intermediate or high risk for advanced fibrosis (FIB-4 > 1.3) was present in 19% of the total sample with FIB-4 information (34 of 177) and in 17% in those with prevalent NAFLD (7 out of 41).

Table 1 presents the characteristics of participants with and without NAFLD. Those with NAFLD were more likely to be men and to have diabetes, insulin resistance, and hypertension. In addition, compared to individuals without NAFLD, those with NAFLD had higher BMI and visceral adipose tissue volume, as well as increased systolic blood pressure and higher levels of total and HDL cholesterol, serum C-reactive protein and ALT and AST. There were no significant differences in liver fibrosis index between those with and without NAFLD (Table 1). Additionally, individuals with intermediate or high risk for advanced fibrosis (FIB-4 > 1.3) were older, had lower C-reactive protein levels, and as expected, had higher ALT and AST, lower platelets levels and higher fibrosis score compared to those with lower risk for advanced fibrosis (Supplementary Table 3).

### The association between NAFLD prevalence and PET Aβ and tau

In our sample, there were no differences in Aβ and tau PET deposition between participants with and without NAFLD (Table 2).

### The association between liver fibrosis and PET Aβ and tau in the general sample

After adjusting for all the study's covariates, each 1-unit increment in FIB-4 index was significantly associated with increased tau deposition in the inferior temporal (β = 0.80 ±

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0.31; $p = 0.01$), parahippocampal (β = 0.88 ± 0.32; $p = 0.01$), entorhinal (β = 0.82 ± 0.35; $p = 0.02$), and rhinal brain regions (β = 1.03 ± 0.33; $p = 0.002$). The association with Rhinal tau remained also after accounting for multiple comparisons (Table 3, Model 2). No significant differences were observed when individuals with increased risk for fibrosis (FIB-4 > 1.3) were compared with those with lower risk. In a sensitivity analysis restricting the sample to participants who had information on NAFLD we found similar findings ($n = 116$; Supplementary Table 4). Results were also similar after controlling for visceral adipose tissue in addition to previously mentioned covariates (Supplementary Table 5). There was no significant relationship between liver fibrosis and Aβ deposition overall and in the inferior temporal, parahippocampal and entorhinal regions (Table 3, Supplementary Tables 4 and 5). In addition, no significant associations were found between liver fibrosis and Aβ deposition in the caudal anterior cingulate, isthmus cingulate, medial orbitofrontal, posterior, and rostral cingulate regions (Supplementary Table 6).

### The association between liver fibrosis and PET Aβ and tau in persons with prevalent NAFLD

After adjustment for age, sex, time between assessment of fibrosis and PET and camera, increased FIB-4 index was significantly associated with higher levels of amyloid in FLR (β = 1.93 ± 0.47; $p < 0.001$), inferior temporal amyloid (β = 1.59 ± 0.38; $p < 0.001$), parahippocampal regions (β = 1.52 ± 0.54; $p = 0.008$), and tau in inferior temporal (β = 2.01 ± 0.47; $p < 0.001$), parahippocampal (β = 1.60 ± 0.53; $p = 0.007$), entorhinal (β = 1.59 ± 0.47; $p = 0.003$), and rhinal regions (β = 1.60 ± 0.42; $p = 0.001$) in individuals with prevalent NAFLD (Table 4). Similarly, increased Aβ and tau levels were observed in individuals with intermediate/high versus low risk for advanced fibrosis (β = 1.33 ± 0.44; $p = 0.005$, β = 1.10 ± 0.36; $p = 0.005$, β = 2.57 ± 0.56; $p < 0.001$, β = 1.72 ± 0.70; $p = 0.02$, β = 1.97 ± 0.58; $p = 0.003$, and β = 1.59 ± 0.59; $p = 0.01$, for FLR amyloid, interior temporal amyloid, inferior temporal tau, parahippocampal tau, entorhinal tau and rhinal tau, respectively). Visceral adipose tissue volume was strongly correlated with BMI (r = 0.67; $p < 0.001$), and similar results were obtained after further adjustment for visceral adipose tissue volume (Supplementary Table 7).

## DISCUSSION

Our study explored the association of NAFLD and non-invasive liver fibrosis index with Aβ and tau deposition in the brain. We observed no associations between NAFLD and brain Aβ and tau. However, in the total sample, high risk for advanced liver fibrosis was significantly related to tau deposition in the rhinal brain region. Among individuals with NAFLD, advanced liver fibrosis was related to tau pathology in the inferior temporal, parahippocampal, entorhinal and rhinal regions as well as to Aβ deposition overall and in the inferior temporal and parahippocampal brain regions.

In line with our findings, a recent review of the available evidence for cognitive dysfunction in NAFLD concluded that despite insufficient evidence on the link between the whole NAFLD spectrum and cognitive dysfunction, simple steatosis may not be an independent risk factor for cognitive dysfunction and that a more severe NAFLD, with involvement of

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fibrosis and hepatitis may be required to affect cognition [13]. This includes a previous study from our group, which found no significant association between NAFLD and cognitive function, yet risk for advanced liver fibrosis assessed using the NAFLD fibrosis score was associated with poorer executive function and abstract reasoning among individuals with NAFLD [23]. Findings are also consistent with regard to dementia risk. Indeed, a cohort study with histological data demonstrated no association between NAFLD and incident dementia, yet, histological indicators of fibrosis improved dementia risk prediction beyond that of conventional dementia risk factors [16]. Accordingly, a community-based cohort study conducted in Germany found no significant link between NAFLD and all-cause or vascular dementia [49], while in the Italian longitudinal study, NAFLD fibrosis score among the general population (i.e., not restricted to NAFLD patients) was related to increased dementia risk in those who were physically frail. Although the latter studies did not assess NAFLD and fibrosis together, they imply that liver fibrosis rather than NAFLD per se is a risk factor for dementia. Lastly, few studies exist showing that the presence of white matter hyperintensities is associated with the fibrosis severity among patients with NAFLD [50, 51], which again, consistent with our report, highlights the importance of liver fibrosis to brain health.

The pathophysiology of both NAFLD and AD are complex and multi-factorial, and the mechanistic links between them are speculative. Moreover, it should be noted that the assessment of liver fibrosis in our study is based on a non-invasive score that may indicate liver function in general rather than liver fibrosis specifically. Because the liver is responsible for activation, clearance, and processing of multiple molecules, there may be various mechanisms in which liver dysfunction may affect the brain. For example, liver damage may lead to change in cholesterol catabolism through its conversion to primary bile acids, which in turn may be linked with increased AD risk, both in animals [52] and humans [53].

One major hypothesis for the link between NAFLD and dementia to date was that dementia risk in NAFLD is driven primarily by vascular forms. This hypothesis was based on studies showing a direct association of NAFLD, and particularly NAFLD with advanced fibrosis, with subclinical vascular damage including atherosclerosis, endothelial dysfunction, arterial stiffness, vascular calcification [54–56], and cerebral small vessel disease [50, 51]. However, recent explorations of AD temporal dynamics suggest that accumulation of Aβ and tau precedes cerebral small vessel disease [57, 58]. Thus, the strong link between fibrosis and AD pathology in our study may highlight the additional contribution of other pathophysiological mechanisms. One possible pathway for the link of liver fibrosis with Aβ and tau deposition in our study may be through systemic inflammation, which characterizes advanced liver fibrosis in particular [59]. Indeed, a network clustering and pathways enrichment pointed to the Interleukin signaling pathway as a key pathway underlying both NAFLD and AD [60]. Furthermore, according to preclinical studies, systemic inflammation may increase dementia risk not only through elevation in atherosclerosis and cerebral small vessel disease [20, 61], but also by triggering neuroinflammation that promotes cerebral Aβ accumulation directly [62, 63]. Further support for this hypothesis arrives from the Atherosclerosis Risk in Communities (ARIC) - PET Study, which demonstrated a link between systemic inflammation and cerebral Aβ deposition, albeit only in specific race and

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sex groups [64]. Moreover, a recent study among the Alzheimer's Disease Neuroimaging initiative (ADNI) participants with mild cognitive impairment demonstrated an association between elevated inflammatory markers and tau, but not Aβ pathology [65]. These results are in accordance with the link of liver fibrosis with tau but not Aβ pathology in our general sample, and may highlight the importance of inflammation as an underlying mechanism. An additional explanation for our findings may be that hepatic dysfunction and insulin resistance lead to insufficient Aβ clearance in the blood, possibly through reduced expression of Low-density lipoprotein receptor–related protein 1 (LRP1) [66], which in turn contributes to brain Aβ accumulation [67]. Lastly, toxic metabolites produced in the injured liver may cross the blood brain barrier and lead to Aβ and tau pathology. For example, ceramides, bioactive sphingolipids, are activated in the course of NAFLD [68] and liver fibrosis [69], and were recently linked with incident AD and Aβ load on PET in the Framingham Study [70].

We show that in the general sample (i.e., not restricting to those with NAFLD), higher risk of advanced fibrosis is coupled with tau but not with Aβ deposition. Although previous studies demonstrated that amyloidosis is required for the subsequent elevation of tauopathy [71, 72], recent PET assessments confirm autopsy studies [73] by highlighting the contribution of the initial medial temporal tau deposition to AD natural history [40]. Specifically, the first signal of tau PET is thought to appear independently of Aβ burden in the rhinal cortex, which in our study showed strong association with FIB-4 index. Subsequent to the tau deposition in the rhinal cortex, tau is thought to spread to temporal neocortex but this stage is dependent on the primary tau accumulation and on global Aβ load [40]. Thus, our findings may advance the literature by showing that liver fibrosis is linked with early markers of AD, upstream to the development of vascular pathology and Aβ aggregation. Furthermore, tau deposition in these brain regions are highly correlated with early AD clinical phenotypes [72, 74–76].

Focusing on a subsample with confirmed NAFLD yielded a small sample size and therefore limited power to adjust for the whole set of confounders. However, after adjustment for age, sex, time between exposure and PET and camera, we found additional associations of advanced fibrosis with overall Aβ load as well as in the inferior temporal and parahippocampal regions. Due to between-studies heterogeneity in Aβ detection techniques (e.g., PET versus autopsy) as well as in population characteristics and study settings, the spatiotemporal ordering of Aβ deposition is not fully understood [47]. Yet, recent PET studies suggest that Aβ accumulates initially in the medial frontal and cingulate regions [47] which, in our sensitivity analysis, were not related to liver fibrosis. In contrast, overall Aβ load and its deposition in brain regions that were linked with liver fibrosis in our study (e.g., middle temporal) are considered to accumulate in relatively later AD stages [77, 78]. However, these accumulations of Aβ may be needed for the observed spread of tau beyond the middle temporal lobe to neocortical regions [40, 72].

The inconsistency in results between the general sample and the NAFLD subsample (i.e., associations of FIB-4 with tau in the first and with tau and Aβ in the latter) may imply that Aβ deposition is influenced by a synergistic effect of liver steatosis and fibrosis. Alternatively, the different findings may reflect the differential capacity of the FIB-4 index

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to non-invasively identify advanced fibrosis in these two samples. Of note, FIB-4 has been extensively studied in ethnically diverse NAFLD populations where it showed good ability to discriminate advanced versus non-advanced fibrosis [79, 80]. On the other hand, it may have limited ability to estimate risk of advanced fibrosis in the general population [81]. In addition, the FIB-4 cutoff for advanced fibrosis has been determined among persons with NAFLD [45], which is in line with our findings that dichotomous FIB-4 using this cutoff is related to AD markers only in the NAFLD subsample. Thus, despite the small sample of participants who had both PET and NAFLD information, the significant link between FIB-4 and both Aβ and tau may more specifically indicate the consequences of advanced liver fibrosis.

The strengths of our study include the predominantly middle-aged sample with information on Aβ and tau PET imaging, the CT-based ascertainment of NAFLD and the well-characterized cohort of individuals with a wide variety of metabolic and lifestyle covariates, including visceral adipose tissue. We also acknowledge several limitations of our study: first, this is a cross-sectional design that does not allow inference on temporal relationship between liver conditions and AD pathology. Second, liver fibrosis was assessed using a non-invasive index with limited validity compared to liver biopsy as the gold standard, and we lacked imaging information such as magnetic resonance elastography or ultrasound elastography (i.e., FibroScan) [82]. However, FIB-4 has the advantage of being a simple tool composed of routinely collected biochemical variables. Third, we lacked information on fibrosis etiology other than NAFLD such as hepatotoxic medications, viral hepatitis, autoimmune liver disease, or alcohol abuse (due to underreporting of alcohol consumption). A fourth limitation is the restricted external validity of our results as the sample was predominantly of European ancestry, from one geographic area and of a relatively high socioeconomic status. Lastly, the statistical power was limited due to small number of persons in some subgroup analyses, and residual confounding could exist due to incomplete adjustment.

In conclusion, our study highlights the importance of liver fibrosis severity to extrahepatic health conditions by demonstrating a link between liver fibrosis severity and early stages of AD. If validated in other studies, these findings suggest that FIB-4 may improve risk stratification models in AD and may help identifying individuals at risk for tau and Aβ accumulation, who may benefit from preventive strategies. The public health implications of these findings may be particularly significant because liver fibrosis is prevalent and yet often underdiagnosed. In addition, liver fibrosis can be managed through lifestyle modifications as well as through existing therapeutics and numerous drugs that are currently under development [18]. Future investigations are necessary to explore whether therapeutic strategies that target liver fibrosis can lead to decreased AD burden and to clarify the underlying mechanisms linking liver fibrosis to AD.

## Supplementary Material

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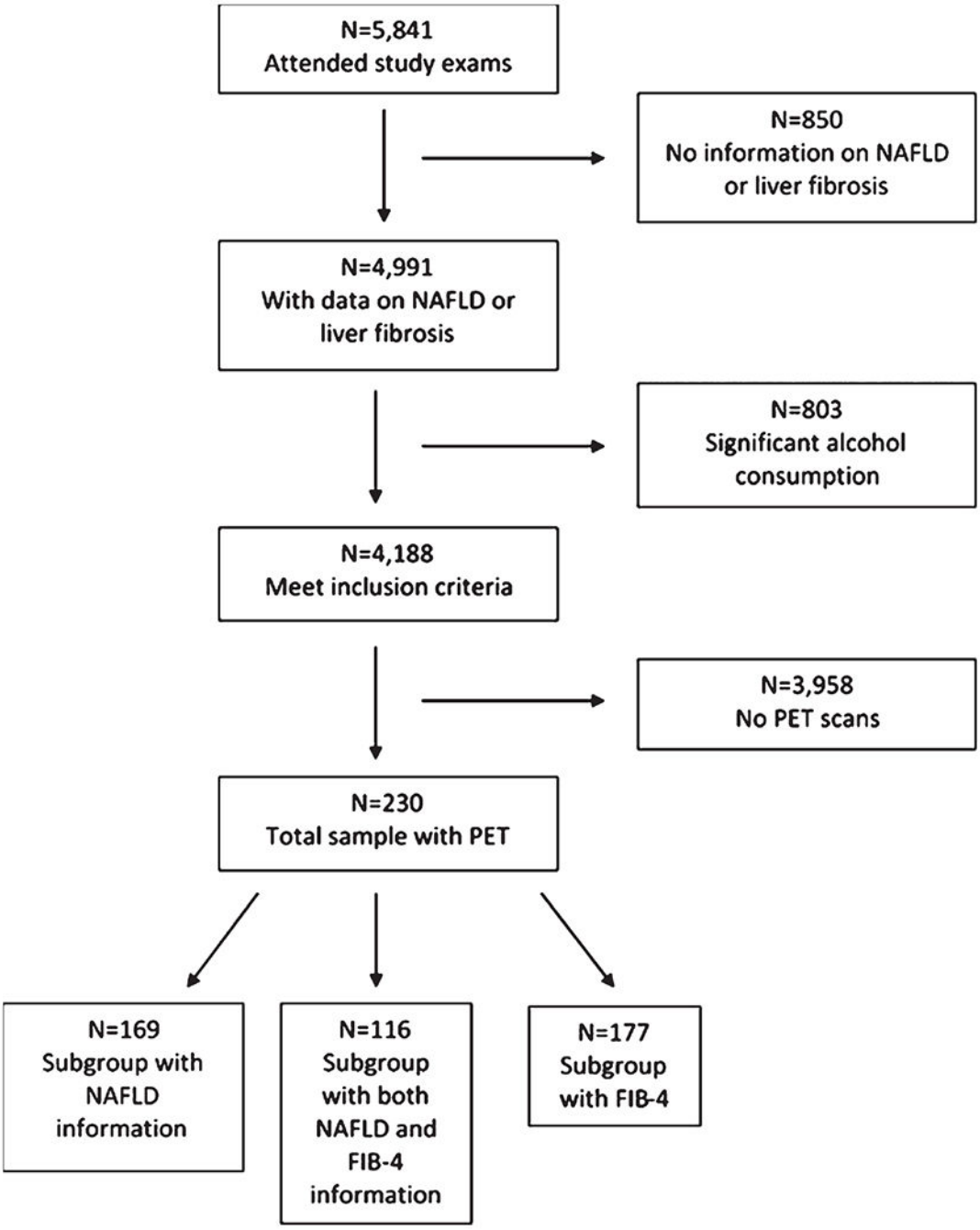


**Fig. 1.**
Flow chart of the study sample.

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**Table 1**

Study sample characteristics according to presence of NAFLD

| General characteristics | N | Total sample (N = 169) | No NAFLD (N = 112) | Prevalent NAFLD (N = 57) | *p* |
|---|---|---|---|---|---|
| Age (y) | 169 | 52(9) | 52(8) | 53(9) | 0.40 |
| Male, n(%) | 169 | 96(57%) | 57(51%) | 39(68%) | **0.03** |
| Time between CT and PET(y) | 169 | 8(1) | 8(1) | 8(1) | 0.79 |
| Time between exam and PET(y) | 169 | 8(1) | 8(1) | 8(2) | 0.61 |
| Generation 3 Cohort, n(%) | 169 | 132(78%) | 88(79%) | 44(77%) | 0.84 |
| Body Mass Index(kg/m$^2$), Median[IQR] | 169 | 28[25, 32] | 27[24, 29] | 32[27, 36] | **<0.001** |
| Alcohol consumption (drinks per day), Median[IQR] | 169 | 2[0, 5] | 3[0, 5] | 1[0, 4] | 0.16 |
| Systolic Blood Pressure(mg/dL) | 169 | 117(13) | 115(10) | 122(15) | **0.001** |
| Smoker, n(%) | 169 | 6(4%) | 2(2%) | 4(7%) | 0.18 |
| Cardiovascular disease, n(%) | 169 | 9(5%) | 3(3%) | 6(11%) | 0.06 |
| Physical Activity Index, Median[IQR] | 168 | 35[32, 39] | 35[32, 39] | 34[30, 37] | 0.18 |
| Serum c-reactive protein(mg/L), Median[IQR] | 167 | 1.2[0.6, 3.0] | 0.9[0.5, 2.3] | 2.1[1.0, 4.2] | **<0.001** |
| Total cholesterol(mg/dL) | 169 | 184(36) | 188(36) | 176(36) | **0.03** |
| High-density lipoprotein cholesterol(mg/dL) | 169 | 57(17) | 61(17) | 50(13) | **<0.001** |
| Volume of visceral adipose tissue(cm$^3$) | 167 | 2311(1394) | 1840(1117) | 3269(1419) | **<0.001** |
| HOMA-IR, Median[IQR] | 168 | 2.5[1.5, 3.9] | 2.1[1.3, 2.9] | 4.0[2.6, 5.9] | **<0.001** |
| Diabetes, n(%) | 168 | 15(9%) | 5(5%) | 10(18%) | **0.008** |
| Hypertension, n(%) | 169 | 51(30%) | 21(19%) | 30(53%) | **<0.001** |
| ALT(U/L), Median[IQR] | 168 | 23[17, 31] | 21[15, 26] | 29[20, 36] | **<0.001** |
| AST (U/L), Median[IQR] | 168 | 21[18, 25] | 20[17, 24] | 24[18, 26] | **0.02** |
| Platelets (×10$^9$/L) | 117 | 250(64) | 247(67) | 255(57) | 0.55 |
| **Liver Function Characteristics** | | | | | |
| Fibrosis-4 Score(FIB-4), Median[IQR] | 116 | 0.9[0.8, 1.3] | 0.9[0.8, 1.4] | 1.0[0.8, 1.2] | 0.92 |
| Intermediate/Advanced Fibrosis (FIB-4> 1.3), n(%) | 116 | 27(23%) | 20(27%) | 7(17%) | 0.24 |

NAFLD, non-alcoholic fatty liver disease; CT, computed tomography; PET, positron emission tomography; IQR, intra-quartile range; HOMA-IR, homeostatic model assessment for insulin resistance; ALT, alanine aminotransferase; AST, aspartate transaminase. Bold values indicate *p*-value< 0.05.

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**Table 2**

Association between NAFLD and PET Amyloid-β and tau (N = 169)

| | Model 1 | | Model 2 | |
|---|---|---|---|---|
| | **ß±SE** | ***p*** | **ß±SE** | ***p*** |
| Amyloid FLR[a] | 0.02±0.16 | 0.92 | 0.06±0.19 | 0.75 |
| Inferior temporal amyloid[a] | −0.07±0.13 | 0.60 | 0.03±0.16 | 0.83 |
| Parahippocampal amyloid | 0.05±0.17 | 0.78 | 0.13±0.20 | 0.50 |
| Entorhinal amyloid | 0.16±0.16 | 0.34 | 0.20±0.19 | 0.30 |
| Inferior temporal tau | 0.15±0.19 | 0.42 | 0.18±0.22 | 0.42 |
| Parahippocampal tau | −0.13±0.20 | 0.53 | −0.05±0.24 | 0.84 |
| Entorhinal tau | 0.04±0.21 | 0.85 | −0.01±0.25 | 0.96 |
| Rhinal tau | 0.07±0.20 | 0.71 | −0.10±0.24 | 0.69 |

NAFLD, non-alcoholic fatty liver disease; PET, positron emission tomography; SE, standard error; FLR, frontal, lateral, and retrosplenial. Model 1: Adjusted for age, sex, time between exposure and PET, and camera. Model 2: Adjusted for age, sex, time between exposure and PET, camera, body mass index, alcohol consumption, smoking, cardiovascular disease, c-reactive protein, total to HDL-cholesterol ratio, diabetes, and hypertension.

[a]log transformed.

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**Table 3**

Association of liver fibrosis with PET Amyloid-β and tau (N=177)

| | Fib-4 score[a] | | | | Fib-4 > 1.3 | | | |
|---|---|---|---|---|---|---|---|---|
| | Model 1 | | Model 2 | | Model 1 | | Model 2 | |
| | ß±SE | *p* | ß±SE | *p* | ß±SE | *p* | ß±SE | *p* |
| Amyloid FLR[a] | 0.06±0.24 | 0.82 | 0.10±0.27 | 0.71 | 0.09±0.20 | 0.66 | 0.17±0.22 | 0.43 |
| Inferior temporal amyloid[a] | −0.04±0.19 | 0.82 | −0.11±0.21 | 0.59 | 0.05±0.16 | 0.75 | 0.11±0.17 | 0.52 |
| Parahippocampal amyloid | −0.12±0.24 | 0.62 | −0.20±0.26 | 0.44 | −0.01±0.20 | 0.96 | 0.06±0.21 | 0.76 |
| Entorhinal amyloid | −0.34±0.24 | 0.16 | −0.52±0.26 | 0.05 | −0.16±0.20 | 0.42 | −0.13±0.21 | 0.55 |
| Inferior temporal tau | 0.52±0.27 | 0.06 | 0.80±0.31 | **0.01** | 0.16±0.28 | 0.56 | 0.28±0.28 | 0.33 |
| Parahippocampal tau | 0.65±0.29 | **0.03** | 0.88±0.32 | **0.01** | 0.32±0.30 | 0.28 | 0.44±0.30 | 0.13 |
| Entorhinal tau | 0.39±0.30 | 0.20 | 0.82±0.35 | **0.02** | 0.20±0.31 | 0.51 | 0.39±0.31 | 0.22 |
| Rhinal tau | 0.72±0.30 | **0.02** | 1.03±0.33 | **0.002** *b* | 0.12±0.30 | 0.68 | 0.26±0.30 | 0.39 |

PET, positron emission tomography; FIB-4, Fibrosis-4 score; SE, standard error; FLR, frontal, lateral, and retrosplenial. Model 1: Adjusted for age, sex, time between exposure and PET, and camera. Model 2: Adjusted for age, sex, time between exposure and PET, camera, body mass index, alcohol consumption, smoking, cardiovascular disease, c-reactive protein, total to HDL-cholesterol ratio, diabetes, and hypertension.

[a] log transformed.

[b] Significant at FDR-corrected α=0.05 level. Bold values indicate *p*-value<0.05.

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**Table 4**

Association of liver fibrosis with PET Amyloid-β and tau in subjects with prevalent NAFLD(N=41)

| | Fib-4 score[a] | | Fib-4 > 1.3 | |
|---|---|---|---|---|
| | **ß±SE** | ***p*** | **ß±SE** | ***p*** |
| Amyloid FLR[a] | 1.93±0.47 | **<0.001** *b* | 1.33±0.44 | **0.005** *b* |
| Inferior temporal amyloid[a] | 1.59±0.38 | **<0.001** *b* | 1.10±0.36 | **0.005** *b* |
| Parahippocampal amyloid | 1.52±0.54 | **0.008** *b* | 1.00±0.49 | 0.05 |
| Entorhinal amyloid | 0.89±0.44 | 0.05 | 0.61±0.39 | 0.13 |
| Inferior temporal tau | 2.01±0.47 | **<0.001** *b* | 2.57±0.56 | **<0.001** *b* |
| Parahippocampal tau | 1.60±0.53 | **0.007** *b* | 1.72±0.70 | **0.02**[b] |
| Entorhinal tau | 1.59±0.47 | **0.003** *b* | 1.97±0.58 | **0.003** *b* |
| Rhinal tau | 1.60±0.42 | **0.001** *b* | 1.59±0.59 | **0.01** *b* |

PET, positron emission tomography; NAFLD, non-alcoholic fatty liver disease; FIB-4, Fibrosis-4 score; SE, standard error; FLR, frontal, lateral, and retrosplenial. Adjusted for age, sex, time between exposure and PET, and camera.

*a*log transformed.

*b*Significant at FDR-corrected α=0.05 level. Bold values indicate *p*-value <0.05.