

Non-alcoholic fatty liver disease and increased risk of fatal and nonfatal cardiovascular events: an updated systematic review and meta-analysis

Dr. Alessandro Mantovani, MD¹, Dr. Alessandro Csermely, MD¹, Dr. Graziana Petracca, MD¹,
Dr. Giorgia Beatrice, MD¹, Prof. Kathleen E. Corey, MD², Dr. Tracey G. Simon, MD^{2,3}, Prof.
Christopher D. Byrne, MB BCh, PhD^{4,5}, Prof. Giovanni Targher, MD¹

¹Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy

²Liver Center, Division of Gastroenterology, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

³Clinical and Translational Epidemiology Unit (CTEU), Massachusetts General Hospital, Boston, MA, USA

⁴Nutrition and Metabolism, Faculty of Medicine, University of Southampton, UK

⁵Southampton National Institute for Health Research Biomedical Research Centre, University Hospital Southampton, Southampton General Hospital, Tremona Road, Southampton, UK

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Address for correspondence:

Prof. Giovanni Targher, MD
Section of Endocrinology, Diabetes and Metabolism
Department of Medicine
University and Azienda Ospedaliera Universitaria Integrata
Piazzale A. Stefani, 1
37126 Verona, Italy
Phone: +39-045-8123110
E-mail: giovanni.targher@univr.it

SUMMARY

Background: Studies have reported a significant association between non-alcoholic fatty liver disease (NAFLD) and increased incidence of cardiovascular disease (CVD). However, the magnitude of the risk and whether this risk changes with the severity of NAFLD remains uncertain. We performed a meta-analysis of observational studies to quantify the magnitude of the association between NAFLD and risk of incident CVD events.

Methods: We systematically searched PubMed, Scopus and Web of Science from database inception to July 1, 2021 to identify eligible observational studies, in which NAFLD was diagnosed by imaging, International Classification of Diseases codes, or liver biopsy. The primary outcomes were CVD death, nonfatal CVD events, or both. Data from selected studies were extracted, and meta-analysis was performed using random-effects models to obtain summary hazard ratios (HRs) with 95% CIs. The quality of the evidence was assessed with the Cochrane risk of bias tool. This study is registered on Open Science Framework, number osf.io/5z7gf.

Findings: We identified 36 longitudinal studies with aggregate data on 5,802,226 middle-aged individuals of different countries and 99,668 incident cases of fatal and nonfatal CVD events over a median follow-up of 6.5 years. NAFLD was associated with a moderately increased risk of fatal or nonfatal CVD events (pooled random-effects HR 1.45, 95% CI 1.31-1.61; $I^2=86.2\%$). This risk markedly increased across the severity of NAFLD, especially the stage of fibrosis (pooled random-effects HR 2.50, 95% CI 1.68-3.72; $I^2=73.8\%$). All risks were independent of age, sex, adiposity measures, diabetes and other common cardiometabolic risk factors. Sensitivity analyses did not modify these results. Funnel plot did not show any significant publication bias.

Interpretation: NAFLD is associated with a ~1.5-fold increased long-term risk of fatal or nonfatal CVD events. CVD risk is further increased with more advanced liver disease, especially with higher fibrosis stage. These results provide evidence that NAFLD may be an independent risk factor for CVD morbidity and mortality.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver diseases worldwide. NAFLD affects up to ~30% of the world's adults and its global prevalence is expected to rise dramatically in the next decade.^{1,2} The clinical burden of NAFLD is not only restricted to its liver-related complications, but also includes an increased risk of cardiovascular disease and other extra-hepatic manifestations, such as cardiac arrhythmias, chronic kidney disease, type 2 diabetes and some types of extra-hepatic cancers, that exert a substantial socioeconomic impact on healthcare systems.³⁻⁵ This strongly supports the view that NAFLD is a “multisystem” disease that affects multiple organ systems and requires a multidisciplinary and holistic approach.⁶

To our knowledge, there are only three previous meta-analyses (published in 2016 and 2019, respectively) that have examined the association between NAFLD and risk of CVD mortality and morbidity.⁷⁻⁹ These three meta-analyses showed that NAFLD is associated with an increased risk of incident CVD (mostly nonfatal CVD events), whereas the available data on its association with CVD mortality are conflicting. However, these meta-analyses included a relatively low number of observational studies (ranging from 7 to 16 longitudinal studies) with a relatively modest sample size. In addition, these meta-analyses did not definitively address the question of whether the strength of any association between NAFLD and CVD events was affected by severity of NAFLD.⁷⁻⁹ Notably, over the last 2-3 years more than a dozen large cohort studies in adults from the Europe, United States and Asia have examined the association between NAFLD and the risk of fatal and nonfatal CVD events. Furthermore, as discussed in detail below, some of these cohort studies also used liver biopsy (i.e., the gold standard) for diagnosing and staging NAFLD.

We undertook a systematic review and meta-analysis of observational studies to quantify the long-term risk of fatal and nonfatal CVD events in individuals with NAFLD. We also aimed to examine whether the severity of NAFLD was associated with an increased risk of adverse CVD outcomes. Clarification of the magnitude of CVD risk associated with the different stages of

liver disease in NAFLD may directly impact the development of primary prevention strategies for CVD across the spectrum of liver disease.

METHODS

Search strategy and selection criteria

We systematically searched PubMed, Scopus and Web of Science from database inception to July 1, 2021 to identify observational studies examining the risk of incident CVD events amongst adult (age ≥ 18 years) individuals with and without NAFLD. Search free text terms were “nonalcoholic fatty liver disease” (“NAFLD” OR “fatty liver” OR “nonalcoholic steatohepatitis” OR “NASH”) AND risk of “cardiovascular disease” OR “CVD” OR “incident CVD events” OR “incidence of CVD events”. Searches were restricted to human studies. Studies in languages other than English were also excluded. Additionally, we reviewed references from relevant original papers and review articles to identify further eligible studies not covered by the original database searches. We performed the systematic review according to the updated Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.¹⁰ Because the included studies were observational in design, we followed the reporting proposed by the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) for the meta-analysis of these studies.¹¹

Studies were included in the meta-analysis if they met the following criteria: 1) observational longitudinal studies examining the association between NAFLD and risk of developing fatal and/or nonfatal CVD events; 2) studies reporting hazard ratios (HRs) with 95% confidence intervals (95% CIs) values for the outcome of interest; and 3) studies diagnosing NAFLD with liver biopsy, imaging techniques or International Classification of Diseases, 9th revision (ICD-9) or ICD-10 codes, in the absence of significant alcohol consumption (i.e., usually defined in the studies as either alcohol consumption >20 g/day for both sexes, or >30 g/day for men and >20 g/day for women, respectively) or other competing causes for hepatic steatosis. Study participants included in the meta-analysis were of either sex without any restriction in terms of race or ethnicity.

Criteria for exclusion of the studies from the meta-analysis were as follows: 1) congress abstracts, case reports, reviews, commentaries, editorials, practice guidelines, and cross-sectional studies; 2) studies where NAFLD diagnosis was based exclusively on serum aminotransferase concentrations or other surrogate markers of NAFLD (for example, the fatty liver index); 3) studies which did not exclude individuals with significant alcohol consumption; 4) studies which did not specifically report any HR and 95% CIs for the outcome of interest; 5) studies conducted in patients with type 1 diabetes mellitus, advanced chronic kidney disease or those who received liver transplantation; and 6) studies performed in the paediatric population (age<18 years).

Data extraction and quality assessment

Data from studies eligible for the aggregate data meta-analysis were extracted by two authors independently (A.M. and G.T.). Any disagreements were resolved by consensus and a third author if needed (A.C.).

We extracted data on publication year, study design, study country, sample size, population characteristics, methods used for NAFLD diagnosis, length of follow-up, outcomes of interest, matching and confounding factors included in multivariable regression analyses. In case of multiple publications, we included the most up-to-date or comprehensive information. We did not contact any corresponding author of the eligible studies in order to obtain additional information for the meta-analysis.

Quality of the studies included in the aggregate data meta-analysis was assessed using the Newcastle-Ottawa scale (NOS) by two independent authors (A.M. and G.T.). Any disparities in scoring were reviewed and consensus obtained following discussion. The NOS uses a star system to evaluate a study in three domains: selection of participants (assigning a maximum of four stars), comparability of study groups (assigning a maximum of two stars), and ascertainment of outcomes of interest (assigning a maximum of three stars). Therefore, nine

stars reflect the highest quality. We judged studies that received a score of 9 stars to be at low risk of bias, studies that scored 7 or 8 stars to be at medium risk, and those that scored ≤ 6 stars to be at high risk of bias. We recorded the review authors' judgments about the three NOS domains into the risk of bias tool of the Review Manager software of the Cochrane collaboration.¹²

Data synthesis and analysis

The primary outcome of the meta-analysis was the incidence of fatal and/or nonfatal CVD events among individuals with NAFLD compared to NAFLD-free controls. In particular, the pooled primary analysis included clinical CVD events, stratified into CVD death, nonfatal CVD events (i.e., angina, myocardial infarction, ischaemic/haemorrhagic strokes or coronary revascularization procedures), or both. The HRs and 95% CIs were considered as the effect size for each eligible study. In the case of studies reporting HRs with varying degrees of covariate adjustment, those that reflected the maximum extent of adjustment for potentially confounding factors were extracted. The adjusted HRs of all eligible studies were then pooled, and an overall estimate of effect size was calculated using a random effects meta-analysis with the DerSimonian and Laird method.¹²

Visual inspection of the forest plot was used to assess statistical heterogeneity. This was also assessed with the I^2 -statistics, which provides an estimate of the percentage of variability across eligible studies that is due to heterogeneity rather than chance alone.¹³ The risk of publication bias was examined using the funnel plot and the Egger's regression test.¹²

To explore the possible sources of (expected) heterogeneity among the studies and to test the robustness of the observed associations, we performed subgroup analyses by study country, publication year, length of follow-up, methodology used for diagnosing NAFLD, severity of NAFLD (based on either the severity of hepatic steatosis on ultrasonography or the severity of liver fibrosis by histology and/or non-invasive fibrosis biomarkers, such as NAFLD fibrosis score [NFS]), whether the studies had 8 or 9 stars on the NOS scale (i.e. the "high-quality" studies), or whether they had full adjustment for traditional CVD risk factors, i.e., arbitrarily

defined as those studies adjusting *at least* for age, sex, adiposity measures, smoking history, hypertension (or systolic blood pressure), dyslipidaemia (or plasma lipid profile) and pre-existing diabetes (or fasting glucose or haemoglobin A1c levels). We also performed univariable meta-regression analyses to test the impact of specific moderator variables (i.e., age, sex, body mass index, smoking status, plasma LDL-cholesterol levels and percentages of hypertension or diabetes at baseline) on the effect size for the incidence of NAFLD-related CVD events. Finally, we tested for possible excessive influence of individual studies using a meta-analysis influence test that eliminated each of the included studies one at a time. All statistical tests were two sided and used a significance level of $p < 0.05$. For analyses we used STATA® 16.1 (StataCorp, College Station, Texas, USA) and its meta-analysis package and R software (version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria) with “meta” and “metafor” packages. This systematic review is registered on Open Science Framework, number osf.io/5z7gf.

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RESULTS

Characteristics of included studies

Figure 1 shows the results of the literature research and study selection. Based on the titles and abstracts of 2,626 selected citations (after excluding duplicates), we initially identified 46 potentially eligible studies from PubMed, Scopus or Web of Science databases prior to July 1, 2021.¹⁴⁻⁵⁸ In **appendix p9** syntax used is specified as are the records identified through database search. After examining the full text of these 46 potentially eligible studies, we excluded 10 studies, mainly due to unsatisfactory inclusion criteria or unsatisfactory outcome measures (see **appendix p10**).⁴⁹⁻⁵⁸ As a consequence of this exclusion, we identified 36 unique, observational studies for inclusion in the meta-analysis.

The main characteristics of the 36 studies are summarized in **appendix p11-16**. Overall, these studies had aggregate data on 5,802,226 middle-aged individuals (49.8% men; mean age 53 years; mean BMI 27.7 kg/m²) with a total of 335,132 individuals with NAFLD at baseline and 99,668 incident cases of fatal or nonfatal CVD events over a median follow-up period of 6.5 years (interquartile range: 5-10 years). Most of these studies recruited participants either from general populations, large health examination check-up programs or from outpatient cohorts of individuals with type 2 diabetes, in which NAFLD was diagnosed by liver biopsy, imaging techniques (mostly ultrasonography), or ICD codes. Ten studies reported data only on CVD death, 13 studies reported data only on nonfatal CVD events, and 11 studies reported data on the combined CVD outcome. Thirteen studies were carried out in Asia (South Korea, China, Japan and Turkey); one study was carried out in Africa (Egypt); fourteen studies were carried out in Europe (Sweden, Germany, Italy, Netherlands, Denmark, Finland, and UK) and eight studies were carried out in the United States. Twenty-seven studies used imaging techniques (mostly ultrasonography) for the diagnosis of NAFLD, five studies used liver biopsy and four studies used ICD codes.

As reported both in **appendix p2-3** and in **appendix p17**, nineteen studies received at least 8 stars on the NOS (i.e., studies at relatively low risk of bias), eight studies received 7 stars (studies at medium risk of bias) and nine studies received 6 or 5 stars (studies at high risk of bias), thus indicating an overall medium-low risk of bias.

NAFLD and risk of CVD events

The distribution of eligible studies by estimate of the association between NAFLD and risk of incident CVD events is plotted in **Figure 2**. Thirty-three studies (involving a total of 5,790,329 middle-aged individuals with 98,955 incident cases of fatal or nonfatal CVD events) provided data suitable for the pooled primary analysis. We excluded three eligible studies from this analysis, which were used only for examining the association between the severity of NAFLD and risk of CVD events (see below).^{22,25,30}

The presence of NAFLD was associated with a moderately increased risk of fatal or nonfatal CVD events (n=33 studies; pooled random-effects 1.45, 95% CI 1.31-1.61, $p<0.0001$; $I^2=86.2\%$). Notably, since we have always used the fully adjusted HR estimates for each study (as specified in **appendix p11-16**), this pooled random-effects HR was independent of age, sex, smoking, adiposity measures and other common cardiometabolic risk factors. As also shown in **Figure 2**, the association between NAFLD and risk of CVD events was consistent even when the comparison was stratified by outcome, i.e., analyzing separately the published studies that had either nonfatal CVD events, or fatal CVD events, or both as primary outcomes. In particular, the presence of NAFLD was significantly ($p<0.0001$ for all) associated with fatal CVD events (n=10 studies; pooled random-effects HR 1.30, 95%CI 1.08-1.56; $I^2=86.1\%$), as well as with both an increased risk of nonfatal CVD events alone (n=13 studies; pooled random-effects HR 1.40, 95%CI 1.20-1.64; $I^2=87.7\%$) and an increased risk of fatal and nonfatal CVD events considered together (n=10 studies; pooled random-effects HR 1.81, 95%CI 1.39-2.36; $I^2=78.1\%$).

Subgroup analyses and meta-regressions

To explore the possible sources of heterogeneity across the eligible studies, we undertook subgroup analyses (**Table 1**). Notably, the association between NAFLD and risk of fatal or nonfatal CVD events was consistent in all subgroups. In particular, the pooled random-effects HRs were essentially comparable after stratification by modality of NAFLD diagnosis, study country, follow-up duration, publication year, NOS quality scale or degree of covariate adjustment.

As reported in **appendix p4-6**, the results of univariable meta-regression analyses to examine the effect of potential moderator variables showed a significant positive association between the proportion of patients with pre-existing type 2 diabetes ($p=0.001$, panel D) or mean plasma LDL-cholesterol concentrations ($p=0.041$, panel G) and the risk of NAFLD-related CVD events. Conversely, meta-regression analyses did not show any significant effects of age, male sex, body mass index, smoking or hypertension on the association between NAFLD and risk of CVD events (see panels A, B, C, E and F).

We also tested for the possibility of excessive influence of individual studies using an influence test that eliminated each of the included studies one at a time. Notably, eliminating each of the studies from our pooled primary analysis did not have any significant effect on the overall risk of CVD events (see **appendix p7**).

Appendix p8 shows that there was no significant asymmetry of the funnel plot, thus suggesting that publication bias was unlikely.

NAFLD severity and risk of CVD events

The distribution of eligible studies by estimate of the association between the severity of NAFLD and risk of incident CVD events is plotted in **Figure 3 (panel A)**. Eleven studies (involving a total of 187,604 individuals and 16,382 incident cases of fatal or nonfatal CVD events) provided data suitable for this pooled secondary analysis. The severity of NAFLD was defined by ultrasonographic scores in three studies; by presence of NAFLD on ultrasonography *plus* elevated serum gamma-glutamyltransferase concentrations or increased 18F-fluoro-deoxyglucose uptake on positron emission tomography in two studies; by severity of liver fibrosis as assessed by histology in four studies or by NFS in three studies. It should be noted that these studies did not always have a comparator control group without NAFLD, which was the case for the studies included in **Figure 2**. That said, this pooled secondary analysis showed that the risk for fatal and nonfatal CVD events was even greater amongst individuals with more “severe” NAFLD (n=11 studies included; pooled random-effects HR 2.29, 95%CI 1.74-3.03; $I^2=57.9\%$). This result was consistent even when we analyzed separately the published studies that had either fatal CVD events, or nonfatal CVD events, or both as primary outcomes (with pooled HRs ranging from 2.03 for fatal CVD to 2.54 for fatal and nonfatal CVD events combined). As shown in **Figure 3 (panel B)**, similar results were observed when we restricted the statistical analysis only to studies that explored the association between the severity of liver fibrosis (as assessed either by liver histology or by NFS) and risk of fatal and nonfatal CVD events (n=7 studies; random-effects HR 2.50, 95% CI 1.68-3.72, $p<0.001$; $I^2=73.8\%$).

DISCUSSION

This updated meta-analysis of 36 observational studies (involving a total of about 5.8 million people from different countries with ~100,000 cases of incident CVD events over a median follow-up of 6.5 years) provides substantive evidence that NAFLD confers a hazard risk of ~1.5 for fatal and nonfatal CVD events (pooled random-effects HR 1.45, 95% CI 1.31-1.61, $p < 0.0001$; $I^2 = 86.2\%$). The magnitude of this risk remained essentially unchanged when the comparison was stratified by study country, publication year, follow-up duration, modality of NAFLD diagnosis, degree of covariate adjustment, NOS quality scale, or when we analyzed separately the published studies that had either fatal CVD events, or nonfatal CVD events, or both as primary outcomes. Furthermore, the risk of fatal and nonfatal CVD events appeared to increase further with greater severity of NAFLD (especially the severity of fibrosis, as assessed by liver histology or non-invasive fibrosis markers: pooled random-effects HR 2.50, 95% CI 1.68-3.72, $p < 0.001$; $I^2 = 73.8\%$) and, most importantly, remained significant in those studies where statistical analysis was adjusted for age, sex, smoking, adiposity measures, pre-existing diabetes and other common cardiometabolic risk factors. Our meta-regression analyses also show for the first time a positive association between the proportion of patients with pre-existing diabetes at baseline and the risk of NAFLD-related CVD events. This could represent further evidence of a “vicious cycle” that exists between type 2 diabetes and NAFLD to further increase the risk of CVD.^{6,59} Conversely, meta-regression analyses did not reveal any significant effect of age, sex, body mass index, smoking and hypertension on the association between NAFLD and risk of CVD events.

To our knowledge, this meta-analysis examining the relationship between NAFLD and the long-term risk of fatal and nonfatal CVD events is the largest and most comprehensive assessment to date. Our findings corroborate and further extend the results of three previous smaller meta-analyses published in 2016 and 2019.⁷⁻⁹ In the first meta-analysis that incorporated 16 longitudinal studies (involving a total of ~34,000 individuals), Targher et al. reported that biopsy-proven or imaging-defined NAFLD was associated with a nearly 65% increased risk of fatal or nonfatal CVD events (pooled random-effects HR 1.64, 95% CI 1.26-2.13; $I^2 = 86.0\%$).⁷ In this pooled primary analysis, the association of NAFLD appeared to be

stronger for studies that analysed nonfatal CVD events alone or fatal and nonfatal CVD events combined than for those studies analysing CVD mortality alone. Patients with more 'severe' NAFLD were also more likely to develop fatal and nonfatal CVD events (n=6 studies included; random-effects HR 2.58, 95% CI 2.58; 1.78-3.75).⁷ However, at variance with our meta-analysis, in this previous meta-analysis, the severity of NAFLD was principally assessed by imaging techniques or non-invasive fibrosis biomarkers (only one cohort study used biopsy for staging liver fibrosis).⁷ In the second meta-analysis that incorporated 8 longitudinal cohort studies, Wu et al. reported that NAFLD was associated with a nearly 40% increased risk of nonfatal CVD events (n=3 studies; pooled random-effects HR 1.37, 95% CI 1.10-1.72; $I^2=55.1\%$), although it was not significantly associated with CVD mortality (n=5 studies; random-effects HR 1.10, 95%CI 0.86-1.41; $I^2=64.9\%$).⁸ Finally, in the third meta-analysis including 7 cohort studies, Liu et al. reported that NAFLD significantly predicted increased all-cause mortality, but not CVD mortality (pooled random-effects HR 1.13; 95% CI 0.92-1.38; $I^2=57.5\%$).⁹

Collectively, therefore, compared to data of the aforementioned smaller meta-analyses, we have increased the number of eligible studies (by including nearly 20 new large cohort studies published from 2019 to July 2021); and we have increased the sample size more than 160 times, increasing the total number of individuals included from almost 34,000 in our previous meta-analysis published in 2016 to about 5.8 million individuals with aggregate data on nearly 100,000 incident cases of fatal and nonfatal CVD events. In addition, in the present meta-analysis, we have also included new large cohort studies that used liver biopsy for staging NAFLD, thereby providing a more reliable estimate of the association between the severity of NAFLD (especially the stage of fibrosis) and risk of fatal and nonfatal CVD events. Our results are in agreement with previous meta-analyses reporting a significant association between the presence and severity of NAFLD and the risk of developing liver-related complications, chronic kidney disease, cardiac arrhythmias or other extra-hepatic complications.⁶⁰⁻⁶⁴

There is intense scientific debate about the independent contribution of steatotic/inflamed or fibrosing liver to the pathophysiology of CVD in people with NAFLD.^{53,65-70} It is beyond the

scope of this meta-analysis to discuss in detail the putative underlying mechanisms by which NAFLD can be involved in CVD development. Although further intervention and mechanistic studies are needed for establishing a causal relationship between NAFLD and risk of CVD events, there is accumulating evidence of biological plausibility that NAFLD may increase risk of incident CVD. There are probably several pathophysiological mechanisms by which NAFLD increases the risk of CVD and other cardiac complications; e.g., NAFLD (especially NASH with increasing amounts of liver fibrosis) exacerbates insulin resistance, promotes atherogenic dyslipidaemia and releases a variety of pro-inflammatory cytokines and pro-atherogenic mediators.^{6,67,69} Some genetic polymorphisms, such as the *patatin-like phospholipase domain-containing protein 3* (PNPLA3-I148M) and *trans-membrane 6 super family-2* (TM6SF2-E167K) variants, may worsen liver disease, but also attenuate the strength of the association between NAFLD and CVD risk, possibly via their effects on lipoprotein metabolism.^{67,71-73} However, further studies are needed for better elucidate this issue.

Our meta-analysis has some important limitations, which are inherent to the design of the eligible studies. Firstly, the observational design of the studies precludes us from assessing causality. Secondly, although most of the eligible studies adjusted their results for age, sex, adiposity measures, diabetes and other cardiometabolic risk factors, the possibility of residual confounding by unmeasured factors cannot be ruled out. Thirdly, although we used a random-effects model, the interpretation of some results of our meta-analysis (like previously published meta-analyses⁷⁻⁹) requires some caution, given the significant heterogeneity observed in the pooled primary analysis. We systematically explored and identified possible sources of heterogeneity using stratified analyses, meta-regressions and sensitivity analyses. It is possible that the significant heterogeneity likely reflects differences in the characteristics of study populations, in methods used for NAFLD diagnosis, in follow-up duration, as well as in severity of NAFLD. However, a more detailed analysis of the possible sources of heterogeneity would require collaborative pooling of individual participant data from future cohort studies. Finally, in this meta-analysis we included four large cohort studies that used ICD-9 or ICD-10 codes for diagnosing NAFLD. In these four studies (see appendix p11-16), the rates of recorded NAFLD diagnoses were much lower than expected, suggesting under-diagnosis, potential misclassification and under-recording. That said, in our subgroup analyses we

showed that diagnosis of NAFLD, identified by using ICD codes, was also associated with a higher risk of incident CVD events, especially CVD mortality (Table 1).

Notwithstanding these limitations, our meta-analysis has important strengths. This study incorporates data from large cohort studies from different countries that are likely to be an accurate reflection of people with NAFLD routinely seen in clinical practice. The large number of both individuals with NAFLD at baseline and incident cases of CVD events at follow-up provides sufficiently high statistical power to quantify the magnitude of the association between the presence and severity of NAFLD and risk of CVD mortality and morbidity. In addition, the quality of studies included in the meta-analysis was acceptable, suggesting an overall medium-low risk of bias, according to the NOS scale. Finally, we did not extensively search for “grey literature” by also searching the Embase database. Therefore, a selective reporting bias of studies cannot be definitely excluded, although we believe that our comprehensive search has made it unlikely that any published studies were missed. Moreover, visual inspection of the funnel plot and formal statistical tests (i.e., the Egger’s regression test) did not show any significant asymmetry of the funnel plot, thus supporting the notion that publication bias was unlikely.

In conclusion, this comprehensive and updated meta-analysis provides evidence for a significant association between the presence of NAFLD and the long-term risk of fatal and nonfatal CVD events. The magnitude of this risk parallels the underlying severity of NAFLD (especially the stage of liver fibrosis). The findings of this meta-analysis also emphasise that clinicians should have a high index of suspicion that individuals with NAFLD may also have co-existing CVD. Early recognition of NAFLD can help identify people, who may benefit from specific CVD risk factor modification or emerging pharmaco-therapies to prevent progression to CVD and liver-related complications. We believe that the complex interplay between the liver and cardiometabolic risk factors in NAFLD highlights an urgent need for a person-centred, multidisciplinary and holistic approach to manage both liver disease and cardiometabolic risk. Future research is required to decipher the existing but complex links

between NAFLD and CVD, and to elucidate whether improvement or resolution of NAFLD attenuates development and progression of CVD.

Contributors: AM and GT designed the study. AM, AC, GP, GB and GT did the literature search, with arbitration by AC. AM and GT analysed the data and did the figures. KEC, TGS, and CDB interpreted the data. AM, CDB and GT wrote the manuscript. All authors reviewed and approved the final manuscript.

Conflicts of Interest Statement: The authors have no competing financial interests to declare.

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Table 1. Subgroup analyses - Associations between NAFLD and risk of fatal or nonfatal CVD events, stratified by modality of NAFLD diagnosis, study country, median length of follow-up, publication year, Newcastle-Ottawa quality scale, or degree of covariate adjustment.

	Random-effects Hazard Ratio (95% Confidence Intervals)	Number of studies included	Number of subjects included	<i>I</i> ² -statistics
CVD mortality *				
NAFLD diagnosis				
Liver biopsy	1.36 (1.27-1.45)	2	60,692	0.0%
Imaging techniques	1.22 (0.87-1.71)	7	340,273	87.8%
ICD-9/10 codes	1.70 (1.52-1.90)	1	134,368	Not applicable
Study country				
USA	0.87 (0.68-1.12)	2	11,708	0.0%
Asia	1.64 (0.94-2.85)	2	321,548	74.3%
Europe	1.33 (1.08-1.64)	6	202,077	89.1%
Follow-up length				
Follow-up length ≤7 years	1.71 (1.34-2.17)	4	457,720	78.3%
Follow-up length >7 years	1.50 (0.82-1.34)	6	77,613	78.1%
Publication year				
Publication year <2015	1.27 (0.78-2.07)	5	20,996	91.5%
Publication year ≥2015	1.30 (1.08-1.56)	5	514,337	74.9%
Newcastle-Ottawa scale (NOS)				
NOS <8	1.73 (1.41-2.14)	3	137,921	34.6%
NOS ≥8	1.15 (0.92-1.46)	7	397,412	86.8%
Degree of covariate adjustment §				
Minimal adjustment	1.24 (0.85-1.81)	7	22,278	88.9%
Maximal adjustment	1.42 (1.18-1.71)	3	513,055	81.1%
Nonfatal CVD events				
NAFLD diagnosis				
Liver biopsy	1.54 (1.30-1.83)	1	6,872	Not applicable
Imaging techniques	1.76 (1.29-2.40)	9	419,463	87.8%
ICD-9/10 codes	1.09 (0.86-1.39)	3	4,814,260	93.1%
Study country				
USA	0.94 (0.80-1.11)	2	23,490	0.0%
Asia/Africa	1.84 (1.29-2.62)	7	414,676	89.7%
Europe	1.32 (1.06-1.64)	4	4,802,429	89.2%
Follow-up length				
Follow-up length ≤7 years	1.60 (1.13-2.27)	7	4,993,443	91.3%
Follow-up length >7 years	1.35 (1.19-1.53)	6	247,152	66.5%
Publication year				
Publication year <2015	2.50 (1.15-5.44)	3	7,199	77.6%
Publication year ≥2015	1.23 (1.08-1.40)	10	5,233,396	82.0%
Newcastle-Ottawa scale (NOS)				
NOS <8	1.46 (1.14-1.87)	8	4,829,156	91.4%
NOS ≥8	1.31 (1.06-1.62)	5	411,439	70.2%
Degree of covariate adjustment §				
Minimal adjustment	1.46 (1.14-1.87)	8	4,829,156	91.4%
Maximal adjustment	1.31 (1.06-1.62)	5	411,439	70.2%
Fatal and nonfatal CVD events (combined)				
NAFLD diagnosis				
Liver biopsy	None	-	-	-
Imaging techniques	1.81 (1.39-2.37)	10	14,401	78.1%
ICD-9/10 codes	None	-	-	-
Study country				
USA	1.49 (1.26-1.77)	3	8,761	0.0%
Asia	1.88 (0.79-4.48)	3	1,465	92.4%
Europe	2.22 (1.76-2.81)	4	4,175	0.0%
Follow-up length				
Follow-up length ≤7 years	1.88 (1.22-2.90)	6	3,435	84.4%
Follow-up length >7 years	1.83 (1.46-2.29)	4	10,966	32.6%
Publication year				
Publication year <2015	2.15 (1.66-2.79)	2	3,091	0.0%
Publication year ≥2015	1.74 (1.28-2.37)	8	11,310	78.7%
Newcastle-Ottawa scale (NOS)				

NOS <8	2.03 (0.96-4.27)	4	2,363	89.8%
NOS ≥8	1.74 (1.44-2.10)	6	12,038	36.6%
Degree of covariate adjustment §				
Minimal adjustment	1.37 (0.97-1.94)	4	2,008	74.0%
Maximal adjustment	2.17 (1.58-2.97)	6	12,393	66.4%

*NB: In this table, we analyzed separately the published studies that had either CVD mortality, or nonfatal CVD events, or both as primary outcomes.

§ Maximal covariate adjustment included studies that have adjusted the results *at least* for the following traditional CVD risk factors: age, sex, adiposity measures (body mass index or waist circumference), smoking history, hypertension (or systolic blood pressure), dyslipidaemia (or plasma lipid profile) and pre-existing type 2 diabetes mellitus (or fasting plasma glucose or haemoglobin A1c levels). Minimal covariate adjustment included all other eligible studies that have adjusted the results for a lower number of traditional CVD risk factors compared to those listed above.

FIGURE LEGENDS

Figure 1. The PRISMA flow diagram for search and selection processes of the meta-analysis.

Figure 2. Forest plot and pooled estimates of the effect of NAFLD on the risk of fatal and nonfatal CVD in 33 eligible studies, stratified by outcome.

Figure 3. (A) Forest plot and pooled estimates of the effect of severity of NAFLD on the risk of fatal and nonfatal CVD in 11 eligible studies, stratified by outcome. (B) Forest plot and pooled estimates of the effect of severity of liver fibrosis (assessed by histology or NAFLD fibrosis score [NFS]) on the risk of fatal and nonfatal CVD in 7 eligible studies.

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