Nonalcoholic Fatty Liver Disease and Risk of Incident Diabetes Mellitus: An Updated Meta-Analysis of 501,022 Adult Individuals

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ABSTRACT

Objective: Follow-up studies have shown that nonalcoholic fatty liver disease (NAFLD) is associated with an increased risk of incident diabetes, but currently it is uncertain whether this risk changes with increasing severity of NAFLD. We performed a meta-analysis of relevant studies to quantify the magnitude of the association between NAFLD and risk of incident diabetes.

Design: We systematically searched PubMed, Scopus and Web of Science databases from January 2000 to June 2020 using predefined keywords to identify observational studies with a follow-up duration of at least 1 year, in which NAFLD was diagnosed by imaging techniques or biopsy. Meta-analysis was performed using random-effects modelling.

Results: 33 studies with 501,022 individuals (30.8% with NAFLD) and 27,953 cases of incident diabetes over a median of 5 years (interquartile range: 4.0-19 years) were included. Patients with NAFLD had a higher risk of incident diabetes than those without NAFLD (n=26 studies; random-effects hazard ratio [HR] 2.19, 95%CI 1.93-2.48; I^2=91.2%). Patients with more 'severe' NAFLD were also more likely to develop incident diabetes (n=9 studies; random-effects HR 2.69, 95%CI 2.08-3.49; I^2=69%). This risk markedly increased across the severity of fibrosis (n=5 studies; random-effects HR 3.42, 95%CI 2.29-5.11; I^2=44.6%). All risks were independent of age, sex, adiposity measures and other common metabolic risk factors. Sensitivity analyses did not alter these findings. Funnel plots did not reveal any significant publication bias.

Conclusion: This updated meta-analysis shows that NAFLD is associated with a ~2.2-fold increased risk of incident diabetes. This risk parallels the underlying severity of NAFLD.

Key-words: NAFLD; fatty liver; diabetes risk; meta-analysis

SUMMARY BOX

What is already known about this subject: Observational studies have shown that nonalcoholic fatty liver disease (NAFLD) is associated with an increased incidence of type 2 diabetes, but currently it is uncertain whether risk changes with increasing severity of NAFLD.

What are the new findings: This updated meta-analysis of 501,022 middle-aged individuals of different countries provides strong evidence that NAFLD is associated with a 2.2-fold increased risk of developing diabetes. This risk parallels the underlying severity of NAFLD, especially fibrosis stage.

How might it impact on clinical practice in the foreseeable future: Health care professionals should be aware that risk of developing diabetes is increased ~2 fold in patients with NAFLD, and that there is an even greater increase in risk in those with advanced liver fibrosis. We recommend that blood glucose and haemoglobin A1c levels be monitored to identify NAFLD patients who develop diabetes.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a growing global health problem, affecting up to a quarter of the adult population. NAFLD is a metabolic liver disease that is closely associated with obesity and type 2 diabetes, and its prevalence is increasing worldwide at approximately the same rate as the global epidemics of obesity and diabetes [1-3].

It is well established that type 2 diabetes and NAFLD are two pathologic conditions that frequently coexist and act synergistically to increase risk of adverse clinical outcomes. Diabetes is one of the strongest clinical risk factors for faster progression of NAFLD to nonalcoholic steatohepatitis (NASH), cirrhosis or hepatocellular carcinoma [1-4]. To date, however, it is becoming increasingly clear that the link between NAFLD and diabetes is more complex than previously believed [4-6]. Indeed, accumulating evidence suggests that the relationship between NAFLD and diabetes is mutual and bidirectional, and that NAFLD may also precede and/or promote the development of type 2 diabetes [4-6].

To our knowledge, there are only three previous meta-analyses that examined the association between NAFLD and risk of incident diabetes [7-9]. However, it is important to note that two of these meta-analyses (published in 2011 and 2016, respectively) have a relatively modest sample size and have included a large number of observational studies in which the diagnosis of NAFLD was based on abnormal serum liver enzyme levels [7,8], which are only surrogate markers of NAFLD [10]. In addition, and most importantly, none of these three previous meta-analyses has included studies where the diagnosis of NAFLD was made by liver biopsy [7-9], which is the 'reference' method for diagnosing and staging NAFLD [10]. Presently, there is intense scientific debate on the impact of NAFLD on the long-term risk of incident diabetes, and it remains uncertain whether this risk parallels the underlying severity of NAFLD.

We therefore carried out an updated systematic review and meta-analysis of observational cohort studies examining the association between NAFLD (as detected by liver biopsy or imaging methods) and the risk of developing diabetes. Our aim was to gauge the nature and magnitude of the relationship between NAFLD and risk of new-onset diabetes. We have also examined whether the severity of NAFLD is associated with a modified risk of diabetes; since risk of diabetes may change

with alterations in hepatic glucose metabolism and insulin sensitivity that occur as liver disease progresses [5]. We believe that clarification of the magnitude of risk of incident diabetes associated with the different stages of liver disease within the spectrum of NAFLD might also have important clinical implications for future strategies in the prevention and treatment of type 2 diabetes.

METHODS

Registration of review protocol

The protocol for this systematic review was registered in advance with Open Science Framework registries (no: osf.io/ed346).

Data sources and searches

We conducted a systematic literature search from January 1, 2000 to June 30, 2020 (date last searched) of PubMed, Scopus and Web of Science databases for all observational studies of individuals assessing the association between NAFLD and risk of incident diabetes. Search free text terms were “fatty liver” (OR “NAFLD” OR “nonalcoholic fatty liver disease” OR “nonalcoholic steatohepatitis” OR “hepatic steatosis”) AND “risk of diabetes” OR “diabetes incidence” OR “incident diabetes”. Searches were restricted to human studies. No language restrictions were imposed. 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 a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (http://www.prisma-statement.org). Because the included studies were observational in design, we followed the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines for the meta-analysis of these studies [11].

Study selection

The inclusion criteria of the meta-analysis were as follows: 1) observational longitudinal (prospective or retrospective) cohort studies with a follow-up duration of at least 1 year that explored the association between NAFLD and risk of incident diabetes; 2) all studies should

reported hazard ratios (HRs) or odds ratios (ORs) with 95% confidence intervals (95% CIs) values for the outcome of interest; 3) the diagnosis of NAFLD was based on either imaging methods or liver biopsy in the absence of significant alcohol consumption and other competing causes of hepatic steatosis; and 4) the diagnosis of incident diabetes was based on a self-reported history of disease or use of any anti-hyperglycaemic drugs, and in most cases, it was also based on a fasting glucose level ≥7.0 mmol/L and/or a haemoglobin A1c level ≥6.5% (≥48 mmol/mol). Study participants included in the meta-analysis were of either sex without any restriction in terms of age, race or ethnicity.

Criteria for exclusion of the selected studies from this meta-analysis were as follows: 1) congress abstracts, case reports, theses, reviews, practice guidelines, commentaries and editorials; 2) studies with a follow-up duration less than 1 year; 3) studies where NAFLD diagnosis was based exclusively on serum liver enzymes or other surrogate markers of NAFLD (e.g., fatty liver index); 4) studies which did not exclude individuals with significant alcohol consumption and other competing causes for steatosis; 5) studies which did not specifically report any HR (or OR) and 95% confidence intervals for the outcome measure of interest; 6) studies performed in human immunodeficiency virus-infected patients; and 7) studies conducted in paediatric population (<18 years old).

Data extraction and quality assessment

Two investigators (GP and GB) independently examined all titles and abstracts, and obtained full texts of potentially relevant papers. Working independently and in duplicate, we read the papers and determined whether they met inclusion criteria. Discrepancies were resolved by consensus, referring back to the original article, in consultation with a third author.

For all studies, we extracted information on study design, sample size, study country, population characteristics, methods used for NAFLD diagnosis, length of follow-up, outcome of interest, and covariates adjusted in multivariable regression analyses. In the case of multiple publications, we included the most up-to-date or comprehensive information.

Two authors assessed the risk of bias independently. Since all the included studies were non-randomised and had a cohort design, the Newcastle-Ottawa Scale (NOS) was used to judge study quality, as recommended by the Cochrane Collaboration [12]. This scale uses a star system (with a maximum of nine stars) to evaluate a study in three domains: selection of participants, comparability of study groups, and the ascertainment of outcomes of interest [12]. We judged studies that received a score of nine stars to be at low risk of bias, studies that scored seven or eight stars to be at medium risk, and those that scored six or less to be at high risk of bias [12].

Data synthesis and analysis

The primary outcome measure was the development of incident diabetes among individuals with NAFLD compared to their counterparts without NAFLD. The HRs (or ORs) with their 95% confidence intervals were considered as the effect size for all eligible studies. When studies had several adjustment models, we extracted those that reflected the maximum extent of adjustment for potentially confounding factors. The adjusted HR/ORs of all eligible studies were then pooled, and an overall estimate of effect size was calculated using a random-effects model, as this methodology considers any differences between studies even if there is no statistically significant heterogeneity [12].

Visual inspection of the forest plots was used to investigate the possibility of statistical heterogeneity. Statistical heterogeneity was assessed by the I^2-statistics, which provides an estimate of the percentage of variability across studies that is due to heterogeneity rather than chance alone. A rough guide to interpretation is as follows: I^2-values of approximately 25% represent low heterogeneity; approximately 50% represent medium heterogeneity; and approximately 75% represent high heterogeneity [13]. The possibility of publication bias was evaluated using the funnel plot and the rank correlation Begg's test [12,14].

To explore the possible sources of the (expected) heterogeneity among the eligible studies and to test the robustness of the associations, we conducted stratification-sensitivity analyses by study country, study design, definition used for diagnosing NAFLD, length of follow-up (<5 vs. ≥5 years), severity of NAFLD (based on ultrasonographic severity of steatosis, or severity of liver fibrosis by

histology and/or fibrosis biomarkers, such as NAFLD fibrosis score [NFS] or FIB-4 index), whether the studies had eight or nine stars on the NOS scale (i.e., the "high-quality" studies), or whether they had full adjustment for common diabetes risk factors (i.e., arbitrarily defined as those studies adjusting *at least* for age, sex, body mass index [or waist circumference], family history of diabetes, fasting glucose, lipids, hypertension, smoking and physical activity). We also performed a meta-regression analysis for the association of age, sex and adiposity measures with HRs of incident diabetes. Finally, we tested for possibly excessive influence of individual studies using a meta-analysis influence test that eliminated each of the included studies at a time.

All statistical tests were two sided and used a significance level of $p<0.05$. We used STATA® 14.0 (StataCorp, College Station, Texas, USA) for all statistical analyses.

RESULTS

Characteristics of included studies

Figure 1 summarizes the results of the literature research and study selection. Based on the titles and abstracts of 2,627 citations, we initially identified 42 potentially relevant studies from three electronic databases prior to June 30, 2020 (date last searched). After examining the full text of these 42 publications, we excluded 9 studies [15-23], because of unsatisfactory inclusion criteria or unsatisfactory outcome measures as specified in the flow diagram. Therefore, 33 longitudinal studies were eligible for inclusion in the meta-analysis and were assessed for quality.

The main characteristics of the included studies are summarized in **supplementary Table S1**. All studies had an observational retrospective or prospective design. Most of them recruited participants from approximately general populations in which NAFLD was diagnosed by imaging methods (mostly ultrasonography), and incident diabetes was diagnosed by self-reported disease history, drug treatment use or biochemistry (fasting glucose or haemoglobin A1c levels). Four liver biopsy cohort studies were also available and were used for examining the association between the histologic severity of NAFLD and diabetes risk (in the study of Önnerhag et al. the authors enrolled

patients with biopsy-proven NAFLD but used only non-invasive fibrosis biomarkers for staging NAFLD).

Overall, in the 33 eligible studies included in the meta-analysis there were 501,022 middle-aged individuals (62.1% men; mean age 47 years, mean BMI 24 kg/m^2) with a total of 154,314 (30.8%) participants with NAFLD at baseline and 27,953 cases of incident diabetes over a median follow-up of 5 years (inter-quartile range: 4.0-19 years). Most of these studies (n=27) were carried out in Asia (South Korea, China, Taiwan, Japan and Sri Lanka); two studies were carried out in the United States and four studies were carried out in Europe (Sweden and Spain). As reported in **supplementary Table S2**, 17 studies received eight or nine stars at the NOS (indicating an overall low risk of bias), 15 studies received seven or six stars (indicating an overall medium risk of bias), and 1 study received less than six stars at the NOS (indicating an overall high risk of bias).

NAFLD and risk of incident diabetes

The distribution of eligible studies by estimate of the association between NAFLD and risk of incident diabetes is plotted in **Figure 2**. Twenty-six studies provided data suitable for the pooled primary analysis (involving a total of 418,564 with 22,267 cases of incident diabetes). We excluded seven studies from this primary analysis because these studies did not provide any HRs for incident diabetes among individuals with NAFLD pooled together or did not include subjects without NAFLD (e.g. the four liver biopsy cohort studies); these studies were used in a secondary analysis for examining the association between the severity of NAFLD and diabetes risk (see below).

Presence of NAFLD was associated with an increased risk of incident diabetes (random-effects HR 2.19, 95%CI 1.93-2.48; I^2=91.2%). Notably, since we always used the fully adjusted HR estimates for each eligible study (as detailed in **supplementary Table S1**), this random-effects HR was independent of a (relatively) large number of common metabolic risk factors and potential confounders. As also shown in the figure, when the comparison was stratified by study country, the association between NAFLD and diabetes risk was significant in all countries, but it appeared to be (slightly) stronger in Japan.

Subgroup/sensitivity analyses and meta-regressions

To explore possible sources of heterogeneity across the included studies, we carried out some subgroup/sensitivity analyses (**Table 1**). Notably, the association between NAFLD and diabetes risk was consistent in all subgroups considered. In particular, the random-effects HRs were significant and essentially comparable when the comparison was stratified by study design, length of study follow-up, NOS quality scale, degree of covariate adjustment or modality of NAFLD diagnosis. In addition, as reported in **supplementary Figure S1,** the results of univariable meta-regression analyses did not show any significant effects of age, sex or body mass index on the association between NAFLD and risk of diabetes.

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. Interestingly, eliminating each of the eligible studies from the analysis had no significant effect on the overall risk of incident diabetes (**supplementary Figure S2, panel A**).

As shown in **supplementary Figure S3 (panel A),** the rank correlation Begg's test did not show any statistically significant asymmetry of the funnel plot, thus suggesting that publication bias was unlikely.

Severe NAFLD and risk of incident diabetes

The distribution of studies by estimate of the association between severity of NAFLD and risk of diabetes is plotted in **Figure 3**. Nine studies (involving a total of 116,105 individuals with 8,683 cases of incident diabetes) reported data on the severity of NAFLD, defined either by severity of hepatic steatosis (by increasing ultrasonographic steatosis scores), or by severity of liver fibrosis (by histology and/or non-invasive fibrosis biomarkers).

Presence of 'severe' NAFLD was associated with an increased risk of incident diabetes (n=9 studies; random-effects HR 2.69, 95%CI 2.08-3.49; I^2=69.0%). This risk increased across the ultrasonographic steatosis scores (n=4 studies; random-effects HR 2.40, 95%CI 2.08-2.77; I^2=2.6%) and the severity of liver fibrosis (n=5 studies; random-effects HR 3.42, 95%CI 2.29-5.11; I^2=44.6%). As specified in the

figure, it is important to point out that the liver biopsy studies enrolled only patients with NAFLD and did not have a comparator control group without NAFLD, which was the case for all the studies shown in **Figure 2**. Rather, the liver biopsy studies compared the risk of incident diabetes in patients with advanced fibrosis with the risk in those with either F0 or F0-F2 fibrosis. Thus, these data show that the risk of diabetes was greater in patients with more severe liver disease.

Eliminating each of the included studies from the analysis had no effect on the overall risk of incident diabetes (**supplementary Figure S2, panel B**).

As also shown in **supplementary Figure S3 (panel B),** the Begg's test did not show statistically significant asymmetry of the funnel plot, thus suggesting that publication bias was unlikely, although it should be noted that the numbers of included studies (n=9) was relatively small.

DISCUSSION

Our updated meta-analysis involves a total of 33 observational cohort studies with aggregate data on more than half a million middle-aged individuals of different countries (30.8% with imaging-defined or biopsy-proven NAFLD) and nearly 28,000 cases of incident diabetes followed-up over a median period of 5 years (interquartile range: 4-19 years).

We found that the presence of NAFLD conferred a hazard ratio of ~2.2 for incident diabetes (random-effects HR 2.19, 95%CI 1.93-2.48). The magnitude of this risk remained unchanged when the analysis was stratified by study design, length of follow-up, NOS quality scale, degree of covariate adjustment or modality of NAFLD diagnosis. Furthermore, the risk of diabetes appeared to increase further with greater severity of NAFLD (especially the severity of liver fibrosis: random-effects HR 3.42, 95%CI 2.29-5.11) and, most importantly, remained significant in those studies where analysis was fully adjusted for age, sex, adiposity measures, family history of diabetes, fasting glycaemia (or prediabetes status), dyslipidaemia, hypertension, smoking and physical activity.

To our knowledge, this meta-analysis assessing the association between NAFLD and the long-term risk of diabetes is the largest and most comprehensive assessment to date. Our findings corroborate and extend the results of two previous small meta-analyses by Musso et al. published in 2011 (n=3 studies with ultrasound data; random-effects OR 3.51, 95%CI 2.28-5.41; I^2=70%) and Ballestri et al. published in 2016 (n=9 studies with ultrasound data; random-effects OR 1.86, 95%CI 1.76-1.95; I^2=86.5%) that incorporated observational studies using either ultrasonography or, in most cases, abnormal serum liver enzymes to diagnose NAFLD [7,8]. Both of these two meta-analyses showed that the presence of biochemistry-defined or imaging-detected NAFLD significantly increased the risk of developing incident diabetes [7,8]. Notably, the results of the present meta-analysis also corroborate and extend the findings of our previously published meta-analysis exploring the association between imaging-detected NAFLD and risk of new-onset diabetes that incorporated 19 observational studies (published up to July 2017), involving a total of ~295,000 individuals (random-effects HR 2.22, 95% CI 1.84-2.60; I^2=79.2%); no liver biopsy studies were available for the analysis [9]. In particular, compared with the results of this latter meta-analysis [9], we have almost doubled the number of eligible studies (by including new 14 follow-up studies published from July 2017 to June 2020), the overall sample size (increasing the total number of individuals from nearly 295,000 to more than 500,000), as well as the number of studies that examined the association between the severity of NAFLD and risk of diabetes (mostly by including liver biopsy cohort studies that were *not* included in our previously published meta-analysis). The issue of whether the increase in NAFLD-associated risk of diabetes is restricted to patients with more severe NAFLD or applies to all patients with NAFLD, is particularly relevant in view of the disease burden that NAFLD represents. Our meta-analysis also by including these new liver biopsy cohort studies (that compared the diabetes risk in patients with advanced fibrosis vs. absent or moderate fibrosis) showed that risk of incident diabetes appeared to increase further with greater severity of liver fibrosis. Although further studies in cohorts of well-characterized patients with NAFLD are needed to better elucidate this issue, our meta-analysis suggests that the magnitude of risk of incident diabetes parallels the underlying severity of NAFLD, particularly the severity of liver fibrosis. This finding is also in line with the conclusion of previous studies and meta-analyses supporting a link between the severity of liver fibrosis and risk of developing not only liver-related morbidity and mortality in patients with NAFLD, but also extra-hepatic complications, such as adverse cardiovascular outcomes, chronic kidney disease and colorectal tumours [3,24-27].

Our meta-analysis has some important limitations (strictly inherent to the design of the included studies) that should be mentioned. First, the observational design of the eligible studies does not allow establishing a causal association between NAFLD and diabetes risk. Second, although the large majority of the eligible studies have adjusted the results for age, sex, adiposity measures, family history of diabetes, dyslipidaemia and other common metabolic risk factors, the possibility of residual confounding by some unmeasured factors cannot be ruled out. Another potential limitation of the meta-analysis is that although we used a random-effects model, the interpretation of some results of this meta-analysis (like all previously published meta-analyses [7-9]) requires some caution, given the high heterogeneity observed in the overall primary analysis (**Figure 2**). It is possible that this high heterogeneity likely reflects differences in the demographic characteristics of study populations, in the length of study follow-ups, in the methodology used for NAFLD diagnosis as well as in the severity of NAFLD. We systematically explored and identified possible sources of statistical heterogeneity using stratified analyses, meta-regressions and sensitivity analyses. Although we found significant heterogeneity between studies when investigating associations in the overall primary analysis, it is noteworthy that there was low heterogeneity between studies, and stronger associations between NAFLD and diabetes risk, when we restricted our statistical analyses to studies with only the more 'severe' forms of NAFLD (**Figure 3**). In addition, it should also be noted that the overall quality of studies included in the meta-analysis was relatively good, suggesting a medium-low risk of bias according to the NOS scale. That said, we think more detailed analyses of the causes of heterogeneity will require collaborative pooling of individual participant data from large studies as these become available over time. Finally, since the diagnosis of diabetes was not always consistent among the eligible studies, some inaccuracy in the estimated risk of diabetes and in the identification of diabetic subtypes may not be excluded, although the large majority of incident cases were likely to be type 2 diabetes. Despite important research advancements in NAFLD, our understanding of sex differences in NAFLD remains insufficient [28,29]. Some liver biopsy studies reported that the prevalence of NASH was not different in both sexes, whereas the severity of liver fibrosis showed a marked difference between men and women [30]. Our meta-regression analyses did not reveal any significant effect of sex on the association between NAFLD and diabetes risk. However, the eligible studies lacked an adequate consideration of sex differences and sex hormones/menopausal status in the analysis. In particular, no separate analyses for men and women were available. We believe

that in future epidemiological studies, sex-specific and sex/age-specific analyses should be performed, and sex and menopausal status should be also collected when possible and considered as potential effect modifiers [28].

Despite these limitations, our meta-analysis has several important strengths. As discussed previously, this meta-analysis provides the most comprehensive and updated assessment to date on the prognostic role of imaging-defined or biopsy-proven NAFLD on the long-term risk of diabetes. These results, obtained by including more than half a million middle-aged individuals (30.8% with imaging-defined or biopsy-confirmed NAFLD) and nearly 28,000 cases of incident diabetes (incorporating data from large cohort studies from Asia, United States and Europe that are likely to be an accurate reflection of patients with NAFLD commonly observed in clinical practice), provide strong evidence that NAFLD at least doubles the long-term risk of incident diabetes, irrespective of age, sex, adiposity measures and other common metabolic risk factors. Finally, although a selective reporting bias of eligible studies could be not definitely excluded, we also searched for 'grey' literature in Web of Science and Scopus databases and made every effort to rule out very low-quality studies by using stringent inclusion criteria. We believe that our comprehensive search has made it unlikely that any published reports were missed, and visual inspection of funnel plots and formal statistical tests demonstrated no evidence of any publication bias.

It is beyond the scope of this meta-analysis to deeply discuss the putative underlying mechanisms by which NAFLD may contribute to the development of diabetes. To date, however, there is convincing evidence of biological plausibility that NAFLD may increase risk of incident diabetes. Indeed, NAFLD (especially NASH with varying levels of liver fibrosis) may exacerbate hepatic insulin resistance and causes the release of a myriad of lipid metabolites, proinflammatory cytokines and hepatokines (e.g., fetuin A, fetuin B and angiopoietin-like protein) that may promote the development of diabetes [4,31-34]. It is known that high fat diets and adipose tissue dysfunction with excessive lipolysis supply the liver with chylomicron remnants and non-esterified fatty acids. Elevated hepatic lipid availability combined with inadequate adaptation of mitochondrial function may induce the hepatic production of diacylglycerols (DAG that activates the novel protein kinase C [nPKC]ε) and certain ceramides, which affect insulin sensitivity and progression of NAFLD [35,36]. Studies suggest a critical role of particular lipid species, such as C18:1-DAG and sn-1,2-

DAG, and their localization in the plasma membrane, for both nPKC translocation and insulin resistance [35]. Emerging evidence from Mendelian randomization studies (using risk alleles in patatin-like phospholipase domain-containing protein-3 [*PNPLA3*], trans-membrane 6 superfamily-2 [*TM6SF2*] and other NAFLD-related genetic variants) also suggests that genetically-driven NAFLD may causally increase the risk of developing insulin resistance and new-onset type 2 diabetes [37,38]. Finally, it is worth noting that some observational cohort studies, mostly performed in Asian individuals, have also reported that the incidence of diabetes appeared to diminish over time following the improvement or resolution of NAFLD on ultrasonography, irrespective of changes in body weight [39-41]. However, caution is needed in interpreting these results, because these studies are not controlled trials focussing on treatment of NAFLD. That said, to further emphasize the strong link between NAFLD and diabetes, an international consensus of experts has recently proposed the new definition of "metabolic dysfunction-associated fatty liver disease" (MAFLD) instead of NAFLD [42]. Although the proposal to change the terminology from NAFLD to MAFLD is under discussion, the proposed change in terminology is influenced by the link of this liver disease with diabetes and underlying metabolic dysfunction. The findings of our meta-analysis strongly emphasise that there is a real need now to include outcomes, such as incident diabetes, in future randomised controlled trials focussed on examining the efficacy of novel therapies for liver disease in NAFLD. This might also have important implications for future strategies in the prevention and treatment of diabetes and other cardiometabolic diseases.

In conclusion, this large and updated meta-analysis provides clear evidence for a significant positive association between the presence of imaging-defined or biopsy-proven NAFLD and the long-term risk of incident diabetes. The magnitude of this risk parallels the underlying severity of NAFLD (especially the stage of liver fibrosis). However, it should be noted that the observational design of the eligible studies does not allow for proving causality, and further studies are certainly required in both Asian and non-Asian populations to draw any firm conclusions about the independent hepatic contribution to the increased risk of incident diabetes observed among patients with NAFLD. Moreover, mechanistic studies are also needed to better understand the link between NAFLD and diabetes risk.

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Table 1. Subgroup analyses – Association between imaging-defined NAFLD and risk of incident diabetes, stratified by study design, length of study follow-up, Newcastle-Ottawa Scale (NOS) category, degree of covariate adjustment or modality of NAFLD diagnosis.

Study design	
Prospective design	Random-effects HR 2.18 (95% CI 1.87-2.55) I^2= 89.0% Number of studies: 6 N= 252,678
Retrospective design	Random-effects HR 2.22 (95% CI 1.86-2.66) I^2= 91.1% Number of studies: 20 N= 165,886
Length of study follow-up	
Follow-up <5 years	Random-effects HR 1.96 (95% CI 1.67-2.29) I^2= 90.1% Number of studies: 11 N= 198,455
Follow-up ≥5 years	Random-effects HR 2.37 (95% CI 2.01-2.80) I^2= 86.2% Number of studies: 15 N= 220,109
NOS category	
NOS ≥8 stars	Random-effects HR 2.09 (95% CI 1.86-2.37) I^2= 85.2% Number of studies: 12 N= 297,769
NOS <8 stars	Random-effects HR 2.33 (95% CI 1.81-2.96) I^2= 93.0% Number of studies: 14 N= 120,795
Degree of adjustment*	
Maximal covariate adjustment	Random-effects HR 2.51 (95% CI 2.11-2.99) I^2= 91.4% Number of studies: 13 N= 274,955
Minimal covariate adjustment	Random-effects HR 1.88 (95% CI 1.61-2.20) I^2= 85.5% Number of studies: 13 N= 143,609
Methods of NAFLD diagnosis	
Ultrasonography	Random-effects HR 2.19 (95% CI 1.93-2.79) I^2= 91.8% Number of studies: 24 N= 414,771
Computed tomography	Random-effects HR 2.09 (95% CI 1.56-2.79) I^2= 0% Number of studies: 2 N= 3,793

NB: In these subgroup analyses, we analyzed all the eligible studies that were included in the Figure 2 (n=26 studies).

* Maximal adjustment includes studies that have adjusted the results at least for the following covariates: age, sex, adiposity measures (body mass index and/or waist circumference), family history of diabetes, fasting glucose (or HbA1c), lipids, hypertension (or systolic blood pressure), smoking history, alcohol consumption and physical activity.

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 incident diabetes in 26 eligible studies, stratified by study country.

Figure 3. Forest plot and pooled estimates of the effect of the severity of NAFLD (stratified either by the ultrasonographic severity of steatosis, or by the severity of fibrosis, which was based on histologic stages of liver fibrosis and/or increased non-invasive fibrosis scores) on the risk of incident diabetes in 9 eligible studies.

Supplementary Figure S1. Univariable meta-regression analyses. A meta-analysis of the association of age (panel A), body mass index (panel B) and male sex (panel C) with the risk of incident diabetes (for the 26 studies included in Figure 2).

Supplementary Figure S2. Meta-analysis estimates, given named study is omitted. Panel A for the 26 eligible studies included in Figure 2. Panel B for the 9 eligible studies included in Figure 3. The effect size was expressed as random-effects HRs and 95% confidence intervals for all studies.

Supplementary Figure S3. Panel A: Funnel plot of standard error by log-hazard ratio for the risk of incident diabetes (for the 26 studies included in Figure 2). Panel B: Funnel plot of standard error by log-hazard ratio for the risk of incident diabetes (for the 9 studies included in Figure 3). P-values by the rank correlation Begg's test.