Nonalcoholic Fatty Liver Disease and Risk of Incident Type 2 Diabetes Mellitus: a Meta-Analysis.

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ABSTRACT

Objective: Several studies explored the impact of nonalcoholic fatty liver disease (NAFLD) on risk of incident diabetes. However, the extent to which NAFLD may confer risk of incident diabetes remains uncertain. We performed a meta-analysis of relevant studies to quantify the magnitude of the association between NAFLD and risk of incident diabetes.

Research Methods and Design: We searched PubMed, Scopus and Web of Science using pre-defined keywords to identify large observational studies with a follow-up duration >1 year, in which NAFLD was diagnosed by imaging methods and that were published up to July 2017. Data from selected studies were extracted, and meta-analysis was performed using random-effects modeling.

Results: A total of 19 observational studies with 296,439 individuals (30.1% with NAFLD) and nearly 16,000 cases of incident diabetes over a median of 5 years were included in the final analysis. Patients with NAFLD had a greater risk of incident diabetes than those without NAFLD (random-effects hazard ratio [HR] 2.22, 95% CI 1.84-2.60; I^2=79.2%). Patients with more 'severe' NAFLD were also more likely to develop incident diabetes; this risk increased across the ultrasonographic scores of steatosis (n=3 studies), but it appeared to be even greater among NAFLD patients with high NAFLD fibrosis score (n=1 study; random-effects HR 4.74, 95% CI 3.54-5.94). Sensitivity analyses did not alter these findings. Funnel plot and Egger's test did not reveal significant publication bias.

Conclusions: NAFLD is associated with a two-fold increased risk of incident diabetes. However, the observational design of the eligible studies does not allow for proving causality.

Keywords: NAFLD; fatty liver; diabetes risk; meta-analysis

Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease in high-income countries (affecting up to one third of adults in the Europe and United States), and its prevalence is expected to rise further in the near future.[1,2]

NAFLD has been traditionally considered as the simple 'hepatic manifestation' of the metabolic syndrome.[2,3] Moreover, it is also well known that patients with type 2 diabetes have a high prevalence of NAFLD (up to 70-75%) and that these patients are also at higher risk of developing nonalcoholic steatohepatitis (NASH), and a twofold to fourfold higher risk of developing serious liver-related complications (cirrhosis, liver failure and hepatocellular carcinoma).[4-6]

However, it is now becoming increasingly clear that the link between NAFLD and type 2 diabetes is more complex than previously believed.[7-9] NAFLD and type 2 diabetes share multiple cardiometabolic risk factors and pathophysiological (proinflammatory and profibrotic) pathways. In addition, increasing epidemiological evidence suggests that there is a bi-directional relationship between NAFLD and type 2 diabetes, and that NAFLD may precede and/or promote the development of type 2 diabetes.[3,4,7-9]

To our knowledge, there are only two previously published meta-analyses that have shown that NAFLD is associated with an increased risk of incident diabetes.[10,11] However, both of these meta-analyses (published in 2011 and 2016, respectively) have also included a large number of observational studies in which the diagnosis of NAFLD was based on abnormal serum liver enzyme levels, which are thought to be only surrogate markers of NAFLD.[12] Currently, there is intense debate about the prognostic role of NAFLD *per se* on the long-term risk of incident diabetes. As will be discussed in detail below, over the last year, numerous large observational studies have been published in which the diagnosis of NAFLD was based on ultrasonography or computed tomography, which are the most widely used non-invasive methodologies to diagnose NAFLD in clinical practice.[1-3,12]

We herein report the results of a comprehensive systematic review and meta-analysis of observational cohort studies that has investigated the association between NAFLD (as detected by ultrasonography or other imaging methods) and the risk of incident diabetes. Our aim was to gauge precisely the nature and magnitude of the association between NAFLD and risk of incident diabetes. We have also investigated whether the severity of NAFLD (in studies using either ultrasonographic scoring systems or non-invasive fibrosis markers) is associated with an even greater risk of incident diabetes. Clarification of the magnitude of risk of incident diabetes associated with the different stages of liver disease within the spectrum of NAFLD may have relevant clinical implications for the diagnosis, prevention and treatment of type 2 diabetes.

RESEARCH METHODS AND DESIGN

Registration of review protocol

The protocol for this systematic review was registered in advance with PROSPERO (International Prospective Register of Systematic Reviews, no. CRD42017072305).

Data sources and searches

Studies were included if they were observational cohort studies that reported the incidence rates of type 2 diabetes in adult individuals (>18 years old) with NAFLD as compared with those without NAFLD. Study participants were of either sex with no restrictions in terms of ethnicity and comorbidities. We included only large (n ≥500) observational studies with a follow-up of at least 1 year in which the diagnosis of NAFLD was based on either imaging or histology in the absence of competing causes of hepatic steatosis. Based on data from the eligible studies, 'severe' NAFLD was defined either by presence of increasing ultrasonographic steatosis scores or high NAFLD fibrosis score (NFS), which is a reliable non-invasive marker of advanced NAFLD fibrosis.[12] In these eligible studies, the diagnosis of incident diabetes was

based on a self-reported history of disease or use of hypoglycemic drugs, and in most cases, it was also based on a fasting plasma glucose level ≥7.0 mmol/l or a HbA1c level ≥6.5% (≥48 mmol/mol).

Exclusion criteria of the meta-analysis were as follows: 1) reviews, editorials, abstracts, case reports, practice guidelines and cross-sectional studies; 2) studies that used only serum liver enzyme levels, fatty liver index or other surrogate markers to diagnose NAFLD; 3) studies with a sample size of less than 500 individuals or with a follow-up duration less than 1 year; 4) studies conducted in paediatric population (<18 years old); and 5) studies which did not report any hazard ratio and 95% confidence interval for the outcome of interest (incident diabetes).

Included and excluded studies were collected following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. Additionally, because included studies were observational in design, we followed the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines for the meta-analysis of observational studies.

Data extraction and quality assessment

Relevant studies were identified by systematically searching PubMed, Scopus and Web of Science from January 1, 1990 to July 15, 2017 (date last searched) using the free text terms "fatty liver" (OR "NAFLD" OR "nonalcoholic fatty liver disease" OR "nonalcoholic steatohepatitis") AND "diabetes risk" OR "diabetes incidence" OR "incident diabetes". No language restriction was applied. Reference lists of relevant papers and previous review articles were hand searched for other relevant studies. Two investigators (AM and GT) 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, study size, source of data, population characteristics, duration of follow-up, outcome of interest, matching and confounding factors. Additionally, in the case of multiple publications, we included the most up-to-date or comprehensive information.

Two authors (AM and GT) assessed the risk of bias independently. Since all the included studies were nonrandomised and had a cohort design, the Newcastle–Ottawa Scale (NOS) was used to judge study quality, as recommended by the Cochrane Collaboration.[13] 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. 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.

Data synthesis and analysis

The outcome measure of this meta-analysis was the occurrence of incident diabetes among individuals with NAFLD compared to incidence of diabetes among those without NAFLD. When possible, we pooled adjusted hazard ratios (or odds ratios), with their 95% confidence intervals. In the case of studies reporting hazard ratios with varying degrees of adjustment, we always used the fully adjusted hazard ratio estimate. Visual inspection of the forest plots was used to investigate the possibility of statistical heterogeneity. Statistical heterogeneity was assessed by the I^2 statistic, which provides an estimate of the percentage of variability across studies that is due to heterogeneity rather than chance alone. According to Higgins and Thompson,[14] 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.

The results of the eligible studies were pooled, and an overall estimate of effect size (ES) was calculated using a random-effects model, as this methodology takes into account any

differences between studies even if there is no statistically significant heterogeneity.[15] Publication bias was evaluated using the funnel plot and Egger's regression test.[16]

Given the expected heterogeneity of the eligible studies, sensitivity analyses were also carried out to relate the primary outcome (*i.e.*, incident diabetes) with the individual study design characteristics. In particular, based on data from the eligible studies, the prognostic impact of NAFLD on risk of incident diabetes was assessed by stratifying the studies according to the duration of follow-up, the study country, the study design, the 'severity' of NAFLD (based on ultrasonographic scoring systems or the NFS), or whether the studies had eight or nine stars on the NOS scale (*i.e.*, the 'high-quality' studies) and whether the studies had full adjustment for covariates (*i.e.*, those studies adjusting at least for age, sex, body mass index [or waist circumference], family history of diabetes, fasting glucose levels [or impaired fasting glycaemia], lipids, hypertension [or blood pressure values], smoking and physical activity). Additionally, 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

Based on the titles and abstracts of 2,072 citations, we identified 36 potentially relevant studies. Of these, we excluded 17 studies for the reasons specified in the MOOSE flow diagram (**supplementary Figure S1**). Thus, 19 unique, observational cohort studies were eligible for inclusion in the meta-analysis and were assessed for quality.

As summarized in **Table 1**, all the eligible studies had an observational retrospective or prospective design.[17-35] The eligible studies recruited participants from approximately general

populations in which NAFLD was diagnosed by imaging methods (mainly ultrasonography), and incident diabetes was diagnosed by biochemistry (fasting glucose levels or HbA1c), clinical history or drug treatment. No studies with biopsy-proven NAFLD were available for the analysis.

Overall, in the 19 observational cohort studies included in the meta-analysis there were 296,439 adult individuals (30.1% with NAFLD; n=89,123) with 15,751 cases of incident diabetes over a median follow-up period of 5 years (interquartile range: 4.6-9.1 years). Most of these studies were carried out in Asia (China, Taiwan, South Korea, Sri Lanka and Japan); two community-based studies were carried out in the United States. Most of these studies included middle-aged subjects predominantly of male sex. Of the 19 included studies, 10 studies received eight stars at the NOS, seven studies received six or seven stars and two studies received fewer than six stars, indicating an overall medium risk of bias (**supplementary Table S1**).

NAFLD and risk of incident diabetes

The distribution of studies by estimate of the association between NAFLD and risk of incident diabetes is plotted in **Figure 1**. Sixteen studies provided data suitable for the pooled primary analysis (n=214,805 with 10,356 cases of incident diabetes). We excluded three studies from this primary analysis because the authors did not provide any HR for incident diabetes among individuals with NAFLD pooled together;[21,24,32] these three studies were used in a secondary analysis for examining the association between NAFLD severity and diabetes risk (see below).

NAFLD was significantly associated with an increased risk of incident diabetes (random-effects HR 2.22, 95%CI 1.84-2.60; I^2=79.2%). Notably, since we always used the fully adjusted HR estimates for each eligible study (as specified in **Table 1**), this random-effects HR was independent of a relatively large number of common risk factors and potential confounders. As also shown in the figure, when the comparison was stratified by the study country, the

association of NAFLD with risk of incident diabetes was significant in all study countries, but it appeared to be stronger in Japan, China and Taiwan than in the United States and in other Asian countries (South Korea and Sri Lanka).

As shown in **Figure 2**, when the comparison was stratified by the length of follow-up period, the association of NAFLD with the risk of incident diabetes appeared to be stronger in those studies with more than 5 years of follow-up (n=16 studies; random-effects HR 2.60, 95%CI 1.92-3.29; I^2=74.6%).

As shown in **supplementary Figure 2**, when the comparison was stratified by the study design, the association between NAFLD and the risk of incident diabetes was consistent in prospective studies (n=3 studies; random-effects HR 2.25, 95%CI 1.93-2.58; I^2=0%) and in retrospective studies (n=13 studies; random-effects HR 2.26, 95%CI 1.80-2.72; I^2=81.0%).

Limiting the analysis to 'high-quality' studies and limiting to studies with adjustment for multiple covariates provided overall estimates consistent with the pooled primary analysis (n=10 studies; random-effects HR 1.85, 95%CI 1.47-2.22; I^2=68.3%). Finally, eliminating each of the included studies from the analysis had no effect on the overall risk of incident diabetes (data not shown).

As shown in **supplementary Figure S3,** the Egger's regression test did not show statistically significant asymmetry of the funnel plot (p=0.31), thus suggesting that publication bias was unlikely.

Severe NAFLD and risk of incident diabetes

Four cohort studies reported data on patients with 'severe' NAFLD, defined either by ultrasonographic severity of steatosis or by high NFS. The distribution of studies by estimate of

the association between severe NAFLD and risk of incident diabetes is plotted in **Figure 3**. Compared with the non-NAFLD group, the presence of more 'severe' NAFLD was significantly associated with an increased risk of incident diabetes (n=4 studies; random-effects HR 2.63, 95%CI 1.57-3.70; I^2=82.4%). This risk increased across the ultrasonographic scores of hepatic steatosis (n=3 studies; random-effects HR 2.15, 95%CI 1.72-2.58; I^2=0%), but it appeared to be even greater among NAFLD patients with high NFS (n=1 study; random-effects HR 4.74, 95%CI 3.54-5.94). However, only a single study assessed NAFLD severity by using NFS, and no studies involving non-Asian individuals were available for this analysis, thus limiting the generalizability of the finding.

DISCUSSION

Our meta-analysis provides evidence for a significant association between imaging-diagnosed NAFLD and the long-term risk of incident diabetes. Indeed, this meta-analysis involves a total of 19 unique, observational studies with aggregate data on 296,439 adult individuals (30.1% with NAFLD) and nearly 16,000 cases of incident diabetes followed-up over a median period of 5 years.

We found that the presence of imaging-diagnosed NAFLD conferred a HR of 2.2 for incident diabetes, a risk that appeared to increase further with greater 'severity' of NAFLD (assessed in four observational studies using either the ultrasonographic severity of steatosis or the NFS) and remained statistically significant in those studies where analysis was fully adjusted for potentially confounding factors (n=10 studies; random-effects HR 1.85, 95%CI 1.47-2.22; I^2=68.3%). In addition, when the analysis was stratified either by follow-up duration or by study country, the association between NAFLD and diabetes risk appeared to be stronger in studies with a follow-up duration longer than 5 years, and in studies performed in Japanese

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and Chinese populations compared to those conducted in the United States and in other Asian countries.

Unfortunately, most of the published studies that used liver biopsies to diagnose NAFLD (*i.e.*, the 'gold standard' method for diagnosing and staging NAFLD) did not have a control group and cannot, therefore, be included in this meta-analysis. To date, however, very little is known about how long-term NAFLD or its histologic features may affect risk of incident diabetes. A retrospective cohort study of 396 patients with biopsy-confirmed NAFLD, who did not have diabetes at baseline, reported that a significantly higher proportion of patients with fibrosis stages 3-4 developed incident diabetes than those with fibrosis stages 0-2 (51% *vs*. 31%) over a mean follow-up of 18.4 years.[36] Interestingly, for patients with fibrosis stages 0-2, fat score was also independently associated with incident diabetes.[36] The issue of whether the increased diabetes risk is confined 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 and might impact on the healthcare resources needed to survey and manage these patients adequately. The results of our meta-analysis (based exclusively on studies using ultrasonographic scoring systems or the NFS) suggest that it is more advanced NAFLD that carries a greater diabetes risk. This is also consistent with the conclusion of another comprehensive meta-analysis supporting a link between NAFLD severity and risk of fatal and nonfatal cardiovascular events.[37] However, this question remains largely unsolved, and further follow-up studies in larger cohorts of both Asian and non-Asian individuals with biopsy-confirmed NAFLD (with an adequate group of control individuals) are needed in order to definitely establish whether NAFLD severity differentially affects risk of incident diabetes.

This is the largest and most updated meta-analysis aimed at investigating the prognostic role of imaging-diagnosed NAFLD on the long-term risk of incident diabetes. Collectively, our findings confirm and extend on a sample size at least three times greater the results of two previous meta-analyses that incorporated studies using both abnormal serum liver enzymes and imaging techniques to diagnose NAFLD.[10,11] In the first meta-analysis, Musso *et al*. in 2011

reported that ultrasound-diagnosed NAFLD (only three studies included) was associated with an increased risk of incident diabetes (random-effects OR 3.51, 95%CI 2.28-5.41; I^2=70%).[10] In the second meta-analysis, Ballestri *et al*. confirmed that NAFLD (defined by ultrasonography; only nine studies included in total) significantly increased the risk of incident diabetes over a follow-up of nearly 5 years (random-effects OR 1.86, 95%CI 1.76-1.95; I^2=86%).[11]

Notably, our meta-analysis is the first to show that the association between NAFLD and the risk of incident diabetes is stronger in some ethnic groups (especially in Japanese individuals) and in studies with longer follow-up duration, and that the more 'severe' forms of NAFLD seem to be associated with an even greater risk of developing diabetes. This latter finding is also indirectly supported by the results of the study of Ma *et al.* demonstrating that among the Framingham Heart Study participants, baseline hepatic fat content (per standard deviation increase) was independently associated with increased odds of incident diabetes over approximately 6 years of follow-up.[33]

There is now convincing evidence of biological plausibility that NAFLD may increase risk of incident type 2 diabetes.[3,4,7,8] Indeed, NAFLD, especially NASH with varying levels of hepatic fibrosis, exacerbates hepatic insulin resistance and causes the release of multiple pro-inflammatory mediators and pro-diabetogenic hepatokines (*e.g*., fetuin-A, fetuin-B, fibroblast growth factor-21, retinol binding protein-4 and selenoprotein P) that may promote the development of diabetes.[38-40] Among the hepatokines particularly data about fetuin-A action in mice and in humans supports a causative relationship of NAFLD with incident diabetes.[38,41,42] However, whether improvement or resolution of NAFLD could decrease risk of incident diabetes remains uncertain. Some evidence suggests that the risk of diabetes appears to diminish over time following the resolution or improvement of NAFLD.[26,43] However, as these two studies are not randomized controlled trials of NAFLD management, these results should be interpreted cautiously.

Although our meta-analysis of observational studies provides support for the existence of a significant association between NAFLD and increased risk of incident diabetes, it remains to be definitely proven that improving the liver condition in NAFLD decreases risk of developing diabetes. It should also be noted that there may be a dissociation between NAFLD and insulin resistance in humans carrying some genetic variants, such as the patatin-like phospholipase 3 gene.[44] Experimental evidence (mainly derived from animal studies) also indicates that specific manipulation of liver fat is insufficient to affect insulin sensitivity/glycemia.[45-47] In addition, it is known that there are ethnic differences in liver fat content (as well in the amount of lipid accumulated in skeletal muscle and abdominal cavity) and risk of diabetes. For example, compared to the white individuals, obese black individuals exhibit a lower prevalence of NAFLD but similar type 2 diabetes prevalence.[48-50] That said, our data strongly emphasise that there is a real need now to include outcomes such as incident diabetes and changes in HbA1c and insulin sensitivity in randomised placebo-controlled trials focussed on testing the efficacy of novel therapies for liver disease in NAFLD. Improved understanding of these features and precise phenotyping of NAFLD could help to improve stratification of cardiometabolic risk. This might also have important implications for future strategies in the prevention and treatment of type 2 diabetes and other cardiometabolic diseases in clinical practice.[51,52]

Our meta-analysis has some important limitations (strictly inherent to the nature of the included studies) that should be mentioned. Although we used a random-effects model, the interpretation of the results of this meta-analysis requires some caution, given the (expected) high heterogeneity ($I^2 > 75\%$) observed in the overall primary analysis. It is plausible to assume that this high heterogeneity likely reflects differences in the demographic and ethno-racial characteristics of study populations, in the length of follow-up, in the design of the study as well as in the severity of NAFLD. We systematically explored and identified all these possible sources of statistical heterogeneity using stratified analyses and sensitivity analyses (as detailed in the Results section). Although we found significant heterogeneity between studies when investigating associations in the overall analysis, it is noteworthy that there was very low heterogeneity between studies, and stronger associations between NAFLD and diabetes

risk, when we restricted the statistical analyses to studies with only the more 'severe' forms of NAFLD on ultrasonography, studies with a prospective design (compared to retrospective ones) or studies performed in the United States population. However, we believe that more detailed analyses of the causes of heterogeneity will require collaborative pooling of individual participant data from large prospective studies as these become available over time.

Another potential limitation of the meta-analysis (strictly inherent to the observational nature of the included studies) is that information about the temporal changes of some important variables (e.g., medication use and lifestyle changes) that may impact NAFLD and incidence diabetes is often missing, and that the varying degree of confounder adjustment across the individual studies hampered a systematic assessment of the impact of known risk factors on the outcome of interest. As shown in **Table 1**, some studies reported incomplete adjustments for established risk factors and potential confounders (*e.g*., waist circumference or insulin resistance); as such, it was not possible to combine models in studies that adjusted for the same set of potential confounding factors. Another limitation of the meta-analysis was that none of the eligible studies used liver biopsy for the diagnosis of NAFLD. Conversely, most of the eligible studies used ultrasonography, which is the recommended first-line imaging method for detecting NAFLD in clinical practice, and it enables a reliable and accurate detection of mild-to-moderate hepatic steatosis compared with liver histology. Furthermore, the results regarding the association between the severity of NAFLD and diabetes risk derived from very few studies, and only a single study assessed NAFLD severity by using the NFS. Additionally, since the diagnosis of diabetes was not always consistent among the included studies, some inaccuracy in the estimated incidence of diabetes and in the identification of diabetic subtypes may not be excluded, although the vast majority of diabetic cases were likely to be type 2. Despite that both fasting plasma glucose and HbA1c levels were available for the majority of the eligible studies, the diagnosis of diabetes was based on HbA1c or fasting glucose measurements, without further systematic confirmation by a second determination on a separate day; however, this is an intrinsic limitation of all large observational studies, in which the confirmation of diabetes diagnosis, on at least two

separate occasions, has been never made. Finally, in none of the published studies, except for the studies by Shibata *et al*. and by Ming *et al*.,[18,27] was the diagnosis of diabetes based on 2-hour post-load plasma glucose levels.

Notwithstanding these limitations, the present meta-analysis has several important strengths. As discussed previously, this meta-analysis provides the most comprehensive assessment to date on the independent prognostic impact of NAFLD on the long-term risk of incident diabetes. These results, obtained by analyzing more than 15,000 new cases of incident diabetes among nearly 300,000 individuals (incorporating data from observational cohort studies that are likely to be an accurate reflection of NAFLD patients commonly seen in routine clinical practice), provide clear evidence that diabetes risk of individuals with NAFLD is significantly higher than that of individuals without NAFLD (with a high level of heterogeneity for the pooled primary analysis and a medium-low quality of the mainly retrospective available studies). Moreover, it is important to underline that we employed standardized risk estimates from all eligible studies to allow a consistent combination of estimates across studies. The large number of incident cases of diabetes provided high statistical power to quantitatively assess the association between NAFLD and diabetes risk. Finally, selective reporting bias of studies was not a concern in our analyses, as our comprehensive search and contact with investigators made it unlikely that any published report was missed and visual inspection of plots and formal tests demonstrated no statistical evidence of publication bias.

Currently, there are no approved pharmacological agents for the treatment of NAFLD. Most interventions evaluated for NAFLD treatment are those commonly used for the treatment of type 2 diabetes and exert a rather indirect effect on the liver, through improvements in both insulin sensitivity and insulin action; decreases in free fatty acid levels and improvements in glucose uptake.[4,8,53] These pharmacological interventions have also been to date the most effective treatments for NAFLD, which is perhaps not surprising, considering the high degree of interplay between these two diseases.

In conclusion, this largest and most comprehensive meta-analysis to date showed that imaging-diagnosed NAFLD is associated with an approximate doubling of risk of incident diabetes, and that this risk seems to be even greater in presence of more 'severe' liver disease (in the few available cohort studies using ultrasonographic scoring systems or non-invasive fibrosis markers). Because no studies with biopsy-proven NAFLD were available for the analysis, the findings of this meta-analysis pave the way for future large, prospective, histologically-based studies. It remains uncertain whether NAFLD causally increases diabetes risk or is a marker of other shared risk factors. Further studies are also needed in non-Asian populations, as most of the published studies have been conducted in Asian populations, where large populations undergo regular health check-ups, including liver ultrasonography. Finally, additional studies are also required to establish whether adding NAFLD (or the different components of liver disease in NAFLD) to the currently available algorithms will improve risk prediction for diabetes. Despite the above-mentioned caveats, current clinical guidelines do recommend routine screening for diabetes in patients with NAFLD[53] and, therefore, there is a need to clarify the magnitude of risk of incident diabetes that is associated with the stages of liver disease in NAFLD.

REFERENCES

1. Lonardo A, Bellentani S, Argo CK, Ballestri S, Byrne CD, Caldwell SH, Cortez-Pinto H, Grieco A, Machado MV, Miele L, Targher. Epidemiological modifiers of non-alcoholic fatty liver disease: focus on high-risk groups. Dig Liver Dis. 2015; 47: 997-1006.
2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease - Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016; 64: 73-84.
3. Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. Nat Rev Gastroenterol Hepatol 2013; 10: 330-344.
4. Bril F, Cusi K. Management of nonalcoholic fatty liver disease in patients with type 2 diabetes: a call to action. Diabetes Care. 2017; 40: 419-430.
5. Wild SH, Morling JR, McAllister DA, Kerssens J, Fischbacher C, Parkes J, Roderick PJ, Sattar N, Byrne CD; Scottish and Southampton Diabetes and Liver Disease Group; Scottish Diabetes Research Network Epidemiology Group. Type 2 diabetes and risk of hospital admission or death for chronic liver diseases. J Hepatol. 2016; 64: 1358-1364.
6. Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. J Hepatol. 2012; 56: 1384-1391.
7. Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol. 2015; 62: S47-S64.
8. Targher G, Marchesini G, Byrne CD. Risk of type 2 diabetes in patients with non-alcoholic fatty liver disease: causal association or epiphenomenon? Diabetes Metab. 2016; 42: 142-156.
9. Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. Gut. 2017; 66: 1138-1153.
10. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011; 43: 617-649.
11. Ballestri S, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, Roverato A, Guaraldi G, Lonardo A. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol. 2016; 31: 936-944.
12. Machado MV, Cortez-Pinto H. Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal. J Hepatol. 2013; 58: 1007-1019.

13. Higgins JPT, Green S, eds. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0* [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.
14. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002; 21: 1539-1558.
15. Furukawa TA, Guyatt GH, Griffith LE. Can we individualize the 'number needed to treat'? An empirical study of summary effect measures in meta-analyses. Int J Epidemiol. 2002; 31: 72-76.
16. Egger M, Smith GD, Phillips AN. Meta-analysis: principles and procedures. BMJ. 1997; 315: 1533-1537.
17. Okamoto M, Takeda Y, Yoda Y, Kobayashi K, Fujino MA, Yamagata Z. The association of fatty liver and diabetes risk. J Epidemiol. 2003; 13: 15-21.
18. Shibata M, Kihara Y, Taguchi M, Tashiro M, Otsuki M. Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men. Diabetes Care. 2007; 30: 2940-2944.
19. Kim CH, Park JY, Lee KU, Kim JH, Kim HK. Fatty liver is an independent risk factor for the development of Type 2 diabetes in Korean adults. Diabet Med. 2008; 25: 476-481.
20. Bae JC, Rhee EJ, Lee WY, Park SE, Park CY, Oh KW, et al. Combined effect of nonalcoholic fatty liver disease and impaired fasting glucose on the development of type 2 diabetes: a 4-year retrospective longitudinal study. Diabetes Care. 2011; 34: 727-729.
21. Park SK, Seo MH, Shin HC, Ryoo JH. Clinical availability of nonalcoholic fatty liver disease as an early predictor of type 2 diabetes mellitus in Korean men: 5-year prospective cohort study. Hepatology. 2013; 57: 1378-1383.
22. Sung KC, Jeong WS, Wild SH, Byrne CD. Combined influence of insulin resistance, overweight/obesity, and fatty liver as risk factors for type 2 diabetes. Diabetes Care. 2012; 35: 717-722.
23. Kasturiratne A, Weerasinghe S, Dassanayake AS, Rajindrajith S, de Silva AP, Kato N, Wickremasinghe AR, de Silva HJ. Influence of non-alcoholic fatty liver disease on the development of diabetes mellitus. J Gastroenterol Hepatol. 2013; 28: 142-147.
24. Chang Y, Jung HS, Yun KE, Cho J, Cho YK, Ryu S. Cohort study of non-alcoholic fatty liver disease, NAFLD fibrosis score, and the risk of incident diabetes in a Korean population. Am J Gastroenterol. 2013; 108: 1861-1868.
25. Choi JH, Rhee EJ, Bae JC, Park SE, Park CY, Cho YK, Oh KW, Park SW, Lee WY. Increased risk of type 2 diabetes in subjects with both elevated liver enzymes and ultrasonographically diagnosed nonalcoholic fatty liver disease: a 4-year longitudinal study. Arch Med Res. 2013; 44: 115-120.
26. Yamazaki H, Tsuboya T, Tsuji K, Dohke M, Maguchi H. Independent association between improvement of nonalcoholic fatty liver disease and reduced incidence of type 2 diabetes. Diabetes Care. 2015; 38: 1673-1679.
27. Ming J, Xu S, Gao B, Liu G, Ji Y, Yang F, Jia Y, Fang Y, Ji Q. Non-alcoholic fatty liver disease predicts type 2 diabetes mellitus, but not prediabetes, in Xi'an, China: a five-year cohort study. Liver Int. 2015; 35: 2401-2407.

28. Li WD, Fu KF, Li GM, Lian YS, Ren AM, Chen YJ, Xia JR. Comparison of effects of obesity and non-alcoholic fatty liver disease on incidence of type 2 diabetes mellitus. World J Gastroenterol. 2015; 21: 9607-9613.

29. Shah RV, Allison MA, Lima JA, Bluemke DA, Abbasi SA, Ouyang P, Jerosch-Herold M, Ding J, Budoff MJ, Murthy VL. Liver fat, statin use, and incident diabetes: The Multi-Ethnic Study of Atherosclerosis. Atherosclerosis. 2015; 242: 211-217.

30. Fukuda T, Hamaguchi M, Kojima T, Hashimoto Y, Ohbora A, Kato T, Nakamura N, Fukui M. The impact of non-alcoholic fatty liver disease on incident type 2 diabetes mellitus in non-overweight individuals. Liver Int. 2016; 36: 275-283.

31. Chen GY, Cao HX, Li F, Cai XB, Ao QH, Gao Y, Fan JG. New risk-scoring system including non-alcoholic fatty liver disease for predicting incident type 2 diabetes in East China: Shanghai Baosteel Cohort. J Diabetes Investig. 2016; 7:206-211.

32. Li Y, Wang J, Tang Y, Han X, Liu B, Hu H, Li X, Yang K, Yuan J, Miao X, Yao P, Wei S, Wang Y, Liang Y, Zhang X, Guo H, Pan A, Yang H, Hu FB, Wu T, He M. Bidirectional association between nonalcoholic fatty liver disease and type 2 diabetes in Chinese population: Evidence from the Dongfeng-Tongji cohort study. PLoS One. 2017; 12: e0174291.

33. Ma J, Hwang SJ, Pedley A, Massaro JM, Hoffmann U, Chung RT, Benjamin EJ, Levy D, Fox CS, Long MT. Bi-directional analysis between fatty liver and cardiovascular disease risk factors. J Hepatol. 2017; 66: 390-397.

34. Chen SC, Tsai SP, Jhao JY, Jiang WK, Tsao CK, Chang LY. Liver Fat, hepatic enzymes, alkaline phosphatase and the risk of incident type 2 diabetes: a prospective study of 132,377 adults. Sci Rep. 2017; 7: 4649.

35. Liu M, Wang J, Zeng J, Cao X, He Y. Association of NAFLD with diabetes and the impact of BMI changes: a 5-year cohort study based on 18,507 elderly. J Clin Endocrinol Metab. 2017; 102: 1309-1316.

36. Björkström K, Stål P, Hultcrantz R, Hagström H. Histologic scores for fat and fibrosis associate with development of type 2 diabetes in patients with non-alcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2017; 15: 1461-1468.

37. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. J Hepatol. 2016; 65: 589-600.

38. Meex RCR, Watt MJ. Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. Nat Rev Endocrinol. 2017; 13: 509-520.

39. Petersen MC, Shulman GI. Roles of diacylglycerols and ceramides in hepatic insulin resistance. Trends Pharmacol Sci. 2017; 38: 649-665.

40. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. Nat Rev Gastroenterol Hepatol. 2017; 14: 32-42.

41. Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, Ray S, Majumdar SS, Bhattacharya S. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat Med. 2012; 18: 1279-1285.

42. Stefan N, Häring HU. Circulating fetuin-A and free fatty acids interact to predict insulin resistance in humans. Nat Med. 2013; 19: 394-395.

43. Sung KC, Wild SH, Byrne CD. Resolution of fatty liver and risk of incident diabetes. J Clin Endocrinol Metab. 2013; 98: 3637-3643.

44. Kantartzis K, Peter A, Machicao F, Machann J, Wagner S, Königsrainer I, Königsrainer A, Schick F, Fritsche A, Häring HU, Stefan N. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. Diabetes. 2009;58:2616-2623.

45. Benhamed F, Denechaud PD, Lemoine M, Robichon C, Moldes M, Bertrand-Michel J, Ratziu V, Serfaty L, Housset C, Capeau J, Girard J, Guillou H, Postic C. The lipogenic transcription factor ChREBP dissociates hepatic steatosis from insulin resistance in mice and humans. J Clin Invest. 2012;122:2176-2194.

46. Moon YA, Liang G, Xie X, Frank-Kamenetsky M, Fitzgerald K, Koteliansky V, Brown MS, Goldstein JL, Horton JD. The Scap/SREBP pathway is essential for developing diabetic fatty liver and carbohydrate-induced hypertriglyceridemia in animals. Cell Metab. 2012;15:240-246.

47. Minehira K, Young SG, Villanueva CJ, Yetukuri L, Oresic M, Hellerstein MK, Farese RV Jr, Horton JD, Preitner F, Thorens B, Tappy L. Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice. J Lipid Res. 2008;49:2038-2044.

48. Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis: an insulin resistance paradox? Hepatology. 2009;49:791-801.

49. Liska D, Dufour S, Zern TL, Taksali S, Calí AM, Dziura J, Shulman GI, Pierpont BM, Caprio S. Interethnic differences in muscle, liver and abdominal fat partitioning in obese adolescents. PLoS One. 2007;2:e569.

50. Nazare JA, Smith JD, Borel AL, Haffner SM, Balkau B, Ross R, Massien C, Alméras N, Després JP. Ethnic influences on the relations between abdominal subcutaneous and visceral adiposity, liver fat, and cardiometabolic risk profile: the International Study of Prediction of Intra-Abdominal Adiposity and Its Relationship With Cardiometabolic Risk/Intra-Abdominal Adiposity. Am J Clin Nutr. 2012;96:714-726.

51. Stefan N, Fritsche A, Schick F, Häring HU. Phenotypes of prediabetes and stratification of cardiometabolic risk. Lancet Diabetes Endocrinol. 2016; 4: 789-798.

52. Stefan N, Schick F, Häring HU. Causes, characteristics, and consequences of metabolically unhealthy normal weight in humans. Cell Metab. 2017; 26: 292-300.

53. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). J Hepatol. 2016; 64: 1388-1402.

FIGURE LEGENDS

Figure 1. Forest plot and pooled estimates of the effect of NAFLD on the risk of incident diabetes in 16 eligible studies, stratified by study country.

Figure 2. Forest plot and pooled estimates of the effect of NAFLD on the risk of incident diabetes in 16 eligible studies, stratified by length of follow-up (based on the median follow-up of the eligible studies).

Figure 3. Forest plot and pooled estimates of the effect of the severity of NAFLD (defined by ultrasonography [US] or high NAFLD fibrosis score [NFS]) on the risk of incident diabetes in 4 eligible studies.

Figure S1. Study flow-chart: the MOOSE flow diagram.

Figure S2. Forest plot and pooled estimates of the effect of NAFLD on the risk of incident diabetes in 16 eligible studies, stratified by study design.

Figure S3. Funnel plot of standard error by log-hazard ratio (HR) for risk of incident diabetes. Egger's regression test: *p*-value=0.31.

Table 1 – Principal observational (retrospective or prospective) cohort studies of the association between NAFLD (as detected by imaging techniques) and the risk of incident type 2 diabetes (ordered by publication year).

Authors, year (ref.)	Study design, Sample size, Population, Follow-up, and NAFLD Diagnostic tool	Diagnosis of Incident Diabetes	No. incident cases of diabetes; % in non-NAFLD vs. NAFLD cases	Adjustments Considered	Main Findings	Newcastle-Ottawa quality assessment scale (NOS)
Okamoto *et al.* 2003 (17)	Retrospective cohort study n=840 (of whom 14.3% with NAFLD) nondiabetic Japanese subjects; 10 years; liver ultrasonography	Fasting glucose >6.1 mmol/l or HbA1c ≥6.5%,	n=82 incident cases 7.6% vs. 22.5%	Age, sex, BMI, family history of diabetes, fasting glucose, HbA1c, alcohol intake, frequency of check-ups, changes of BMI during follow-up	NAFLD was associated with incident diabetes in univariate analysis (OR 2.62, 95% CI 1.6–4.3). This association disappeared after adjusting for potential confounding factors (aOR 1.83, 95% CI 0.9–3.5)	6
Shibata *et al.* 2007 (18)	Retrospective cohort study with a nested case-control analysis n=3,189 (of whom 33.6% with NAFLD) male Japanese workers with normal glucose tolerance, without known chronic liver diseases; 4 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l or 2-h glucose ≥11.1 mmol/l on 75-g OGTT	n=109 incident cases 1.8% vs. 8.1%	Age and BMI (in the whole cohort analysis); age, BMI, smoking history, blood pressure, physical activity, follow-up duration, metabolic syndrome (in the nested case-control analysis)	NAFLD was independently associated with incident diabetes both in the whole cohort (aHR 5.50, 95% CI 3.6–8.5) and in the nested case-control analysis (aHR 4.60, 95% CI 3.0–6.9)	4
Kim *et al.* 2008 (19)	Retrospective cohort study n=5,372 (of whom 33.3% with NAFLD) nondiabetic South Korean subjects without known chronic liver diseases; 5 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, clinical history or drug treatment	n=234 incident cases 2.3% vs. 8.5%	Age, sex, BMI, family history of diabetes, smoking, fasting glucose, HDL-cholesterol, triglycerides, serum ALT	NAFLD was independently associated with incident diabetes (aHR 1.51, 95% CI 1.04–2.2). Moderate/severe NAFLD had higher HRs vs. mild NAFLD. Exclusion of drinkers did not attenuate this association	8

Bae et al. 2011 (20)	Retrospective cohort study n=7,849 (of whom 29.2% with NAFLD) nondiabetic South Korean subjects; 5 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l or HbA1c ≥6.5%, clinical history or drug treatment	n=435 incident cases 3.7% vs. 9.9%	Age, sex, BMI, triglycerides, HDL cholesterol, systolic blood pressure, smoking, physical activity, alcohol intake, IFG status	NAFLD was independently associated with incident diabetes (aHR 1.33, 95% CI 1.1–1.7). This association was much stronger in pre-existing IFG	8
Park et al. 2012 (21)	Prospective cohort study (health check-up) n=25,232 (of whom 35% with NAFLD) nondiabetic South Korean men without known chronic liver diseases; 5 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, HbA1c ≥6.5%, clinical history	n=2,108 incident cases 7% in no-steatosis vs. 9.8% in mild steatosis vs. 17.8% in moderate-severe steatosis	Age, waist circumference, HDL-cholesterol, triglycerides, systolic blood pressure, C-reactive protein, HOMA-IR, serum creatinine, family history of diabetes, physical activity, metabolic syndrome	NAFLD was independently associated with incident diabetes; the HRs (95% CI) were increased in mild steatosis (1.09; 0.8-1.5) and in moderate/severe steatosis (1.73; 1.0-3.0) vs. no-steatosis	8
Sung et al. 2012 (22)	Retrospective cohort study n=12,853 (of whom 27.6% with NAFLD) nondiabetic South Korean subjects; 5 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, clinical history or drug treatment	n=223 incident cases 0.8% vs. 4.3%	Age, sex, BMI, educational status, smoking, physical activity, alcohol intake, HOMA-IR, serum triglycerides, serum ALT	The clustering of increased HOMA-IR, overweight/obesity, and NAFLD markedly increases the odds of developing diabetes, with effects independent of each other and of confounding factors. NAFLD was associated with incident diabetes (aOR 2.42, 95% CI 1.7–3.4)	7
Kasturiratne et al. 2013 (23)	Retrospective cohort study n=2,276 (of whom 40.7% with NAFLD) nondiabetic Sri Lankan individuals without known chronic liver diseases; 3 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, clinical history or drug treatment	n=242 incident cases 10.5% vs. 19.7%	Age, sex, family history of diabetes, BMI, waist circumference, hypertension, serum ALT, dyslipidemia, IFG status	NAFLD was independently associated with incident diabetes (aHR 1.64, 95% CI 1.2-2.2). NAFLD was the only independent predictor of incident diabetes among those with IFG at baseline	5

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Chang et al. 2013 (24)	Retrospective cohort study n=38,291 (of whom 30.4% with NAFLD) nondiabetic South Korean subjects without known chronic liver diseases; 5 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, HbA1c ≥6.5%, or drug treatment	n=2,025 incident cases 3.5% in no-NAFLD *vs.* 7.4% in NAFLD with low fibrosis score (NFS) *vs.* 15.3% in NAFLD with intermediate or high NFS	Age, sex, smoking, alcohol intake, physical activity, family history of diabetes, total cholesterol, triglycerides, HDL-cholesterol, HOMA-IR, C-reactive protein	The aHRs (95% CI) for incident diabetes in NAFLD with low NFS and NAFLD with intermediate or high NFS *vs.* no NAFLD were 2.01 (1.8–2.2) and 4.74 (3.7–6.1), respectively. This association remained significant in subjects with fasting glucose levels <100 mg/dl or with HbA1c <5.8%	8
Choi et al. 2013 (25)	Retrospective cohort study n=7,849 (of whom 29% with NAFLD) nondiabetic South Korean subjects without known chronic liver diseases; 4 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, HbA1c ≥6.5%, or drug treatment	n=435 incident cases 3.5% in controls vs. 4.6% in the increased ALT vs. 7.3% in the steatosis vs. 11.8% in the combined abnormality group	Age, sex, BMI, systolic blood pressure, triglycerides, HDL cholesterol, IFG status, physical activity, smoking, alcohol intake	The HRs (95% CI) of incident diabetes progressively increased across the elevated ALT, the hepatic steatosis, and the combined abnormality groups. Subjects in the combined abnormality group had the highest risk of incident diabetes (aHR of 1.64, 95% CI 1.3-2.1)	8
Yamazaki et al. 2015 (26)	Retrospective cohort study n=3,074 (of whom 23.7% with NAFLD) nondiabetic Japanese subjects without known chronic liver diseases; 11.3 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, HbA1c ≥6.5%, clinical history or drug treatment	n=189 incident cases 3.1% vs. 16.1%	Age, sex, family history of diabetes, BMI, IFG status, dyslipidemia, hypertension, physical activity	NAFLD was independently associated with incident diabetes (aOR 2.37, 95% CI 1.6-3.5). NAFLD improvement was associated with a reduction of incident diabetes (aOR 0.27, 95% CI 0.1-0.6)	8
Ming et al. 2015 (27)	Retrospective cohort study n=508 (of whom 19.1% with NAFLD) nondiabetic Chinese subjects without	Fasting glucose ≥7.0 mmol/l, 2-h glucose ≥11.1 mmol/l on 75-g OGTT or drug treatment	n=20 incident cases 2.4% vs. 10.3%	Age, sex, educational level, smoking, alcohol intake, physical activity, family history of diabetes, BMI, blood pressure, fasting glucose, 2-h glucose,	NAFLD was independently associated with incident diabetes (aHR 4.46, 95% CI 1.9–10.7) but not with incident pre-diabetes (aHR 1.64, 95% CI 0.97-2.8)	6

	known chronic liver diseases; 5 years; liver ultrasonography				triglycerides, HDL cholesterol	
Li *et al.* **2015 (28)**	Retrospective cohort study n=4,736 (of whom 29.8% with NAFLD) nondiabetic Chinese subjects without known chronic liver diseases; 4 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, clinical history or drug treatment	n=380 incident cases 4.1% vs. 17.4%	Age, sex, blood pressure, lipids, serum ALT, uric acid, creatinine	NAFLD was independently associated with incident diabetes (aHR 3.37, 95% CI 2.4–4.3)	7
Shah *et al.* **2015 (29)**	Prospective cohort study n=3,153 US (of whom 24.9% with NAFLD) nondiabetic individuals from the Multi-Ethnic Study of Atherosclerosis without known chronic liver diseases; 9.1 years; liver computed tomography	Fasting glucose ≥7.0 mmol/l, clinical history or drug treatment	n=216 incident cases	Age, sex, race, family history of diabetes, BMI, waist circumference, systolic blood pressure, triglycerides, HDL cholesterol, fasting glucose, C-reactive protein, exercise, statin use	NAFLD (defined as 1st quartile of hepatic attenuation on computed tomography) was independently associated with incident diabetes (aHR 2.06, 95% CI 1.5-2.8, P<0.001)	8
Fukuda *et al.* **2016 (30)**	Retrospective cohort study n=4,629 (of whom 38.4% with NAFLD) nondiabetic Japanese subjects without known chronic liver diseases; 12.8 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, HbA1c ≥6.5%, or drug treatment	n=351 incident cases 3.2% in non-overweight, no-NAFLD vs. 14.4% in non-overweight NAFLD vs. 8.0% in overweight no-NAFLD vs. 26.4% in overweight NAFLD	Age, sex, family history of diabetes, alcohol intake, smoking, regular exercise, HbA1c	aHRs for incident diabetes *vs.* non-overweight without NAFLD group were: 3.59 (95%CI 2.1–5.8) in the non-overweight with NAFLD group, 1.99 (95%CI 1.5–2.7) in the overweight without NAFLD group and 6.77 (95%CI 5.2–8.9) in the overweight with NAFLD group, respectively	7

Chen GY et al. 2016 (31)	Prospective cohort study n=6,542 (of whom 3.2% with NAFLD) nondiabetic Chinese subjects without known chronic liver diseases; 6 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, HbA1c ≥6.5%, or drug treatment	n=368 incident cases	Age, BMI, triglycerides, fasting glucose, IFG status	NAFLD was independently associated with incident diabetes (aHR 2.17, 95% CI 1.6–3.0)	7
Li et al. 2017 (32)	Prospective cohort study n=18,111 (of whom 31.8% with NAFLD) nondiabetic Chinese subjects without known chronic liver diseases; 4.6 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, clinical history or drug treatment	n=1,262 incident cases 4.6% in no-NAFLD vs. 10.6% in mild NAFLD vs. 18.1% in moderate-severe NAFLD	Age, sex, BMI, waist circumference, alcohol intake, smoking, exercise, family history of diabetes, fasting glucose, triglycerides, total cholesterol	aHRs for incident diabetes vs. those without NAFLD group were: 1.88 (95%CI 1.6–2.2) in the mild NAFLD group, 2.34 (95%CI 1.9–3.0) in the moderate-severe NAFLD group, respectively	8
Ma et al. 2017 (33)	Retrospective cohort study n=1,051 (of whom 17.8% with NAFLD) US nondiabetic individuals without known chronic liver diseases; 6.2 years; liver computed tomography	Fasting glucose ≥7.0 mmol/l, clinical history or drug treatment	n=64 incident cases	Age, sex, smoking, exercise, alcohol intake, fasting glucose, systolic blood pressure, BMI, visceral adipose tissue and changes in BMI, visceral adipose tissue and liver fat during follow-up	NAFLD was independently associated with incident diabetes (aOR 2.66, 95% CI 1.2–5.7)	8
Chen SC et al. 2017 (34)	Prospective cohort study n=132,377 (of whom 32% with NAFLD) nondiabetic Taiwanese subjects without known chronic liver diseases; 18 years; liver	Fasting glucose ≥7.0 mmol/l, clinical history or drug treatment	n=6,555 incident cases	Age, sex, BMI, hypertension, family history of diabetes, smoking, alcohol intake, exercise, triglycerides, HDL-cholesterol, total cholesterol, serum AST, ALT, GGT and ALP levels	NAFLD was independently associated with incident diabetes (aHR 2.38, 95% CI 1.6-2.5 for the whole sample; aHR 2.08, 95% CI 1.9–2.2 for men and aHR 2.65, 95% CI 1.4–2.9 for women)	8

	ultrasonography					
Liu *et al.* 2017 (35)	Retrospective cohort study n=18,507 (of whom 18.8% with NAFLD) nondiabetic Chinese elderly men without known chronic liver diseases; 5 years; liver ultrasonography	Fasting glucose ≥7.0 mmol/l, 2-h glucose ≥11.1 mmol/l on 75-g OGTT or clinical history or drug treatment	n=453 incident cases 2.1% vs. 3.7%	Age, BMI, smoking, marital status, alcohol intake, hypertension, dyslipidemia	NAFLD was independently associated with incident diabetes (aHR 1.67, 95% CI 1.4–2.1)	7

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; aHR, adjusted hazard ratio; aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; IFG, impaired fasting glycaemia; OGTT, oral glucose tolerance test.