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**Causality between non-alcoholic fatty liver disease and risk of
cardiovascular disease and type 2 diabetes:
A meta-analysis with bias analysis**

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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

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CONFLICTS OF INTEREST

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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.

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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.

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

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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).

Table 1: Characteristics of studies included in the meta-analysis

First Author (Year)	PubMed ID	Country	Age* (years)	Men (%)	Follow-up* (years)	Non-alcoholic fatty liver disease			
						Yes		No	
						Cases	Participants	Cases	Participants
Cardiovascular Events									
Lauridsen (2018)	29228164	Denmark	55.5	44.8	-	115	633	10782	94075
El Azeem (2013)	24198448	Egypt	51.4	48.9	3.0	136	268	110	479
Pisto (2014)	24650811	Finland	51.2	49.8	17.7	42	144	97	720
Wong (2016)	26406278	Hong Kong	63.0	70.8	6.0	9	356	18	256
Fracanzani (2016)	26803429	Italy	51.7	87.5	10.0	17	125	18	250
Yoshitaka (2017)	28471965	Japan	47.8	46.7	6.0	6	69	5	915
Wild (2017)	29167212	Scotland	62.4	54.0	4.3	41	1452	4428	131209
Ekstedt (2015)	25125077	Sweden	48.8	66.0	26.4	41	229	760	2286
Dunn (2008)	18684196	USA	52.0	-	8.7	26	980	387	6594
Lazo (2011)	22102439	USA	43.9	-	14.5	192	2089	508	8856
Pickhardt (2014)	24660702	USA	51.2	53.9	7.0	28	282	45	768
Zeb (2016)	27102512	USA	63.1	-	7.6	-	728	-	3391
Alexander (2018)^	29529073	USA	64.7	45.0	5.8	-	-	-	-
Type 2 Diabetes Mellitus									
Adams (2009)	19293782	Australia	59.9	68.0	11.1	20	106	15	246
Ming (2015)	25879672	China	45.6	42.3	5.0	10	97	10	411
Li WD (2015)	26327768	China	52.7	66.5	3.9	245	1412	135	3379
Chen GY (2016)	27042272	China	35.3	85.9	6.0	25	196	343	6346
Liu (2017)	28324002	China	71.4	-	5.0	137	3479	316	15028
Li Y (2017)	28350839	China	63.1	41.6	4.6	186	1029	574	12341
Balkau (2010)	20529259	France	47.1	48.8	9.0	77	352	126	3459
Zelber-Sagi (2013)	23656177	Israel	48.8	-	7.0	26	35	51	106
Okamoto (2003)	12587609	Japan	42.7	55.6	10.0	27	120	55	720
Shibata (2007)	17666460	Japan	48.0	100	8.0	65	802	44	2387
Yamada (2009)	19817963	Japan	49.8	54.9	5.0	27	116	72	221
Yamazaki (2015)	26156527	Japan	43.3	60.7	11.3	116	729	73	2345
Kato ^h (2015) ^s	26303223	Japan	42.8	59.7	2.2	-	-	-	-
Fukuda (2016)	26176710	Japan	41.5	59.0	12.8	20	139	91	2850
Mitsuhashi (2017)	28867686	Japan	46.7	58.6	5.1	491	3846	313	13964
Kim (2008)	18346164	Korea	46.8	68.3	5.0	153	1800	81	3572
Ryoo (2016)	27709854	Korea	42.7	100	3.5	218	539	1712	14758
Kastuiratne (2012)	22989165	Sri Lanka	52.5	-	3.0	104	926	138	1350
Chen SC (2017)	28680048	Taiwan	47.2	52.0	18.0	3735	42410	2820	93967
Shah (2015)	26209814	USA	59.0	43.9	9.1	105	785	111	2368

Studies are sorted by outcome, country, and year of publication.

- not available; *mean/median [^]Total events/participants: 572/1589; ^sTotal events/participants: 24/1515

Cardiovascular Events

Type 2 Diabetes Mellitus

liv_13994_f2.eps

liv_13994_f3.eps

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