Fatty liver, cardiometabolic disease and mortality

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Purpose of review
We discuss the findings of the most recent metanalyses on the association between nonalcoholic fatty liver disease (NAFLD), cardiometabolic disease and mortality.

Recent findings
Recent metanalyses have shown that NAFLD is associated with incident type 2 diabetes mellitus (T2DM) and incident cardiovascular disease (CVD). Nonalcoholic steatohepatitis, which can be diagnosed by liver biopsy only in tertiary care centers, is often associated with liver fibrosis, which has been shown by metanalyses to increase both cardiovascular and liver-related mortality. Hyperlipidemia, lipotoxicity and impaired insulin secretion are among the possible mechanisms underlying the association of NAFLD with T2DM and CVD. Metanalyses of the association between NAFLD and mortality in the general population, where risk stratification cannot be performed on the basis of liver biopsy, have given contradictory results.

Summary
To establish conclusively whether NAFLD adds to known prognostic factors of death in the general population will require a shared operational definition of NAFLD, purposefully designed cohort studies, and the use of clinically relevant measures of effect size.

Keywords
cardiovascular disease, fatty liver, mortality, nonalcoholic fatty liver disease, type 2 diabetes mellitus

WHAT IS FATTY LIVER?

The liver metabolizes fatty acids released by adipose tissue during fasting and by chylomicrons after a meal and synthesizes triglycerides that are exported as very low density lipoproteins or stored as lipid droplets. Fatty liver is produced by excessive triglyceride accumulation inside the liver and by reduced VLDL secretion [1] (Fig. 1).

Fatty liver is operationally defined as visible steatosis in more than 5% of hepatocytes at liver biopsy [2▪,3] or as an intrahepatic triglyceride content of at least 5.6% at magnetic resonance spectroscopy (MRS) or MRI [2▪,4]. MRS/MRI quantifies the total amount of triglyceride whereas liver biopsy, which is an 'imperfect gold standard' because of sampling error, reports just the number of hepatocytes with lipid droplets. Liver biopsy can be performed only on selected patients in tertiary care centers [2▪]. Unfortunately, the use of MRI/MRS is currently limited to few research centers because of its cost [2▪]. The method most commonly employed to diagnose fatty liver in both clinical and research practice is, however, liver ultrasonography, which is known to underestimate degrees of fatty liver less than 33% as compared with liver biopsy [2▪]. Another option to diagnose fatty liver suggested by guidelines is the use of surrogate markers, such as the fatty liver index, the SteatoTest and the NAFLD liver fat score [2▪].

The fact that fatty liver can be diagnosed using different methods has important implications for the assessment of the burden of disease associated with it and metanalyses aiming at evaluating such burden have to consider whether it is appropriate or not to 'pool' diagnoses of fatty liver obtained with different methods [5▪▪,6▪].

WHAT IS NONALCOHOLIC FATTY LIVER DISEASE?

Fatty liver is separated into nonalcoholic fatty liver disease (NAFLD) and alcoholic fatty liver disease (AFLD) [2▪]. NAFLD is presently diagnosed when

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KEY POINTS

- Recent metanalyses have shown that nonalcoholic fatty liver disease is associated with incident type 2 diabetes mellitus and incident cardiovascular disease.
- Metanalyses of the association between nonalcoholic fatty liver disease and mortality in the general population, where risk stratification cannot be performed on the basis of liver biopsy, have given contradictory results.
- To establish conclusively whether nonalcoholic fatty liver disease adds to known prognostic factors of death in the general population will require a shared operational definition of nonalcoholic fatty liver disease, purposefully designed cohort studies, and the use of clinically relevant measures of effect size.

ethanol intake is 20 g/day or less in women and 30 g/day or less in men after the exclusion of (at least) hepatitis B infection, hepatitis C infection, and the use of steatogenic drugs [2▪].

The NAFLD vs. AFLD dichotomization may be useful in clinical practice as ethanol is unlikely to be toxic at low quantities but hides the important information that ethanol and obesity interact in determining the burden of liver disease in the general population [2▪]. This is the reason why, in our population studies of fatty liver, we regularly investigate the association of ethanol intake, BMI, and their interaction with fatty liver [7]. Another problem of the NAFLD vs. AFLD dichotomization is that it requires the use of a measuring instrument accurate enough to detect small quantities of ethanol intake. As we discussed in detail elsewhere [8], the measurement error of ethanol intake may be responsible for substantial bias in the separation of NAFLD from AFLD. Also in consideration of this fact, a substantial revision of the traditional NAFLD vs. AFLD dichotomization was recently called for [9].

Most of the current literature on fatty liver, cardiometabolic disease and mortality focuses on NAFLD and this article will review such literature with special attention to metanalyses. When analyzing such literature, one will have to keep in mind the limitations of the NAFLD vs. AFLD dichotomization discussed above. As detected by imaging methods, the prevalence of NAFLD in the general population is 25% and is expected to increase in parallel with the current epidemic of obesity [5▪▪]. Although NAFLD is especially prevalent among obese individuals and is strongly associated with visceral obesity, it can be detected also among

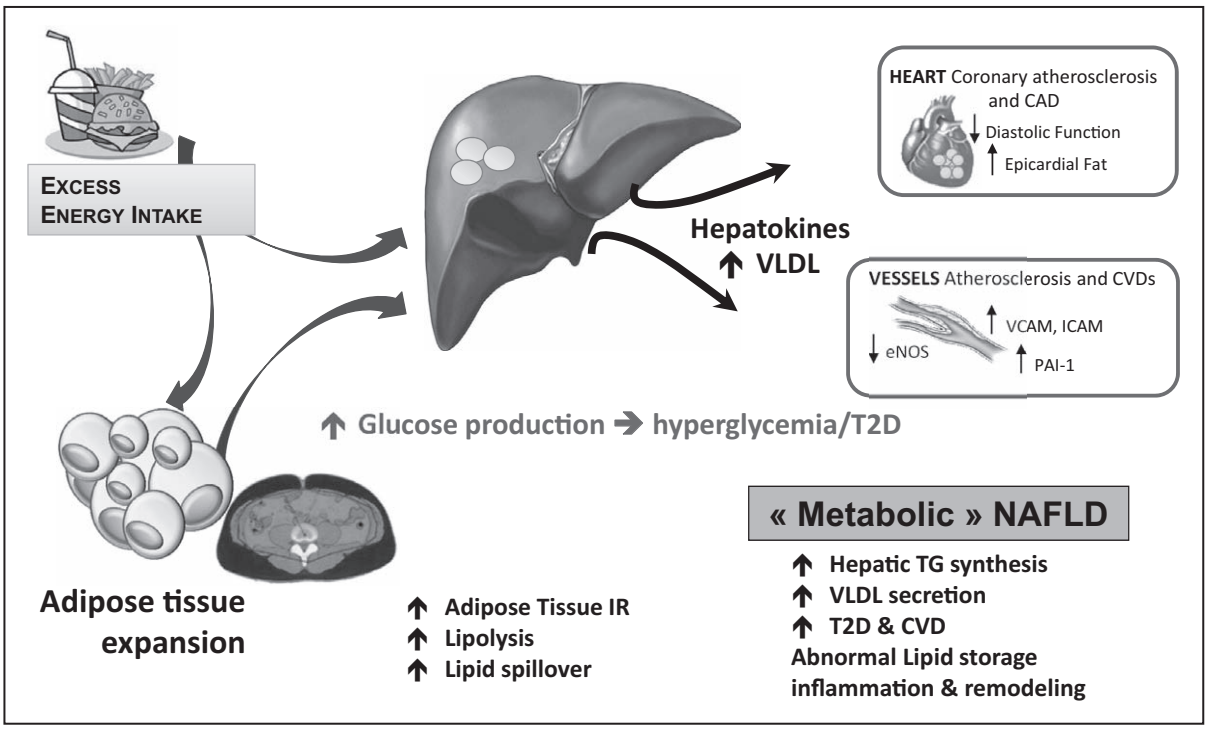

FIGURE 1. An overview of the mechanisms linking fatty liver disease, cardiovascular disease and type 2 diabetes mellitus. CAD, coronary artery disease; CVD, cardiovascular disease; eNOS, endothelial nitric oxide synthase; ICAM, intercellular adhesion molecule; IR, insulin resistance; NAFLD, nonalcoholic fatty liver disease; PAI-1, plasminogen activator inhibitor-1; T2D, type 2 diabetes mellitus; TG, triglycerides; VCAM, vascular cell adhesion molecule.

nonobese individuals and has both genetic and environmental causes. The distinction between 'genetic' and 'metabolic' NAFLD is an useful one provided that one remembers that in most cases the genes will interact with the environment [1,10].

WHAT IS NONALCOHOLIC STEATOHEPATITIS?

Nonalcoholic steatohepatitis (NASH) is a condition of liver inflammation caused by a buildup of fat in the liver. NASH is diagnosed by liver biopsy as the presence of steatosis, inflammation and ballooning as opposed to nonalcoholic fatty liver (NAFL), that is, steatosis with or without ballooning or inflammation [2▪,3].

As liver biopsy cannot distinguish alcoholic steatohepatitis (ASH) from NASH, the NASH vs. ASH dichotomization shares the same dependence of the NAFLD vs. ALFD dichotomization from the measurement of daily ethanol intake [8,9]. Moreover, the decision to perform a liver biopsy in a patient with NAFLD is often taken in the presence of altered aminotransferase levels. However, even patients with normal aminotransferase levels are known to develop clinically relevant histological phenotypes [11]. In patients with NAFLD as detected by liver biopsy, the severity of liver fibrosis is associated with reduced insulin sensitivity and with impaired glucose tolerance independently from obesity [12].

The clinical relevance of NASH stems mostly from its association with liver fibrosis, which is diagnosed separately from NASH [2▪,13▪]. Contrarily to NASH, NAFL carries a generally benign prognosis [2▪]. The fact that NASH must be assessed using liver biopsy implies that it cannot be diagnosed outside tertiary care centers. The available estimates of the prevalence of NASH in the general population obtained by extrapolation from tertiary care centers or from unreliable markers, such as altered liver enzymes should be, therefore, taken with caution [5▪▪,8,14]. Moreover, the case-mix of individuals is very different in the general population and in tertiary care centers so that the umbrella term 'NAFLD' will introduce substantial bias when data from the general population and tertiary care centers are 'pooled' for metanalysis [15▪▪].

Although liver biopsy is the reference method for the diagnosis of liver fibrosis, indirect and less invasive methods are available to detect it, such as transient elastography (Fibroscan), magnetic resonance elastography and acoustic radiation force impulse elastography [16]. Moreover, new MRI-based techniques are undergoing validation [17] and a panel of surrogate markers is also available [16].

WHAT IS THE RELATIONSHIP BETWEEN FATTY LIVER AND CARDIOMETABOLIC DISEASE?

Individuals with most or all the features of the metabolic syndrome and those with insulin resistance are more likely to have NAFLD both in the general population and in tertiary care centers [1,7]. Thus, it is not surprising that NAFLD is associated with incident type 2 diabetes mellitus (T2DM) and CVD [18,19].

Whether NAFLD 'causes' T2DM and CVD is, however, difficult to ascertain as they share common risk factors [15▪▪] and because of the intrinsic limitations of observational epidemiology [20]. However, some findings of current research may be central to the understanding of this relationship (Fig. 1). Insulin resistance, not only in the muscle and the liver but also in the adipose tissue, and decreased insulin secretion are known predictors of incident T2DM [1] and both are present in 'metabolic' NAFLD [12]. Insulin resistance is less common among the 'genetic' variants of NAFLD (mutations of *PNPLA3*, *DGAT* and *TM6SF2* genes and familiar hyperlipoproteinemia) [21]. It is of great interest that individuals with the TM6SF2 mutation, appear to have a decreased risk of CVD despite NAFLD [22]. NAFLD is also associated with endothelial dysfunction and an increased atherogenic profile due mostly to increased VLDL secretion (Fig. 1). A proinflammatory profile is also common in NAFLD, and recently discovered hepatokines are being investigated as possible mechanisms linking NAFLD and cardiometabolic diseases [23].

WHAT IS THE RELATIONSHIP BETWEEN NONALCOHOLIC FATTY LIVER DISEASE AND MORTALITY?

As we stated above, testing whether NAFLD per se is a cause of cardiometabolic disease and, more importantly, death in the general population, is a very difficult task in view of the intrinsic limitations of observational epidemiology [20]. Instead of asking 'Does NAFLD cause death?' one may instead ask the humbler but no less clinically important question 'Does NAFLD add to already known prognostic risk factors of death?'. The verb 'add' is the key here, as the ability of NAFLD to 'prognosticate' death should be evaluated in addition to that afforded by the available tools [15▪▪]. The presently available evidence base does not allow to satisfactorily answer this question [15▪▪]. A shared operational definition of NAFLD, purposefully designed cohort studies, and the use of clinically relevant measures of effect size are needed to answer this question.

With this important methodological premise, we will now comment the most recent metanalysis

on NAFLD and mortality [6▪] to offer a picture of the many methodological problems associated with the task of evaluating whether NAFLD is a prognostic factor of mortality. This should not to be taken as a critique of this metanalysis, other metanalyses, or primary studies but as a way to (hopefully) stimulate the production of data able to answer the question 'Does NAFLD add to already known prognostic risk factors of death?' [15▪▪].

Liu *et al.* [6▪] introduce their metanalysis by pointing out that the available studies on the association between NAFLD and mortality have given conflicting results and that two recent metanalyses [5▪▪,24] have concluded that NAFLD is not associated to all-cause mortality. In detail, one metanalysis [5▪▪] concluded that NAFLD was associated with increased liver-related mortality and similar all-cause mortality, whereas the other metanalysis [24] concluded that NAFLD was associated neither to all-cause nor to cardiovascular mortality. Liu *et al.* [6▪] then proceed to criticize the fact that the two metanalyses [5▪▪,24] pooled data from multiple analyses of the NHANES III study and did not consider all the available studies. Part of the difference in the findings of these metanalyses may of course depend from different inclusion criteria for primary studies.

Comparing individuals with NAFLD to individuals without NAFLD, Liu *et al.* [6▪] reported hazard ratios of 1.34 (95% confidence interval (CI) 1.17–1.54, $N = 12$ studies) for death, 1.13 (0.92–1.38, $N = 7$ studies) for cardiovascular death, 1.05 (0.89–1.25, $N = 5$ studies) for cancer-related death and 2.53 (1.23–5.18, $N = 5$ studies) for liver-related death. On the basis of these findings, Liu *et al.* [6▪] concluded that 'NAFLD [is] associated with an increased risk of all-cause mortality but not cardiovascular disease and cancer mortality'.

More important than this conclusion is how Liu *et al.* [6▪] exemplarily report the limitations of their metanalysis and address the need of further studies, which takes us at the core problems of the current evidence base [15▪▪]. Liu *et al.* write in fact that 'two key questions concerning the association of NAFLD with mortality remain unsolved in the present study' [6▪]. The first question is whether the 'adverse effect of NAFLD on mortality is restricted to patients with NASH or can extend into those with simple hepatic steatosis' [6▪]. Liu *et al.* hypothesize in fact that the 'excess mortality' might be because of liver-related death and quote a subgroup analysis of all-cause mortality where the hazard ratio is 0.96 (0.83–1.12, $N = 3$ studies) in individuals with 'simple hepatic steatosis' vs. 1.37 (0.77–2.43, $N = 3$ studies) in those with 'nonalcoholic steatohepatitis'. As Liu *et al.* [6▪] point out, this hypothesis needs to be tested by further studies, if only because of the use of the umbrella term NAFLD to include studies performed in very different settings varying from the general population to tertiary care centers ($N = 11$ studies). The second question 'remaining unsolved' is 'whether the direction and magnitude of the association between NAFLD and mortality can be modified by sex' as Liu *et al.* estimated a hazard ratio of all-cause death of 1.49 (95% CI 1.15–1.93, $N = 4$ studies) in women vs. one of 1.08 (0.83–1.41, $N = 4$ studies) in men (with the NAFLD × sex interaction reported as not statistically significant.) As Liu *et al.* [6▪] point out, this finding is 'challenging' and needs to be confirmed by further studies. Although similar conclusions about sex were recently reached by a Korean study [25], confounding from age and other factors could not be properly ruled out [26,27]. Liu *et al.* [6▪] close their article by writing: 'Future studies should determine whether the observed association between NAFLD and mortality is limited to patients with NASH or can extend into those with simple hepatic steatosis'. Thus, we are again confronted with the core problem that we cannot separate patients with 'complicated' (ideally 'NASH') from those with 'uncomplicated' NAFLD (ideally 'NAFL'), who are arguably most of the patients with NAFLD in the general population [15▪▪].

WHY IS THE MEASURE OF EFFECT SIZE IMPORTANT?

To close this review, we wish to add a short technical comment on the use of the hazard ratio as measure of effect size [28,29]. First, the hazard ratio is a relative measure of effect size, meaning that, without the underlying survival curves (or other descriptors), one cannot gauge any information about absolute risks. Second, the hazard ratio is a relative rate and not a relative risk and whereas its direction can be used to explain the direction of the relative risk, the same is not true for its magnitude. That is to say, the hazard ratio and the relative risk are not synonyms. Third, and most importantly, studies 'adjusting for different covariates' will generally produce different estimates of hazard ratio even if the underlying causal effect is the same, and this will introduce bias when such 'adjusted HR' are combined in a metanalysis.

CONCLUSION

NAFLD is associated with incident cardiometabolic disease. A shared operational definition of NAFLD, purposefully designed cohort studies and the use of clinically relevant measures of effect size are needed to answer the important question whether NALFD adds to already known prognostic factors of death.

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Conflicts of interest

There are no conflicts of interest.

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- ■ of special interest
- ■■ of outstanding interest

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