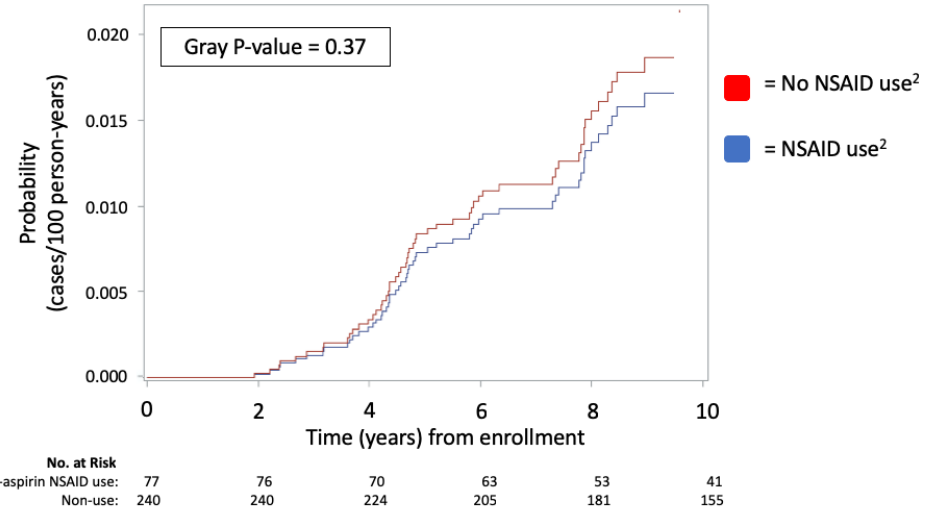
Figure S1. Cumulative Incidence of advanced fibrosis[1] with non-aspirin NSAID use[2], among patients with early-stage NAFLD* at enrollment (n=317)



No. at Risk						
Non-aspirin NSAID use:	77	76	70	63	53	41
Non-use:	240	240	224	205	181	155

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NSAID, nonsteroidal anti-inflammatory drug; No., number
[1]Incident advanced fibrosis was defined by the first recorded Fibrosis-4 (FIB-4) score > 2.67 or NAFLD Fibrosis Score (NFS) > 0.67, or aspartate aminotransferase-to-platelet ratio index (APRI) > 1.0 during study follow-up. Follow-up measurements of each index score was obtained at least annually in all included participants.
[2]Non-aspirin NSAID use was ascertained and verified by trained study staff at enrollment, and prospectively-updated during study follow-up and modeled as a time-varying exposure.
*Early-stage NAFLD defined as baseline fibrosis stage 0-2 on enrollment liver biopsy, confirmed by a blinded, central pathologist.

Supplementary Appendix

Supplementary Methods

Table S1. Association between daily aspirin use and prevalent NAFLD fibrosis in the NAFLD study population (n=361)

Table S2. Cross-sectional association between daily aspirin use and prevalent fibrosis, among patients with NAFLD who did not use non-aspirin NSAID medications (n=284):

Table S3. Association between daily aspirin use and incident advanced fibrosis, among patients with fibrosis stage 0-2 at enrollment (n=317)

Table S4. Aspirin use and risk for developing incident stage 3-4 fibrosis on follow-up liver biopsy, in the sub-group of patients with early-stage NAFLD at enrollment and paired liver biopsies during study follow-up (n=72)

Supplementary Appendix References

Supplementary Methods

Laboratory Testing and Histological Evaluation at Enrollment

On the date of enrollment biopsy, all patients underwent a detailed evaluation, including a complete physical examination and the collection of detailed data regarding personal and family medical history, medication and supplement use, alcohol intake (estimated drinks/week) and detailed laboratory testing. Laboratory testing included liver biochemistries (alanine aminotransferase [ALT] and aspartate aminotransferase [AST], total bilirubin, albumin, alkaline phosphatase); complete blood count; fasting lipids; fasting glucose; fasting insulin; serum ferritin; transferrin saturation; and viral serologies for hepatitis B and C infection. Individuals with abnormal aminotransferase levels also had testing including autoantibodies; alpha-1 antitrypsin levels; and, when appropriate, ceruloplasmin levels. Each NAFLD diagnosis was subsequently confirmed through blinded physician review of the complete medical record.

All liver biopsy slides were read and scored by a blinded, central hepatopathologist, as outlined in the main Methods Section. A threshold of 5% of hepatocytes showing steatosis was required for the histologic diagnosis of NAFLD[1, 2], and NAFLD was graded and staged using validated scoring systems[1]. Histological grade was obtained separately for steatosis (0 to 3), hepatocyte ballooning (0 to 2) and lobular inflammation (0 to 3), on hematoxylin and eosin (H&E) stained slides. We defined NASH as the presence of steatosis and hepatocyte ballooning and lobular inflammation (all grade >0), consistent with prior publications[2]. Fibrosis was staged as follows: no fibrosis (stage 0), fibrosis in only zone 1 or zone 3 (stage 1), fibrosis in both zones 1 and 3 (stage 2), bridging fibrosis (stage 3), and cirrhosis (stage 4)[1]. Stage 1 was further subdivided into

mild fibrosis in zone 3 only (stage 1a), moderate fibrosis in zone 3 only (stage 1b), and any fibrosis in zone 1 only (stage 1c)[1].

Prior Validation and Calculation of FIB-4 Index, NAFLD Fibrosis Score (NFS) and the aspartate aminotransferase-to-platelet ratio index (APRI):

The FIB-4 and APRI were originally developed and validated in populations with chronic hepatitis C virus (HCV) infection[3-5], and were subsequently validated in NAFLD[6, 7], while the NFS was specifically developed and validated in NAFLD[7, 8]. These indices have demonstrated accuracy for identifying advanced NAFLD fibrosis, and for predicting long-term complications, including mortality[7, 9].

- APRI = (AST, in IU/L) / (AST Upper Limit of Normal, IU/L) / (Platelet count [x10^9/L]).
- FIB4 = (Age [years] * AST) / (Platelet count [x10^9/L] * √(ALT))
- NFS = -1.675 + (0.037*age[years]) + (0.094*BMI [kg/m^2]) + (1.13*Impaired glucose tolerance/diabetes [yes=1, no=0]) + (0.99*AST/ALT ratio) – (0.013*Platelet count [x10^9/L]) – (0.66*albumin [g/dl])

Concordance of the FIB-4 Index, NFS and APRI with Enrollment Liver Biopsy:

As outlined in the Methods section, all subjects underwent laboratory testing at the time of enrollment, with sufficient data for calculation of the FIB-4 and NFS and APRI indices. Among those with early-stage NAFLD on enrollment biopsy (i.e. fibrosis stage 0-2, n=317), all patients had NFS, FIB-4 and APRI scores below the validated threshold cut-offs for advanced fibrosis (i.e. all 317 patients had NFS < 0.67 and FIB-4 <= 2.67 and APRI <= 1).

Table S1. Association between daily aspirin use[1] and prevalent NAFLD fibrosis[2] in the NAFLD study population (n=361)

Variable	Cases of prevalent fibrosis[2]	Multivariable-adjusted OR (95% CI) Daily aspirin use[1]		P-interaction[3]
		No	*Yes*	
Age > 50 years at enrollment				
• No	21	1	0.22 (0.01-0.93)	0.12
• Yes	27	1	0.57 (0.24-1.13)	
Sex				
• Male	29	1	0.22 (0.05-0.90)	0.19
• Female	19	1	0.51 (0.25-0.99)	
Diabetes				
• No	18	1	0.45 (0.17-0.82)	0.47
• Yes	30	1	0.58 (0.17-1.04)	
Hispanic ethnicity				
• No	32	1	0.42 (0.19-0.93)	0.56
• Yes	16	1	0.67 (0.22-1.03)	
Any smoking history				
• No	12	1	0.75 (0.43-1.09)	0.35
• Yes	36	1	0.60 (0.34-0.96)	
Any statin use				
• No	29	1	0.46 (0.19-0.94)	0.59
• Yes	19	1	0.41 (0.10-0.85)	
Any non-aspirin NSAID use[4]				
• No	33	1	0.47 (0.24-0.90)	0.26
• Yes	15	1	0.55 (0.37-0.70)	

Abbreviations: NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; NSAID, nonsteroidal anti-inflammatory drug

*All models accounted for age at enrollment liver biopsy (years), sex, number of clinic visits, Hispanic ethnicity (yes v. no), BMI (continuous kg/m^2), diabetes (yes vs. no), hypertension (yes vs. no), hyperlipidemia (yes vs. no), use of statins (yes vs. no), use of metformin (yes vs. no), smoking status (current vs. former vs. never), history of prior CAD (any vs. none), and nonaspirin nonsteroidal anti-inflammatory drug (NSAID) use.

[1]Daily aspirin use was defined as daily use of aspirin, assessed at enrollment. Non-regular aspirin use was defined as less frequent use or never-use.

[2] Prevalent NAFLD fibrosis defined as fibrosis stage ≥1a on baseline liver biopsy

[3]P-interaction obtained using the Wald test in a fully-adjusted multivariable model that also incorporated the relevant interaction term.

[4] Non-aspirin NSAIDs included: ibuprofen, naproxen, ketoprofen, diclofenac, indomethacin, among others, recorded at enrollment.

Table S2. Cross-sectional association between daily aspirin use[1] and prevalent fibrosis, among patients with NAFLD who did not use non-aspirin NSAID medications[2] (n=284):

Histological feature		
	Non-Regular Aspirin Use (ref.; N=158)	**Daily Aspirin use[1] (N=126)**
Fibrosis (any vs. none)		
No. of patients with endpoint:	122	33
Age- and sex-adjusted* OR (95% CI)	1	0.44 (0.24-0.85)
Multivariable Model[¥]; OR (95% CI)	1	0.47 (0.24-0.90)
Fibrosis stage 3-4		
No. of patients with endpoint:	23	14
Age- and sex-adjusted* OR (95% CI)	1	0.37 (0.17-0.80)
Multivariable Model[¥]; OR (95% CI)	1	0.46 (0.20-0.96)

Abbreviations: NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; NSAID, nonsteroidal anti-inflammatory drug

*Model 1: adjusted for age at baseline liver biopsy and sex

[¥]Multivariable Model: Model 1 + Hispanic ethnicity (yes v. no), BMI (continuous kg/m^2), diabetes (yes vs. no), hypertension (yes vs. no), hyperlipidemia (yes vs. no), smoking history (current vs. prior vs. never), prior diagnosis of coronary artery disease (CAD; any vs. none), use of statins (yes vs. no), use of metformin (yes vs. no).

[1]Daily aspirin use was defined as daily use of aspirin. Less frequent use or never-use was defined as non-use. For this analysis, aspirin use data was ascertained and updated at enrollment.

[2]N=77 individuals who reported use of nonaspirin NSAIDs were excluded

Table S3. Association between daily aspirin use[1] and incident advanced fibrosis[2], among patients with fibrosis stage 0-2 at enrollment (n=317)

Variable	Cases of incident advanced fibrosis[2]	Multivariable-adjusted HR (95% CI) Daily aspirin use[1]		P-interaction[3]
		No	*Yes*	
Age > 50 years				
• No	12	1	0.46 (0.27-0.76)	0.38
• Yes	20	1	0.57 (0.37-0.88)	
Sex				
• Male	19	1	0.59 (0.38-0.92)	0.52
• Female	13	1	0.47 (0.28-0.78)	
Diabetes				
• No	14	1	0.50 (0.34-0.73)	0.26
• Yes	18	1	0.67 (0.44-0.95)	
Hispanic ethnicity				
• No	13	1	0.54 (0.39-0.76)	0.36
• Yes	19	1	0.29 (0.08-1.06)	
Any smoking history				
• No	22	1	0.52 (0.35-0.79)	0.52
• Yes	10	1	0.55 (0.29-1.03)	
Any statin use				
• No	20	1	0.48 (0.34-0.68)	0.21
• Yes	12	1	0.19 (0.01-0.95)	
Any non-aspirin NSAID use				
• No	21	1	0.66 (0.37-0.92)	0.45
• Yes	11	1	0.71 (0.40-0.99)	
Baseline fibrosis stage				
• Fibrosis stage 0-1	10	1	0.73 (0.49-0.95)	0.36
• Fibrosis stage 2	22	1	0.50 (0.32-0.77)	

Abbreviations: NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; NSAID, nonsteroidal anti-inflammatory drug

*All models accounted for age at enrollment liver biopsy (years), sex, Hispanic ethnicity (yes v. no), BMI (continuous kg/m^2), diabetes (yes vs. no), hypertension (yes vs. no), hyperlipidemia (yes vs. no), use of statins (yes vs. no), use of metformin (yes vs. no), smoking status (current vs. former vs. never), and history of prior CAD (any vs. none), and use of non-aspirin NSAIDs, with all relevant covariates updated annually during follow-up.

[1]Aspirin use was defined as daily use of aspirin. Less frequent use or never-use was defined as non-use. Aspirin use data was ascertained and updated at each follow-up visit and modeled as a time-varying covariate.

[2]Incident advanced fibrosis was defined as the first recorded FIB-4 Index >=2.67 or NFS >0.67 or APRI>1.0, on follow-up laboratory testing, in an individual with baseline fibrosis stage 0-2.

[3]P-interaction obtained using the Wald test in a fully-adjusted multivariable model that also incorporated the relevant interaction term.

[4] Non-aspirin NSAID use included use of ibuprofen, naproxen, ketoprofen, diclofenac, indomethacin, among others, recorded prospectively and updated annually over study follow-up.

Table S4. Aspirin use[1] and risk for developing incident advanced fibrosis, among those with early-stage NAFLD at enrollment[2] and paired liver biopsies over study follow-up (n=72)

	Non-aspirin use (*ref.*) (N=48)	**Aspirin use[1] (N=24)**
Incident Advanced Fibrosis (stage 3-4)		
No. of patients with endpoint:	16	5
Unadjusted OR (95% CI)	1	0.59 (0.41-0.72)
Model 2[¥]; OR (95% CI)	1	0.64 (0.50-0.80)

Abbreviations: OR, Odd's Ratio; CI, confidence interval; ref., reference; No., number
[¥]Model 2: adjusted for age, sex, baseline fibrosis stage and time (continuous months) between paired biopsies
[1]Aspirin use was defined as daily use of aspirin. Less frequent use or never-use was defined as non-use.
[2]Early-stage NAFLD defined as fibrosis stage 0-2 at baseline (total n=317)

Supplementary Appendix References

1. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-21.
2. Brunt EM, Kleiner DE, Wilson LA, et al. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology 2011;53:810-20.
3. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317-25.
4. Lin ZH, Xin YN, Dong QJ, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. Hepatology 2011;53:726-36.
5. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518-26.
6. Sumida Y, Yoneda M, Hyogo H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. BMC Gastroenterol 2012;12:2.
7. Angulo P, Bugianesi E, Bjornsson ES, et al. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 2013;145:782-9 e4.
8. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846-54.
9. Castera L. Noninvasive Evaluation of Nonalcoholic Fatty Liver Disease. Semin Liver Dis 2015;35:291-303.