

Causality between non–alcoholic fatty liver disease and risk of cardiovascular disease and type 2 diabetes:

A meta–analysis with bias analysis

Amy E Morrison* MBChB ¹

Francesco Zaccardi* PhD ^{1 2}

Kamlesh Khunti PhD ^{1 2}

Melanie J Davies MD ¹

¹ Leicester Diabetes Centre, Leicester General Hospital, Gwendolen Rd, Leicester LE5 4PW, United Kingdom

² Leicester Real World Evidence Unit, Leicester General Hospital, Gwendolen Rd, Leicester LE5 4PW, United Kingdom

* Equally contributed

Corresponding Author

Dr Francesco Zaccardi, PhD
Leicester Diabetes Centre, Leicester General Hospital
Gwendolen Rd, Leicester LE5 4PW, United Kingdom
Email: frazac@fastwebnet.it
Phone: 0044 116 2584322

Word count

Title 123 characters

Title page 100; Abstract 246; Lay summary 96; Manuscript [3841](#)

References: 48

Tables: 1; Figures: 3

Appendix: 5 Tables; [5 Figures](#)

MOOSE checklist

Abbreviations: **CVD**: Cardiovascular disease; **NAFLD**: Non–alcoholic fatty liver disease; **NASH**: Non–alcoholic steatohepatitis; **RCT**: Randomised Controlled Trial; **RR**: Relative Risk; **T2DM**: Type 2 diabetes mellitus

ACKNOWLEDGEMENTS

National Institute for Health Research (NIHR) Leicester Biomedical Research Centre, Leicester, UK to MJD; NIHR Collaboration for Leadership in Applied Health Research and Care—East Midlands to KK; FZ is a clinical research fellow funded with an unrestricted educational grant from the National Institute for Health Research Collaboration for Leadership in Applied Health Research and Care (CLAHRC) East Midlands to the University of Leicester. The funding sources had no role in study design, data collection, data analysis, data interpretation or writing of the report. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

CONFLICTS OF INTEREST

KK: speaker for Novartis, Novo Nordisk, Sanofi–Aventis, Lilly, Servier and Merck Sharp & Dohme. Grants in support of investigator and investigator initiated trials from Novartis, Novo Nordisk, Sanofi–Aventis, Lilly, Pfizer, Boehringer Ingelheim and Merck Sharp & Dohme. Funds for research, honoraria for speaking at meetings and advisory boards for Lilly, Sanofi–Aventis, Merck Sharp & Dohme and Novo Nordisk. MJD: Consultant, advisory board member and speaker for Novo Nordisk, Sanofi–Aventis, Lilly, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca and Janssen; speaker for Mitsubishi Tanabe Pharma Corporation. Grants in support of investigator and investigator initiated trials from Novo Nordisk, Sanofi–Aventis and Lilly.

ABSTRACT

Background & Aims: A causal association of non-alcoholic fatty liver disease (NAFLD) with cardiovascular disease (CVD) and type 2 diabetes (T2DM) remains unproved. We aimed to quantify the likelihood of causality examining the sensitivity of observational associations to possible confounding.

Methods: Studies investigating longitudinal associations of NAFLD with CVD or T2DM were searched on June 5th, 2018. Study-specific relative risks (RRs) were combined in random-effects meta-analyses and pooled estimates used in bias analyses.

Results: Associations of NAFLD with CVD and T2DM were reported in 13 (258743/18383 participants/events) and 20 (240251/12891) studies, respectively. Comparing patients with NAFLD to those without, the pooled RR was 1.48 (95% CI: 0.96, 2.29) for CVD and 2.17 (1.77, 2.65) for T2DM. In bias analyses, for an unmeasured confounder associated to both NAFLD and CVD with a RR of 1.25, the proportion of studies with a true (causal) effect of NAFLD on CVD surpassing a RR of 1.10 (i.e., 10% increased risk of CVD in participants with NAFLD) was 0.67 (95% CI: 0.42, 0.92) while for 75% increase it was 0.36 (0.11, 0.62). Corresponding figures for T2DM were 0.97 (0.91, 1.00) for a 10% increased risk of T2DM in participants with NAFLD to 0.70 (0.49, 0.92) for a 75% increase.

Conclusions: The results of this study are strongly suggestive for a causal relationship between NAFLD and T2DM, while the evidence for a causal link between NAFLD and CVD is less robust. Therapeutic strategies targeting NAFLD are likely to reduce the risk of developing T2DM.

Word count abstract: 246

Keywords: causality; non–alcoholic fatty liver disease; diabetes; cardiovascular disease; meta–analysis; bias analysis

LAY SUMMARY

- Non-alcoholic fatty liver disease has been consistently associated with an increased risk of incident cardiovascular disease and type 2 diabetes. It remains unclear, however, whether these associations are causal.
- Combining the available evidence in a meta-analysis and bias analysis, the results of this study are strongly indicative for a causal relationship between non-alcoholic fatty liver and type 2 diabetes, while less clear is the evidence for a causal link with cardiovascular disease.
- It is likely that interventions improving non-alcoholic fatty liver disease would also result in a reduced risk of future type 2 diabetes.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as hepatic fat accumulation (steatosis) in more than 5% of hepatocytes on histological examination, following exclusion of other causes of chronic liver disease including hepatitis B and C, autoimmune hepatitis, hepatotoxic drugs and excessive alcohol consumption.¹ The prevalence of NAFLD is rising worldwide, with it rapidly becoming the most common cause of chronic liver disease currently affecting 25% of population worldwide.²⁻⁴ Up to one fifth of patients affected with NAFLD will develop non-alcoholic steatohepatitis (NASH), with evidence of hepatocyte injury (ballooning degeneration); NASH could further progress to liver fibrosis, cirrhosis and its complications including hepatocellular carcinoma.⁵

In prospective longitudinal studies, the presence of NAFLD has been associated with an increased risk of cardiovascular disease (CVD) and type 2 diabetes (T2DM);^{6,7} however, it remains unclear whether NAFLD is causally related to these conditions or, rather, it is just an epiphenomenon of other cardiometabolic factors causally related to CVD and T2DM.⁸⁻¹⁰ In fact, the rising prevalence of NAFLD concurs with that of overweight and obesity (particularly visceral fat).^{11,12} Moreover, as excess body weight is also a risk factor for CVD and T2DM,^{13,14} it has been argued that the associations of NAFLD with CVD and T2DM could be spurious (i.e., non-causal) and reflect the link between overweight/obesity with CVD and T2DM.^{8,9} To account for such possible confounding, previous epidemiological studies have adjusted associations for several potential confounders, including body mass index;^{6,7} however, residual confounding resulting from unmeasured factors is possible.

Recently, bias analysis (also known as sensitivity analysis) for single observational studies and meta-analysis has been proposed as a methodology to infer causal associations from nonrandomised, observational data.¹⁵⁻¹⁷ By quantifying the sensitivity of causal conclusions with respect to unmeasured confounding, this methodology allows assessing the strength of a

causal evidence between the exposure and the outcome of interest using pooled estimates obtained from meta-analysis of observational studies.¹⁷

In this view, we conducted a systematic review, meta-analysis and bias analysis to help elucidate the nature of the association of NAFLD with CVD and T2DM by assessing the likelihood of a causal relationship.

MATERIALS AND METHODS

This systematic review and meta-analysis was registered with PROSPERO International Prospective Register of Systematic Reviews (<https://www.crd.york.ac.uk/prospero/>; no. CRD42018086968, registration date February 8th, 2018) and followed standard guidelines for conducting and reporting systematic reviews (MOOSE checklist reported in the appendix).

Data sources and searches

Relevant articles published in English were systematically sought using the databases PubMed, Web of Science and Scopus by two independent investigators from database inception to June 5th, 2018. The search strategy combined keywords related to the exposure (i.e., “non-alcoholic steato-hepatitis” or “NASH”), outcome (i.e., “cardiovascular disease” or “cardiovascular mortality” or “type 2 diabetes”) and study design (i.e., “prospective” or “cohort”). Cardiovascular events and type 2 diabetes have been selected as outcomes given their wider observational literature compared to other outcomes. Details of the search strategy are reported in the appendix Figure S1. Reference lists of retrieved articles (including previous systematic reviews and meta-analyses) were manually scanned to identify any relevant additional study.

Study selection

Following the PICOS (*population, intervention/exposure, comparator, outcome, study design*) framework, we included prospective or retrospective longitudinal observational studies (*study design*) reporting on the associations between the presence of baseline NAFLD (*exposure* and *comparator*) and incident cardiovascular or type 2 diabetes events (*outcome*) in adult patients (>18 years old) (*population*). We did not limit the inclusion of studies according to a specific definition of outcomes or exposures; however, studies were excluded if there was no clear definition of NAFLD or the diagnosis was based only on abnormal liver function test results

without the exclusion of other possible causes. If studies reported associations in overlapping cohort of participants, we selected those reporting the larger number of events (appendix Table S1).

Data extraction and quality assessment

Using standardised, pre-defined forms, data extraction was performed independently by two authors on: first author, year of publication, geographical area (country and continent), total number of participants and outcomes, characteristics of participants (age, gender), exposure and outcome definition and assessment, risk comparison and measurement, and baseline covariates included in the statistical adjustment; study quality was assessed by two authors using the nine-star Newcastle–Ottawa Scale.¹⁸ Disagreement in study selection, data extraction, or quality assessment was solved by consensus or arbitration.

Data synthesis and analysis

All analyses were performed with Stata/IC (version 15) and R (version 3.4.3); two-sided P-value <0.05 was considered statistically significant.

Relative risk (RR) with 95% confidence interval (CI) was used as a measure of association between NAFLD and the outcomes, assuming that hazard ratios, risk ratios, and odds ratio approximate the same measurement of RR across studies included in the meta-analysis following Cornfield's rare disease assumption.¹⁹ Study-specific standard errors were calculated from CIs; RRs were pooled using a restricted maximum-likelihood random-effects model with test statistics and confidence intervals obtained with the Knapp and Hartung method.²⁰ Statistical heterogeneity across studies was quantified using I^2 and τ^2 statistics and random-effects meta-regression was used to investigate possible sources of heterogeneity;²¹ publication bias was examined with funnel plots and Egger's test and bias-corrected estimates were obtained following the methods proposed by Vevea and Hedges.^{22,23} If studies published

more than one adjusted RR, the most adjusted estimate was used. A within–study fixed effect meta–analysis was performed to obtain an overall estimate when RRs were separately reported for men and women.

Bias analyses were performed using the “[ConfoundedMeta](#)” R package and followed the methodology proposed by Mathur and VanderWeele.^{17,24} This analysis allows to quantify the proportion of observational studies with true effects (i.e., causal association) as a function of different strength of the confounding factor; that is, the relative risk of a confounding factor (for example, body mass index in the relationship between NAFLD and type 2 diabetes) that would be capable of “explaining away” the results of the meta–analysis by reducing the proportion of strong causal effects. If the results of a sensitivity analysis indicate that the proportion of studies with effects larger than a meaningful scientific threshold (i.e., 10% increase or relative risk 1.10 of developing type 2 diabetes in the presence of NAFLD compared to non–NAFLD) is small (i.e., 0.05 or 5%), then it is possible to conclude that under the specified strength of unmeasured confounding it could be the case that only 5% of the studies have true effects stronger than a relative risk of 1.10. For both cardiovascular events and type 2 diabetes outcomes, we estimated the proportion of studies with true causal effects for a range of relative risk thresholds: we assumed an increased risk in presence of NAFLD for both outcomes ranging from 5% to two times higher (i.e., RR from 1.05 to 2.00) and a strength of the confounding factor for a range of RR from 1.0 to 9.0.

RESULTS

Characteristics of included studies

Of 7116 potentially relevant articles, the full-text of 74 was evaluated after exclusion of duplicates and screening of title and abstract; following detailed assessments, 33 articles (13 for cardiovascular events and 20 for type 2 diabetes; references are reported in the appendix) were selected for the meta-analyses with 11 studies excluded as they reported estimates for overlapping cohorts (Figure S1 and Table S1). The main characteristics of the included studies are shown in Table 1. For cardiovascular events, the number of participants ranged from 375 to 132661 (50.4% men), and number of events ranged from 11 to 10897. The mean/median age ranged between 43.9 and 64.7 years old, with years of follow up between 3.0 and 26.4; the majority of the studies (5, 38.5%) were conducted in the USA. For T2DM, the majority of the studies were carried out in Asia (16, 80.0%) and corresponding figures were: 141 to 136377 participants (57.8% men); 20 to 6555 events; 35.3 to 71.4 years old; and 2.2 to 18.0 years of follow-up. All studies were published after 2003.

NAFLD was defined and assessed differently across included studies, although the large majority used abdominal ultrasonography (Table S2); the diagnosis of cardiovascular events was mainly ascertained through medical records (including death certificates) using the WHO International Classification of Diseases while biochemical criteria or diabetes medications were used in virtually all studies to identify incident diabetes cases (Table S3). Lastly, the level of adjustment for the analyses included key covariates such as age, gender, and indices of adiposity (mainly body mass index or waist circumference) in most of the studies (Table S4), resulting in a quality assessment score ≥ 8 in 25 studies (75.8%; Table S5).

Meta-analysis and bias analysis

NAFLD and cardiovascular disease

Thirteen studies, including 258743 participants and 18383 incident events, reported on the association between NAFLD and both fatal and non–fatal cardiovascular outcomes (Figure 1); study–specific incident rates ranged from 3 to 169 cardiovascular events per 1000 person–years in participants with NAFLD and from 1 to 77 in participants without (Figure S2). The pooled RR of cardiovascular events combining maximally–adjusted estimates was 1.48 (95% CI: 0.96, 2.29), with significant statistical heterogeneity ($I^2=89.9\%$, 95% CI: 80.6, 97.6; $\tau^2=0.35$, 95% CI: 0.16, 1.61; $p<0.001$) which was not explained by differences in geographical region, calendar year, baseline age of included participants, duration of follow–up, number of events, or level of adjustment (Figure S3). There was no evidence of publication bias ($p=0.775$; Figure S4); based on the Vevea and Hedges publication bias model, which accounts for a publication process that selects preferentially for studies with $p<0.05$, the corrected point estimate was 1.29 (95% CI: 0.75, 2.23).

In the bias analysis, for a RR of an unmeasured confounder of 1.5, the proportion of studies with a true (causal) effect of NAFLD on cardiovascular events surpassing a RR of 1.1 (i.e., a 10% increased risk of CVD in participants with NAFLD) was 0.62 (95% CI: 0.36, 0.87); corresponding proportions were 0.56 (95% CI: 0.30, 0.82) for an increased risk of 20%; 0.41 (95% CI: 0.15, 0.67) for 50%; and 0.32 (0.07, 0.56) for 75% (Figure 2 and 3). Given the large set of covariates adjusted for in the studies, in the more likely situation of a RR for an unmeasured confounder of 1.25 instead of 1.5, corresponding figures were: 0.67 (95% CI: 0.42, 0.92) for a 10% increased risk; 0.61 (95% CI: 0.36, 0.87) for 20%; 0.47 (95% CI: 0.20, 0.73) for 50%; and 0.36 (95% CI: 0.11, 0.62) for 75% (Figure 2 and 3). Results were consistent assuming heterogeneous bias across studies (Figure S5).

NAFLD and type 2 diabetes mellitus

Twenty studies, including 240251 participants and 12891 incident events, reported on the association between NAFLD and incident cases of type 2 diabetes (Figure 1); study–specific

incident rates ranged from 5 to 114 diabetes cases per 1000 person-years in participants with NAFLD and from 2 to 69 in participants without (Figure S2). The random-effects meta-analysis combining maximally-adjusted RR of type 2 diabetes was 2.17 (95% CI: 1.77, 2.65), with significant statistical heterogeneity across the estimates ($I^2=86.6\%$; 95% CI: 76.7, 96.3; $\tau^2=0.11$; 95% CI: 0.06, 0.43; $p<0.001$) which was not explained in meta-regressions by geographical differences, calendar year, baseline age of participants, follow-up duration, number of events, or level of adjustment (Figure S3). There was no evidence of publication bias ($p=0.704$; Figure S4); based on the Vevea and Hedges model, the corrected point estimate was 1.40 (95% CI: 0.48, 4.11), accounting for preferential publication for studies with $p<0.05$. The bias analysis showed, for a RR of an unmeasured confounder of 1.5, the proportion of studies with a true (causal) effect of NAFLD on incident diabetes surpassing a RR of 1.1 (10% increased risk of diabetes in participants with NAFLD) was 0.96 (95% CI: 0.87, 1.00); corresponding proportions were 0.92 (95% CI: 0.80, 1.00) for 20%; 0.78 (95% CI: 0.58, 0.97) for 50%; and 0.61 (0.39, 0.84) for 75% (Figure 2 and 3). Similarly to cardiovascular events, given the large set of covariates adjusted for, a more likely RR for an unmeasured confounder would be 1.25, for which the corresponding figures were: 0.97 (95% CI: 0.91, 1.00) for an increased risk of diabetes of 10% in patients with NAFLD; 0.95 (95% CI: 0.86, 1.00) for 20%; 0.84 (95% CI: 0.67, 1.00) for 50%; and 0.70 (0.49, 0.92) for 75% (Figure 2 and 3). The strength of causal evidence was stronger with heterogeneous bias (Figure S5).

DISCUSSION

The results of this meta-analysis, including more than 240,000 participants reporting around 19,000 incident CVD events and 13,000 incident T2DM cases from 33 longitudinal observational studies, indicated that subjects with NAFLD, compared to those without, have an increased risk of developing T2DM; conversely, there is some evidence but uncertainty of an increased risk of CVD. As observational associations could be biased by confounding factors, most estimates were adjusted in individual studies for several potential confounders. However, due to the possibility of residual confounding by unmeasured factors, we performed a bias analysis to quantify the extent to which associations across studies were robust to unmeasured confounding. We showed that the associations between NAFLD and T2DM were robust to confounding: assuming a potential 75% increased risk of T2DM in patients with NAFLD vs those without, the proportion of studies reporting a true, causal association was 0.70 (i.e. 70%) with a strength of unmeasured confounder of 1.25, which is strongly suggestive of a causal link between NAFLD and T2DM.

Randomised control trials (RCTs) are considered the highest grade of evidence to assess causality.^{25,26} Strict inclusion and exclusion criteria, however, narrow the enrolment to subjects who might not be truly representative of “real-world” patients, limiting the external validity of RCTs findings.²⁷ In recent years, there has been an extensive theoretical and applied research to develop methods allowing to infer causality from observational data.²⁶ Among them, the instrumental variable approach which used genetic variants as instrument (also known as “Mendelian randomisation”) has gained extensive popularity.²⁸ Mendelian randomisation is conceptually similar a RCT where genes are randomly “assigned” to subjects. Like other statistical analysis, Mendelian randomisation relies on important assumptions. Instrumental variables are variables associated with the exposure but with no causal pathway from the instrumental variable to the outcome, other than via the exposure; an important violation is

therefore the situation when genetic variants are associated with multiple, distinct pathways converging to the same outcome (horizontal pleiotropy).^{29,30} In our investigation, this would translate in the assumption that a single gene or a combination of genes affect the risk of CVD or T2DM only through an increased risk of NAFLD. Although analytical methods are available to account for horizontal pleiotropy (based on further assumptions),²⁹ its assessment could be difficult, particularly for a single genetic variant and for complex phenotypical traits such as CVD or T2DM, and a detailed knowledge of the multiple function of the gene(s) is required. An alternative methodology to the Mendelian randomisation is the bias (or sensitivity) analysis: this technique allows quantification of the robustness to confounding of an association for a single observational study or for a meta-analysis of observational studies.¹⁵⁻¹⁷ In a meta-analytical setting, the analysis quantifies the proportion of studies which report a causal association between the exposure and the outcome, even without full control of the exposure-outcome association. The proportions can be estimated over a range of meaningful increases (or reductions) of the risk in subjects exposed compared to non-exposed and, at the same time, over a range of sensitivity values for an unmeasured confounding.^{15,17} In our analysis, we defined a meaningful increase of CVD and T2DM risk in subjects with NAFLD as a RR ranging from 1.05 to 2.00; for the unmeasured confounder, we deemed very likely that it would be associated with both NAFLD and the outcome with a RR of between 1.25 and 3.5, as most of the studies already accounted for several confounders. However, the proportion of true associations are shown in Figure 2 and 3 for multiple values of meaningful increased risk and confounding factor, giving the reader the possibility to evaluate proportions for different combinations of RRs.

Previous systematic reviews and meta-analysis investigating the association between NAFLD and CVD have shown an increased risk in participants with NAFLD compared to those without;^{7,31-33} these findings are quantitatively, but not qualitatively, different from our results, given the large overlap between the confidence interval of this meta-analysis and those

reported in previous ones. However, our analysis included more recent studies, excluded studies with a cross-sectional or case-control design, and used the Knapp and Hartung method which is considered an improved tests for random effects meta-analyses.³⁴ Similarly to CVD, two previous meta-analyses have shown an increased risk of T2DM in participants with NAFLD,^{6,35} in line with our findings. In remarkable contrast with the substantial availability of individual observational analyses and meta-analyses of observational studies, we identified only one investigation which aimed to assess the potential causality between NAFLD and CVD and none between NAFLD and T2DM;³⁶ from this perspective, our study is the first to explore the nature of the relationship between these NAFLD and T2DM conditions. Interestingly, in the only available study (a Mendelian randomisation) exploring causality between NAFLD and CVD,³⁶ the Authors reported an association between NAFLD and CVD which has not been confirmed when using genetic variants as instrument for NAFLD: these findings are in line with our bias analysis and suggest that relatively weak unmeasured confounding could have spuriously produced the association between NAFLD and CVD.

NAFLD increases the risk of T2DM via multiple biological mechanisms, with a pivotal role played by insulin resistance which is key pathophysiologic defects in T2DM.³⁷ An accumulation of free-fatty acids in the hepatocytes impairs the tyrosine phosphorylation of insulin receptor substrate 2 and increases, in parallel, the activity of protein kinase C; both modifications result in impaired hepatocyte insulin signaling and hepatic insulin resistance.⁹ Moreover, pathways involving proinflammatory cytokines, such as interleukin (IL)-6, IL-1 β , IL-18, and tumour necrosis factor- α , are over-activated in NAFLD and also contribute to insulin resistance.³⁸ Although there are no investigations of a causal link between NAFLD and T2DM, a recent Mendelian randomisation has explored the relationship between NAFLD and insulin resistance.³⁷ The Authors found that genetically-determined hepatic fat accumulation is associated to insulin resistance (quantified with HOMA-IR index) in two independent cohorts;³⁹ interestingly, this association was dependent on the hepatocyte damage, suggesting

a pathway from hepatic fat thesaurismosis to hepatocellular damage, inflammation and fibrosis, and insulin resistance. From this perspective, these findings confirm clinical observations about the relevance of liver injury in the pathophysiology of glucose dysregulation⁴⁰ and complement our results, corroborating the hypothesis of a causal link between NAFLD and T2DM.

The less robust evidence of a causal relationship between NAFLD and CVD, compared to T2DM, could suggest that the constellation of the metabolic disturbances related to insulin resistance are more strongly related to T2DM than CVD, while other factors, such as smoking and cardiorespiratory fitness, might be more relevant than insulin resistance in determining CVD risk.^{41,42} In line with this hypothesis, the relationship between body mass index (which is a good epidemiological surrogate of insulin resistance) and risk of CVD death is non-linear, both in subjects with and without T2DM.^{43,44} Moreover, dietary interventions aiming at reducing body weight have been recently shown to induce remission of T2DM while so far there is no clear experimental evidence of a reduced risk of CVD following weight loss interventions.^{45,46}

To our knowledge, this is the first study examining the nature of the relationship between NAFLD and two of its most important associated complications, CVD and T2DM, using a bias analysis. We extensively searched for studies in multiple databases and included only those reporting longitudinal associations, to avoid biases related to cross-sectional and case-control design (i.e., reverse causation). Moreover, estimates from overlapping populations were identified to avoid the inclusion multiple times of associations obtained from the same cohort, as this results in biased pooled estimate.⁶ However, we searched only for published reports and did not extend our research to conferences abstracts or articles published not in English, yet we did not find evidence of publication bias. Furthermore, we explored in meta-regression differences in the associations across study-level characteristics, which could be different compared to those at individual level (i.e., “ecological bias”).²¹ Lastly, for both CVD and T2DM, there was significant statistical heterogeneity across studies’ estimates which was not

explained by geographical region, calendar year, age of participants, study duration, number of events, or level of adjustment. These findings would therefore suggest that other factors, not captured in meta-regressions, could have contributed to the statistical heterogeneity, such as differences in outcome definition and assessment (particularly for CVD), ethnicity, NAFLD severity and dietary or medical interventions. On the other hand, heterogeneity is accounted for in bias analysis.

As there are currently no specific pharmacological interventions for NAFLD, its treatment relies on the optimisation of the associated metabolic risk factors.⁴⁷ There is, however, an extensive research on possible pharmacological candidates, with more than 30 potential therapies under investigation and 200 ongoing RCTs.⁴⁸ The results of these RCTs will represent an important step from both a biomedical research perspective, to understand the nature of the association between NAFLD and CVD or T2DM, and from a patient and public health perspective, given the worldwide epidemic of NAFLD. Our findings would suggest that future pharmacological treatments able to reduce the risk of NAFLD or to limit its progression would also likely reduce the risk of developing T2DM.

Author contributions: FZ conceived the study question and design and undertook the statistical analysis. AM and FZ did literature search and extracted data. AM drafted the first version of the manuscript. All Authors revised the manuscript for important intellectual content.

Acknowledgements: National Institute for Health Research (NIHR) Leicester Biomedical Research Centre, Leicester, UK to MJD; NIHR Collaboration for Leadership in Applied Health Research and Care—East Midlands to KK; FZ is a clinical research fellow funded with an unrestricted educational grant from the National Institute for Health Research Collaboration for Leadership in Applied Health Research and Care (CLAHRC) East Midlands to the University of Leicester.

The funding sources had no role in study design, data collection, data analysis, data interpretation or writing of the report. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Data sharing: Databases are available from the corresponding author; results data and statistical codes are freely available on Mendeley and GitHub repository, respectively [conditional upon acceptance].

REFERENCES

1. European Association for the Study of the L, European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol*. 2016;64(6):1388-1402.
2. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*. 2018;67(1):328-357.
3. 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(1):73-84.
4. Stefan N, Haring HU, Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. *Lancet Diabetes Endocrinol*. 2018.
5. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther*. 2011;34(3):274-285.
6. Mantovani A, Byrne CD, Bonora E, Targher G. Nonalcoholic Fatty Liver Disease and Risk of Incident Type 2 Diabetes: A Meta-analysis. *Diabetes Care*. 2018;41(2):372-382.
7. Mahfood Haddad T, Hamdeh S, Kanmanthareddy A, Alla VM. Nonalcoholic fatty liver disease and the risk of clinical cardiovascular events: A systematic review and meta-analysis. *Diabetes Metab Syndr*. 2017;11 Suppl 1:S209-S216.
8. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? *Diabetologia*. 2008;51(11):1947-1953.
9. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol*. 2017;14(1):32-42.
10. Bonora E, Targher G. Increased risk of cardiovascular disease and chronic kidney disease in NAFLD. *Nat Rev Gastroenterol Hepatol*. 2012;9(7):372-381.
11. Woo Baidal JA, Lavine JE. The intersection of nonalcoholic fatty liver disease and obesity. *Sci Transl Med*. 2016;8(323):323rv321.
12. Li L, Liu DW, Yan HY, Wang ZY, Zhao SH, Wang B. Obesity is an independent risk factor for non-alcoholic fatty liver disease: evidence from a meta-analysis of 21 cohort studies. *Obes Rev*. 2016;17(6):510-519.
13. Wormser D, Kaptoge S, Di Angelantonio E, et al. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet*. 2011;377(9771):1085-1095.
14. Menke A, Rust KF, Fradkin J, Cheng YJ, Cowie CC. Associations between trends in race/ethnicity, aging, and body mass index with diabetes prevalence in the United States: a series of cross-sectional studies. *Ann Intern Med*. 2014;161(5):328-335.
15. Ding P, VanderWeele TJ. Sensitivity Analysis Without Assumptions. *Epidemiology*. 2016;27(3):368-377.
16. VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. *Ann Intern Med*. 2017;167(4):268-274.
17. Mathur MB, VanderWeele TJ. Sensitivity Analysis for Unmeasured Confounding in Meta-Analyses. <https://osf.io/wdmht/>. Last access 21.09.18.
18. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Last access 21.09.18.

19. Cornfield J. A method of estimating comparative rates from clinical data; applications to cancer of the lung, breast, and cervix. *J Natl Cancer Inst.* 1951;11(6):1269-1275.
20. Knapp G, Hartung J. Improved tests for a random effects meta-regression with a single covariate. *Stat Med.* 2003;22(17):2693-2710.
21. Thompson SG, Higgins JP. How should meta-regression analyses be undertaken and interpreted? *Stat Med.* 2002;21(11):1559-1573.
22. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315(7109):629-634.
23. Vevea JL, Hedges LV. A General Linear-Model for Estimating Effect Size in the Presence of Publication Bias. *Psychometrika.* 1995;60(3):419-435.
24. Mathur MB, VanderWeele TJ. ConfoundedMeta. Sensitivity Analyses for Unmeasured Confounding in Meta-Analyses. <https://cran.r-project.org/web/packages/ConfoundedMeta/ConfoundedMeta.pdf>. Last access 21.09.18.
25. Concato J, Shah N, Horwitz RI. Randomized, controlled trials, observational studies, and the hierarchy of research designs. *N Engl J Med.* 2000;342(25):1887-1892.
26. Hernán MA, Robins JM. *Causal Inference.* Boca Raton: Chapman & Hall/CRC, forthcoming. 2018. <https://www.hsph.harvard.edu/miguel-hernan/causal-inference-book/>. Last access 21.09.18.
27. Mansournia MA, Higgins JP, Sterne JA, Hernan MA. Biases in Randomized Trials: A Conversation Between Trialists and Epidemiologists. *Epidemiology.* 2017;28(1):54-59.
28. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol.* 2003;32(1):1-22.
29. Holmes MV, Ala-Korpela M, Smith GD. Mendelian randomization in cardiometabolic disease: challenges in evaluating causality. *Nat Rev Cardiol.* 2017;14(10):577-590.
30. VanderWeele TJ, Tchetgen Tchetgen EJ, Cornelis M, Kraft P. Methodological challenges in mendelian randomization. *Epidemiology.* 2014;25(3):427-435.
31. Lu H, Liu H, Hu F, Zou L, Luo S, Sun L. Independent Association between Nonalcoholic Fatty Liver Disease and Cardiovascular Disease: A Systematic Review and Meta-Analysis. *Int J Endocrinol.* 2013;2013:124958.
32. 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(3):589-600.
33. Wu S, Wu F, Ding Y, Hou J, Bi J, Zhang Z. Association of non-alcoholic fatty liver disease with major adverse cardiovascular events: A systematic review and meta-analysis. *Sci Rep.* 2016;6:33386.
34. Cornell JE, Mulrow CD, Localio R, et al. Random-effects meta-analysis of inconsistent effects: a time for change. *Ann Intern Med.* 2014;160(4):267-270.
35. Ballestri S, Zona S, Targher G, et al. 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(5):936-944.
36. Lauridsen BK, Stender S, Kristensen TS, et al. Liver fat content, non-alcoholic fatty liver disease, and ischaemic heart disease: Mendelian randomization and meta-analysis of 279 013 individuals. *Eur Heart J.* 2018;39(5):385-393.
37. Zaccardi F, Webb DR, Yates T, Davies MJ. Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective. *Postgrad Med J.* 2016;92(1084):63-69.
38. Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. *N Engl J Med.* 2000;343(20):1467-1476.

39. Dongiovanni P, Stender S, Pietrelli A, et al. Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. *J Intern Med.* 2018;283(4):356-370.
40. Zaccardi F, Pitocco D, Martini F, et al. A case of esomeprazole-induced transient diabetes and hepatitis: the role of liver inflammation in the pathogenesis of insulin resistance. *Acta Diabetol.* 2014;51(1):151-153.
41. Yates T, Zaccardi F, Dhalwani NN, et al. Association of walking pace and handgrip strength with all-cause, cardiovascular, and cancer mortality: a UK Biobank observational study. *Eur Heart J.* 2017;38(43):3232-3240.
42. Elagizi A, Kachur S, Lavie CJ, et al. An Overview and Update on Obesity and the Obesity Paradox in Cardiovascular Diseases. *Prog Cardiovasc Dis.* 2018;61(2):142-150.
43. Di Angelantonio E, Bhupathiraju Sh N, Wormser D, et al. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. *Lancet.* 2016;388(10046):776-786.
44. Zaccardi F, Dhalwani NN, Papamargaritis D, et al. Nonlinear association of BMI with all-cause and cardiovascular mortality in type 2 diabetes mellitus: a systematic review and meta-analysis of 414,587 participants in prospective studies. *Diabetologia.* 2017;60(2):240-248.
45. Lean ME, Leslie WS, Barnes AC, et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *Lancet.* 2018;391(10120):541-551.
46. Ma C, Avenell A, Bolland M, et al. Effects of weight loss interventions for adults who are obese on mortality, cardiovascular disease, and cancer: systematic review and meta-analysis. *BMJ.* 2017;359:j4849.
47. Tsochatzis EA, Newsome PN. Non-alcoholic fatty liver disease and the interface between primary and secondary care. *Lancet Gastroenterol Hepatol.* 2018;3(7):509-517.
48. Drew L. Drug development: Sprint finish. *Nature.* 2017;551(7681).

FIGURE LEGENDS

Figure 1: Relative risk for cardiovascular events and type 2 diabetes comparing participants with vs without non-alcoholic fatty liver disease

Figure 2: Estimated proportion of studies with true (causal) relative risk

Proportions (y-axes) of true effects stronger than each threshold of relative risk in participants with vs those without NAFLD (from 1.05 – top navy lines to 2.00 – bottom khaki lines) under various amounts of confounding (from 1.0 to 9.0, x-axes). For example, the proportion of studies reporting a true causal association between NAFLD and T2DM surpassing a relative risk of 1.20 (i.e., 20% increased risk of T2DM in participants with NAFLD; orange line) was 0.23 (y-axis) for confounding strength of 4 (x-axis) and 0.82 for a confounding strength of 2. Proportions with 95% confidence intervals are shown in Figure 3.

Figure 3: Estimated proportion and 95% confidence interval of studies with true (causal) relative risk

Proportions (y-axes) of true effects stronger than each threshold of relative risk (from 1.05 – top left graph to 2.00 – bottom right graph) in participants with vs those without NAFLD under various amounts of confounding (from 1.0 to 3.0, x-axes). Shadow areas indicate 95% confidence intervals of estimated proportions. The green and red dotted lines refer to the estimated proportions described in the text: for example, the proportion of studies reporting a true causal association between NAFLD and T2DM surpassing a relative risk of 1.75 (i.e., 75% increased risk of T2DM in participants with NAFLD) was 0.70 (95% CI: 0.49, 0.92) for a confounding strength of 1.25 (green line) and 0.61 (95% CI: 0.39, 0.84) for a confounding strength of 1.5 (red line).