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Association between non-alcoholic fatty liver disease and subclinical atherosclerosis in Western and Asian cohorts: an updated meta-analysis

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ABSTRACT

Background Non-alcoholic fatty liver disease (NAFLD) is a well-established risk factor for cardiovascular disease, with ethnic and regional differences noted. With the recent surge of research within this field, we re-examine the evidence associating NAFLD with subclinical atherosclerosis, and investigate potential regional differences.

Methods This is a systematic review and meta-analysis. PubMed and EMBASE were systematically searched for publications from January 1967 to July 2020 using standardised criteria. Original, observational studies investigating the association between NAFLD and either carotid intima-media thickness (CIMT) and/or coronary artery calcification (CAC) were included. Key outcomes included differences in mean CIMT, the presence of increased CIMT, the presence of CAC and the development/progression of CAC. Pooled ORs and pooled standard differences in means were calculated using random-effects models. Between-study heterogeneity was quantified using the Q statistic and I^2. Subgroup analyses stratified by region of study (Asian vs Western) were also conducted.

Results 64 studies involving a total of 172 385 participants (67 404 with NAFLD) were included. 44 studies assessed the effect of NAFLD on CIMT, with the presence of NAFLD associated with increased CIMT (OR 2.00, 95% CI 1.56 to 2.56). 22 studies assessed the effects of NAFLD on CAC score, with the presence of NAFLD associated with the presence of any coronary calcification (OR 1.21, 95% CI 1.12 to 1.32), and the development/progression of CAC (OR 1.26, 95% CI 1.04 to 1.52). When stratified by region, these associations remained consistent across both Asian and Western populations (p>0.05). The majority (n=39) of studies were classified as 'high quality', with the remaining 25 of 'moderate quality'.

Conclusions There is a significant positive association between various measures of subclinical atherosclerosis and NAFLD, seen across both Western and Asian populations. These results re-emphasise the importance of early risk evaluation and prophylactic intervention measures to preclude progression to clinical cardiovascular disease in patients with NAFLD.

Key questions

What is already known about this subject?

► Non-alcoholic fatty liver disease (NAFLD) is a significant, independent risk factor for cardiovascular disease (CVD), with recent evidence positing this association to extend to the preclinical stages of CVD. Previous meta-analyses have quantified positive associations between NAFLD and subclinical atherosclerotic markers before, though the majority of included studies were published before 2016. The last 5 years, however, has experienced a large surge of research in this field, especially within large Asian populations that have not been included in previous meta-analyses. Ethnic and regional differences in the associations between NAFLD and subclinical atherosclerosis have been suggested within individual studies, but have yet to be synthesised across the available literature.

What does this study add?

► This meta-analysis serves as a timely update of the existing literature, incorporating the results of over 21 new studies comprising over 100 000 participants (~50 000 with NAFLD) from both Western and Asian regions. The results reinforce the significant positive association between NAFLD and subclinical atherosclerosis (as defined by increased carotid intima-media thickness and coronary artery calcification scores), and further confirm these associations to be consistent across both Western and Asian populations. Lastly, this is the first meta-analysis to demonstrate that the associations between NAFLD and subclinical atherosclerosis are not just cross-sectional but also longitudinal.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of pathological hepatic conditions ranging from simple steatosis to non-alcoholic steatohepatitis, and may ultimately progress to advanced fibrosis, cirrhosis, and end-stage liver disease.[1–3] Over the last 20 years, NAFLD has become the

Key questions

How might this impact on clinical practice?

- This study highlights that NAFLD serves as an important atherogenic risk factor in both Western and Asian populations, and reemphasises the role of early risk evaluation and prophylactic intervention measures to preclude progression to clinical CVD in NAFLD. By confirming a longitudinal association between NAFLD and subclinical atherosclerotic markers, these results also provide potential insight into the causal relationship between NAFLD and subclinical atherosclerosis.

leading cause of chronic liver disease, with an estimated 1 billion people affected worldwide.[4] NAFLD has increasingly been recognised as the hepatic manifestation of the metabolic syndrome (MetS), and is one facet of a multisystem disease, with close relations to abdominal obesity, type 2 diabetes mellitus (T2DM), insulin resistance and hyperlipidaemia.[5–7]

Cardiovascular disease (CVD) is the leading cause of mortality in patients with NAFLD, with a large body of evidence demonstrating NAFLD to be a significant, independent risk factor for CVD.[2 3 5 8] It is now widely hypothesised that NAFLD is not merely a marker of CVD, but may be actively involved in CVD pathogenesis.[3 9–11] This association extends to preclinical CVD, with recent work identifying NAFLD as a risk factor for early subclinical atherosclerosis, and as a strong independent predictor of incident CVD.[3 8 12] This holds highly important implications for the screening and early evaluation of CVD in patients with NAFLD. Carotid intima-media thickness (CIMT) and coronary artery calcification (CAC) are the two most established and widely studied surrogate measures of subclinical atherosclerosis, and a growing body of literature has investigated this relationship between NAFLD and CAC/CIMT over the last decade.[3 12 13]

NAFLD is no longer considered a disease only prevalent in affluent Western countries, with rapidly growing rates of NAFLD reported within Asia in particular.[4 14] Ethnic and regional differences in NAFLD prevalence, severity and outcomes have been identified between Western, Hispanic and Asian populations,[15 16] and have been attributed to factors including lifestyle, environment, insulin resistance, body composition (adipose distribution and muscle bulk) and genetics.[14 15 17 18] Asian populations are especially susceptible with cardiometabolic complications such as NAFLD seen to develop within a much shorter period, within younger patient populations and in those with lower body mass index.[18 19] These disparities may possibly extend to differential associations between NAFLD and subclinical atherosclerosis.[20–22]

Previous meta-analyses have quantified the associations between NAFLD and subclinical atherosclerotic markers before, with the majority of included studies published before 2016.[13 23–25] However, the last 5 years has experienced a large surge in research within this area, especially within large Asian populations that have not been reported in previous meta-analyses.[26–35] We aim to evaluate the relationship between NAFLD and subclinical atherosclerosis including these updated studies, and to further investigate potential regional differences in these associations.

METHODOLOGY

This meta-analysis was conducted and reported according to the Meta-analysis Of Observational Studies in Epidemiology statement[36] and was registered in the International Prospective Register of Systematic Reviews (registration number: CRD42020204784).

Search strategy

A comprehensive literature search was performed via the MEDLINE and EMBASE databases to identify potentially relevant publications in the English language, with a date range from January 1967 to July 2020. The databases were systematically searched using a combination of the following keywords linked with appropriate Boolean logic: (*Fatty Liver OR NAFLD OR Hepatic Steatosis OR Non-alcoholic fatty liver disease) AND ((subclinical atherosclerosis OR Preclinical atherosclerosis) OR (Coronary calcium OR Calcium Score OR Coronary Calcification) OR ("Carotid Intima-media thickness" OR CIMT OR IMT OR "'intima media thickness'"*)). Relevant references identified from the bibliographies of pertinent articles or review papers were also retrieved.

Eligibility (inclusion and exclusion) criteria

The eligibility criteria was based on the PICOS framework as recommended by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.[37]

1. *Participants*: studies had to be conducted on adult participants. Studies conducted on 'special populations' including adolescent/paediatric populations, and those defined by additional pathologies such as HIV, severe CVD or liver transplants were rejected. Populations with existing metabolic conditions such as MetS and diabetes mellitus were accepted.
2. *Exposures (intervention):* studies had to have a defined exposure of 'NAFLD' or 'fatty liver' or 'hepatic steatosis', as diagnosed by either ultrasound (US), liver biopsy, CT, magnetic resonance spectroscopy (MRS) or Fatty Liver Index.
3. *Outcomes*: study outcomes had to report on either the (1) presence (cross-sectional) of CAC (CAC score >0), (2) progression (longitudinal) of CAC score, and/or (3) on CIMT. The presence of calcified coronary artery plaques was accepted as a measure of CAC score>0. Studies had to specify how CAC and CIMT were recorded and defined, and also had to quantitatively assess the association between NAFLD and CAC/CIMT, respectively, either via logistic regression for categorical outcomes or via comparison of means techniques (t-test/analysis of variance (ANOVA)) for continuous outcomes.

4. *Comparison*: studies had to include a 'healthy' control group of participants without NAFLD, preferably from the same population as the exposure group.
5. *Study design*: we included observational studies (cross-sectional, case–control, retrospective, prospective), which reported quantitative outcomes. Descriptive studies, reviews and studies on animals were excluded. Studies with sample sizes <50 were also excluded.

Using our search strategy, a total of 1007 titles were initially identified. Two authors (MYZW and JJLY) assessed the titles independently according to the predefined inclusion and exclusion criteria. Studies were first screened by title and abstract. The full-text articles deemed potentially relevant were then obtained and systematically included after detailed examination. The following data were extracted: (a) study: year, region, design; (b) patients: mean age, gender, sample size; (c) method of NAFLD evaluation: US, CT, MRS, liver biopsy or composite index; (d) outcomes: outcome type (CIMT or CAC) and method of outcome definition; (e) analysis: statistical techniques used, primary outcomes (mean±SD, ORs with 95% CIs), confounders adjustment.

For studies reporting multiple multivariable-adjusted models, we extracted those reflecting the greatest degree of control for potential confounders. Any discrepancies in data quantification were resolved by discussion among the investigators.

Study quality evaluation

The quality of observational studies was assessed using a modified version of the Newcastle–Ottawa Scale (NOS) for cohort and cross-sectional studies.[38–40] The NOS awards a maximum of 9 stars to assess quality based on three main aspects: (a) the selection and representativeness of the participants (maximum 4 stars), (b) the comparability of groups (maximum 2 stars), and (c) the ascertainment of exposure (for case–control) or outcome (for prospective and cross-sectional) (maximum 3 stars). Following previous reviews, studies assigned 0–4, 5–7, and ≥8 stars were considered as low, medium and high quality, respectively.[41–43]

Data synthesis and statistical analysis

Outcomes were broadly grouped according to four main categories:

1. Differences in mean CIMT (continuous).
2. Presence of increased CIMT (categorical).
3. Presence of CAC (categorical).
4. Development/progression of CAC (categorical, longitudinal).

All outcomes were pooled using DerSimonian-Laird random-effects model. The continuous and categorical outcome was reported as pooled standard differences (Std Diff) in means and ORs with 95% CI. We further conducted subgroup analysis to look into regional differences between Asian versus Western populations. We defined 'Western' studies to comprise of studies conducted in North America, Europe and Australia, while 'Asian' studies comprised of those conducted in South Asian, East Asian and Southeast Asian countries. Lastly, additional subgroup analysis on the Std Diff in mean CIMT within the subset of participants with diabetes was conducted.

The heterogeneity of pooled estimates between studies was quantified using the Q statistic and I^2. A value of I^2 of 0%–25% indicates no heterogeneity, 26%–50% low heterogeneity, 51%–75% moderate heterogeneity and 76%–100% high heterogeneity. Funnel plots and Egger's regression test were used to assess publication bias. P<0.05 was considered as statistical significance.

All statistical analyses were conducted using the Comprehensive Meta-Analysis Software V.3.3.

RESULTS

Search strategy and description of studies

The initial search yielded 1007 potentially relevant titles, where 835 articles were excluded on the basis of title and abstract screen. A total of 172 titles underwent full-length review, of which 108 were further excluded (figure 1). A final total of 64 studies, involving 67 404 patients with NAFLD and 104 981 controls were included in the meta-analysis. Tables 1–3 describe the detailed characteristics of the included studies, grouped by study outcome. These included studies were carried out in Asia (n=32), Western Europe (n=15), the Middle East (n=10) and America (n=7; North America: 6, South America: 1). Sixty studies were cross-sectional and four were prospective cohort studies.

Measurement of exposures and outcomes

The presence of NAFLD was largely determined by US (n=46), with other studies using CT (n=8), biopsy (N=8), Fatty Liver Index (n=1) and MRS (n=1). Twenty-two studies investigated the effects of NAFLD on CAC score, with one study using the presence of calcified coronary artery plaques as a proxy for CAC >0. Forty-four studies investigated the effects of NAFLD on CIMT score. CIMT was assessed via B-mode US of bilateral carotid arteries, with majority of studies (n=18) commonly averaging the mean CIMT over six measurements (three on each carotid artery).

Methodological quality

Tables 1–3 and online supplemental table 1 detail the NOS risk of bias evaluation for the various studies. Of the 60 cross-sectional studies, the majority (n=35) were classified as 'high quality' (≥8 stars) with the remaining 25 classified as 'moderate quality' (5–7 stars). All four prospective studies were classified as 'high quality'.

Effect of NAFLD on CIMT

Figures 2 and 3 summarise the studies which investigated the effects of NAFLD on CIMT. Forty-four studies, with a total of 41 189 individuals, assessed the effect of NAFLD on CIMT. Thirty-nine studies investigated the mean differences in CIMT between NAFLD and controls,[22 30 33 35 44–79] while 13 studies used logistic regression to quantify the associations

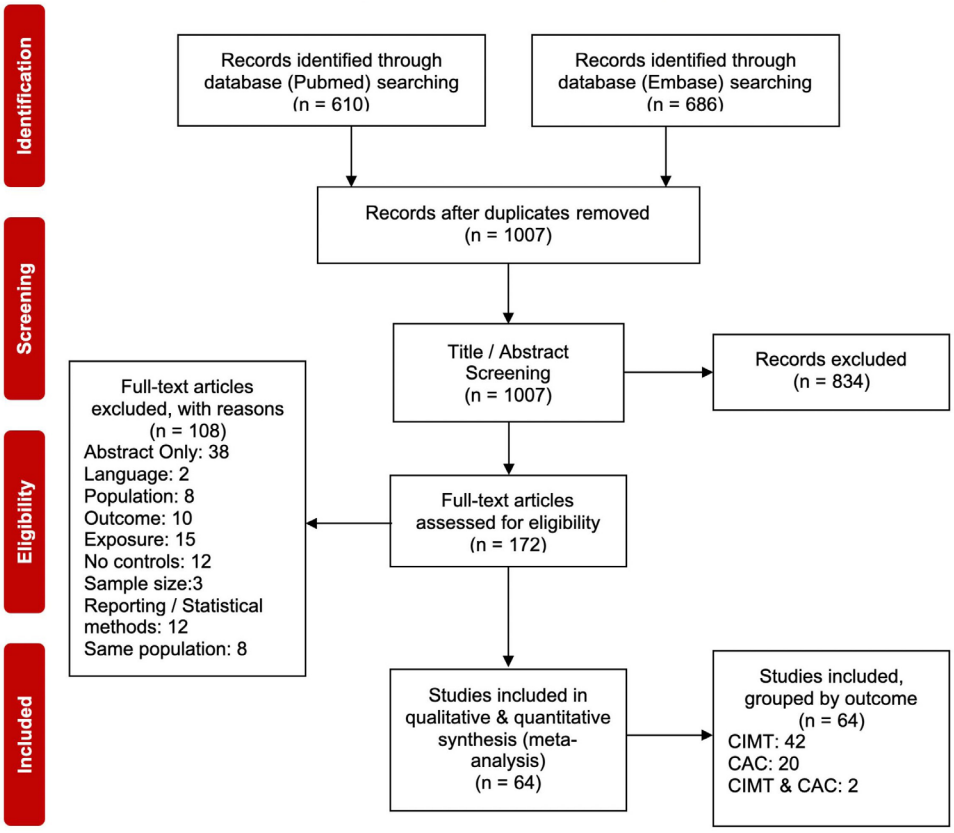

Figure 1 Study selection PRISMA flow diagram. CAC, coronary artery calcification; CIMT, carotid intima-media thickness; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

between NAFLD and an 'increased CIMT'.[22 28 44 45 59 61 62 74 79–83] Increased CIMT was defined as >0.8 mm in six studies, >1.0 mm in two studies and via other stratification methods in the remaining five studies.

Compared with participants without NAFLD, the presence of NAFLD was significantly associated with an increased CIMT, with a pooled OR of 2.00 (95% CI 1.56 to 2.56, $P_{heterogeneity}$<0.001, I^2=81.8%, figure 2). Likewise, subjects with NAFLD had a higher mean CIMT than subjects without, both across studies which adjusted for confounders (pooled Std Diff in means: 1.17, 95% CI: 0.49 to 1.85, figure 3B), and in studies which compared unadjusted means (pooled Std Diff in means: 0.68, 95% CI: 0.44 to 0.91, figure 3A). For all CIMT outcomes, a sensitivity analysis including only studies of 'high quality' was performed, with similar results obtained.

Subgroup analyses

We further stratified the associations between NAFLD and an increased risk of increased CIMT by study region (figure 4A). The pooled ORs for increased CIMT were (OR: 1.63, 95% CI: 1.19 to 2.22, $P_{heterogeneity}$=0.06, I^2=50%, n=7 studies) in Asian populations vs (OR: 2.70, 95% CI: 1.58 to 4.60, $P_{heterogeneity}$<0.001, I^2=93.6%, n=3 studies) in Western populations ($P_{difference}$=0.15). Likewise, the pooled Std Diff in mean CIMT were 0.75 (95% CI: 0.31 to 1.17) in Asian populations (n=12 studies) vs 0.67 (95% CI: 0.25 to 1.09) in Western populations ($P_{difference}$=0.83) (figure 4B). Lastly, when analysing the subset of studies conducted on participants with T2DM, no Std Diff in CIMT means were found between those with and without NAFLD (Std Diff in means: 0.99, 95% CI:−0.21 to 2.20, n=7 studies) (online supplemental figure 1).

Effect of NAFLD on CAC score

Figure 5 summarises the studies investigating the associations between NAFLD and CAC score. Twenty-two studies, with a total of 136 294 individuals, assessed the effect of NAFLD on CAC score. Sixteen studies investigated the cross-sectional associations between NAFLD and the presence of CAC score >0,[22 26 27 29 31 32 47 60 84–91] five studies investigated the cross-sectional associations between NAFLD and the presence of CAC score >100,[60 80 85 92–94] and four studies investigated the longitudinal influence of NAFLD on CAC score progression/development.[31 34 95 96]

Compared with participants without NAFLD, the presence of NAFLD was significantly associated with the presence of both CAC score >0 (pooled OR: 1.21, 95% CI 1.12 to 1.32, $P_{heterogeneity}$=0.018, I^2=47.7%), and CAC score >100 (pooled OR: 1.28, 95% CI 1.01 to 1.63, $P_{heterogeneity}$=0.015, I^2=67.8%), (figure 5A). Likewise, NAFLD was significantly associated with the development/progression of CAC with a pooled OR of 1.26 (95% CI 1.04 to 1.52, $P_{heterogeneity}$=0.34, I^2=10.6%) (figure 5B).

Table 1 Characteristics of included studies which conducted a comparison of carotid-intima media thickness (CIMT) means between those with NAFLD and those without

Name, year	Study region	Study population	Study size	n (%) NAFLD	Age (NAFLD+ vs NAFLD–)	% male (NAFLD+ vs NAFLD–)	NAFLD assessment	Outcome assessment	Confounder adjustment	NOS (max=9)
Oni *et al*[22] 2019	North America	Population based	4123	729 (17.7)	61 vs 63	47.0 vs 44.0	CT, LS ratio <1	Ultrasound, mean IMT (L&R)	–	7
Mohammadzadeh *et al*[44] 2019	Iran	Hospital based	300	150 (50.0)	49.9 vs 52.5	65.3 vs 57.3	Ultrasound	Ultrasound, mean IMT (L&R)	–	6
Yi *et al*[45] 2018	Asia	Outpatient clinic	1981	1888 (95.3)	45.9 vs 44.8	63.4 vs 40.1	Ultrasound	Ultrasound, mean of max IMT (L&R)	–	6
Kim *et al*[30] 2018	Asia	Population (health screen)	819	330 (40.3)	53.4 vs 53.1	64.2 vs 41.5	Ultrasound	Ultrasound, mean IMT (L&R)	–	6
Vanjiappan *et al*[46] 2018	Asia	Hospital based, patients with T2DM	124	73 (58.9)	Overall=53.8	Overall=54.0	Ultrasound	Ultrasound, mean of max IMT (L&R)	–	6
Gummesson *et al*[47] 2018	Europe	Population based	1015	106 (10.4)	58.3 vs 57.5	71.7 vs 52.5	CT, liver HU <40	Ultrasound, mean IMT	–	7
Cetindağlı *et al*[48] 2017	Turkey	Outpatient clinic	120	93 (77.5)	34.5 vs 33.8	100 vs 100	Ultrasound and biopsy	Ultrasound, mean IMT (6 measurements)	Age/sex-matched controls	7
Guo *et al*[33] 2017	Asia	Hospital based, patients with T2DM	8571	4340 (50.6)	57.4 vs 61.9	54.6 vs 55.9	Ultrasound	Ultrasound, mean IMT (6 measurements)	Age	7
Hong *et al*[35] 2016	Asia	Population (health screen)	955	342 (35.8)	53 vs 51 (median)	48.8 vs 42.1	Ultrasound	Ultrasound, mean IMT (99 computer points)	–	7
Zhang *et al*[49] 2016	Asia	Outpatient clinic, patients with T1DM	722	123 (17.0)	47.4 vs 46.0	52.8 vs 51.1	Ultrasound	Ultrasound, mean IMT (6 measurements)	Age, sex, BMI, WC, SBP, DBP, total cholesterol, TAG, LDL, HDL, MetS, ALT, AST, GGT, hsCRP, medications	8
Ozturk *et al*[50] 2015	Turkey	Outpatient clinic, MetS(–)	82	41 (50.0)	32.8 vs 31.8	100 vs 100	Biopsy	Ultrasound, mean IMT (L&R)	–	6
Asakawa *et al*[55] 2014	Asia	Population (health screen)	76	24 (31.6)	61.5 vs 61.0 (median)	91.7 vs 75.0	Ultrasound	Ultrasound, max IMT	–	6
Ayaz *et al*[54] 2014	Turkey	Outpatient clinic	90	60 (66.7)	44.5 vs 39.5 (median)	36.7 vs 26.7	Ultrasound	Ultrasound, mean IMT (8 measurements)	–	6
Kim *et al*[53] 2014	Asia	Population (health screen), MetS(–)	1285	180 (14.0)	55.7 vs 55.7	58.0 vs 36.0	CT, liver minus spleen <5	Ultrasound, mean IMT (4 measurements)	–	7

Continued

Table 1 Continued

Name, year	Study region	Study population	Study size	n (%) NAFLD	Age (NAFLD+ vs NAFLD–)	% male (NAFLD+ vs NAFLD–)	NAFLD assessment	Outcome assessment	Confounder adjustment	NOS (max=9)
Kim *et al*[62] 2014	Asia	Hospital based, patients with T2DM	1211	747 (61.7)	56.7 vs 55.6	51.0 vs 41.8	Ultrasound	Ultrasound, mean IMT (6 measurements)	–	6
Nahandi *et al*[51] 2014	Iran	Hospital based, patients without diabetes	102	50 (49.0)	43.3 vs 43.1	32.0 vs 40.4	Ultrasound	Ultrasound, mean of max IMT (L&R)	HLP, sex, Smk, HT, obesity, walking, liver enzymes	8
Dogru *et al*[58] 2013	Europe	Outpatient clinic	189	115 (60.8)	31 vs 28 (median)	100 vs 100	Liver biopsy	Ultrasound, mean IMT (6 measurements)	–	6
Kucukazman *et al*[67] 2013	Europe	Outpatient clinic	161	117 (72.7)	45.8 vs 45.4	44 vs 32	Ultrasound	Ultrasound, mean IMT (6 measurements)	–	6
Mishra *et al*[66] 2013	Asia	Population based	645	101 (15.7)	31.6 vs 27.1	100 vs 100	Ultrasound	Ultrasound, mean of max IMT (L&R)	–	7
Huang *et al*[62] 2012	Asia	Population based	8632	2590 (30.0)	58.5 vs 58.5	31.4 vs 30.9	Ultrasound	Ultrasound, max IMT (L&R)	–	7
Kang *et al*[61] 2012	Asia	Outpatient (health screen), MetS(–)	413	157 (38.0)	52.0 vs 52.5	51.0 vs 41.8	Ultrasound	Ultrasound, mean IMT (L&R)	–	7
Thakur *et al*[59] 2012	Asia	Hospital based	80	40 (50.0)	42.1 vs 41.9	67.5 vs 67.5	Ultrasound	Ultrasound, mean IMT (6 measurements)	–	7
Colak *et al*[63] 2012	Turkey	Outpatient clinic	87	57 (65.5)	44.2 vs 42.7	45.6 vs 46.7	Liver biopsy	Ultrasound, mean IMT (6 measurements)	–	6
Agarwal *et al*[66] 2011	Asia	Hospital based, patients with T2DM	124	71 (57.3)	57 vs 61	52.5 vs 58.5	Ultrasound	Ultrasound, mean IMT	–	6
Mohammadi *et al*[65] 2011	Iran	Hospital based	335	250 (74.6)	46.6 vs 44.9	55.6 vs 54.1	Ultrasound	Ultrasound, mean IMT (6 measurements)	HT, DM, HLP, hyperglycaemia	8
Poanta *et al*[64] 2011	Europe	Outpatient clinic, patients with T2DM	56	38 (67.9)	59.4 vs 61.5	50.0 vs 83.3	Ultrasound	Ultrasound	–	5
Kilciler *et al*[69] 2010	Europe	Outpatient clinic	114	60 (52.6)	31.7 vs 30.3	100 vs 100	Biopsy	Ultrasound, mean IMT (L&R)	Age-matched controls	6
Salvi *et al*[68] 2010	Europe	Population based	220	92 (41.8)	50.7 vs 49.3	54.3 vs 36.7	Ultrasound	Ultrasound, mean IMT (6 measurements)	–	7
Vlachopoulos *et al*[67] 2010	Europe	Outpatient clinic	51	28 (54.9)	55.4 vs 51.5	52.3 vs 64.3	Biopsy	Ultrasound, mean IMT (L&R)	Age/sex-matched controls	6

Continued

Table 1 Continued

Name, year	Study region	Study population	Study size	n (%) NAFLD	Age (NAFLD+ vs NAFLD–)	% male (NAFLD+ vs NAFLD–)	NAFLD assessment	Outcome assessment	Confounder adjustment	NOS (max=9)
Gastaldelli *et al*[73] 2009	Europe	Population based	842	234 (27.8)	42 vs 45	69.7 vs 24.0	Fatty liver index >60	Ultrasound, mean IMT (10 measurements)	–	6
Karakurt *et al*[72] 2009	Turkey	Not mentioned	66	40 (60.6)	53 vs 53	30.0 vs 42.3	Ultrasound	Ultrasound, mean IMT (6 measurements)	–	5
Petit *et al*[71] 2009	Europe	Hospital based, patients with T2DM	101	61 (60.4)	60.3 vs 60.1	44.2 vs 50.0	MR spectroscopy, liver fat content >5.5%	Ultrasound, mean IMT (6 measurements)	–	6
Ramilli *et al*[70] 2009	Europe	Outpatient clinic	154	90 (58.4)	59.3 vs 60.1	51.1 vs 45.3	Ultrasound	Ultrasound, mean of max IMT (L&R)	Age, sex, BMI, Smk, HT, dyslipidaemia, DM	8
Fracanzani *et al*[74] 2008	Europe	Hospital based	375	125 (33.3)	50.5 vs 52	87.2 vs 87.2	Ultrasound+biopsy	Ultrasound, mean IMT (6 measurements)	–	7
Aygun *et al*[75] 2008	Turkey	Hospital based	80	40 (50.0)	43.2 vs 38.8	47.5 vs 50.0	Biopsy	Ultrasound	Age/sex-matched controls	7
Targher *et al*[77] 2006 1	Europe	Outpatient clinic, patients with T2DM	200	100 (50.0)	55 vs 56	64.0 vs 67.0	Ultrasound	Ultrasound, mean IMT (6 measurements)	Age/sex-matched controls	7
Targher *et al*[76] 2006 2	Europe	Outpatient clinic	245	85 (24.7)	45 vs 45	58.8 vs 59.4	Biopsy	Ultrasound, mean IMT (6 measurements)	Age, sex, BMI, Smk, LDL, HOMA-IR, MetS	8
Brea *et al*[79] 2005	Europe	Hospital based	80	30 (50.0)	53.2 vs 51.6	50.0 vs 50.0	Ultrasound	Ultrasound, mean IMT	–	7
Targher *et al*[78] 2005	Europe	Outpatient clinic	90	50 (55.5)	46 vs 46	60.0 vs 65	Biopsy	Ultrasound, mean IMT (6 measurements)	Age, Sex, HOMA-IR, MetS	8

BMI, body mass index; L&R, left and right; MetS, metabolic syndrome; MR, magnetic resonance; NAFLD, non-alcoholic fatty liver disease; NOS, Newcastle–Ottawa Scale; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

Table 2 Characteristics of included studies which investigated the association between NAFLD and Increased CIMT

Name, year	Study region	Study population	Study size	n (%) NAFLD	Age (NAFLD+ vs NAFLD–)	% male (NAFLD+ vs NAFLD–)	NAFLD assessment	Outcome assessment	Outcome definition	Confounder adjustment	NOS (max=9)
Mohammadzadeh *et al*[44] 2019	Other: Iran	Hospital based	300	150 (50.0)	49.9 vs 52.5	65.3 vs 57.3	Ultrasound	Ultrasound, mean IMT (L&R)	CIMT >0.8	Age, BMI, HLP, HTN, DM	8
Tan *et al*[80] 2019	Asia	Government officials (health screen)	131	84 (64.1)	Overall=47.1	84.0 vs 60.7	Ultrasound (Fibroscan, Controlled attenuation parameter (CAP) ≥263 dB/min)	Ultrasound, mean IMT (6 measurements)	CIMT >0.8	Age, sex, WC, ALT, DM, HT	8
Oni *et al*[22] 2019	North America	Population based	4123	729 (17.7)	61 vs 63	47.0 vs 44.0	CT, LS ratio <1	Ultrasound, mean internal carotid IMT (L&R)	CIMT >1.0	Age, sex, ethnicity, SBP, fasting glucose, lipid-lowering meds, HT meds, LDL, Smk, BMI, logCRP	9
Yi *et al*[45] 2018	Asia	Outpatient clinic	1981	1888 (95.3)	45.9 vs 44.8	63.4 vs 40.1	Ultrasound	Ultrasound, mean of max IMT (L&R)	–	Sex, SBP, FPG, TG, TC, LDL, ALT, AST, GGT, Cr	6
Zheng *et al*[28] 2018	Asia	Population based	4112	1571 (38.2)	56.2 vs 55.6	64.4 vs 35.6	Ultrasound	Ultrasound, max IMT (L&R)	CIMT >0.8	Age, sex, BMI, exercise, Smk, WC, TG, LDL, DM, HT	9
Martínez-Alvarado *et al*[81] 2014	Mexican	Population based	429	122 (28.4)	52.1 vs 54.1	0.0 vs 0.0	CT, LS ratio <1	Ultrasound, mean IMT (10 measurements)	>75th sex/age-specific percentile	Age, HT, hypercholesterolaemia, hyperTAG, HDL, WC, HOMA-IR	9
Lankarani *et al*[82] 2013	Other: Iran	Population based	580	290 (50.0)	46.4 vs 45.4	44.8 vs 40.0	Ultrasound	Ultrasound, mean IMT (6 measurements)	CIMT >0.8	Age, sex, WC, DM, HT, TAG, HDL	9
Huang *et al*[62] 2012	Asia	Population based	8632	2590 (30.0)	58.5 vs 58.5	31.4 vs 30.9	Ultrasound	Ultrasound, max IMT (L&R)	CIMT >0.8	Age, sex, alcohol, Smk, exercise, BMI, LDL, central obesity, FBG, TG, BP, HDL, HOMA-IR	9
Kang *et al*[61] 2012	Asia	Outpatient (health screen), MetS(–) participants	413	157 (38.0)	52.0 vs 52.5	51.0 vs 41.8	Ultrasound	Ultrasound, mean IMT (L&R)	CIMT >1.0	Age, BP, BMI, WC, lipid profile, liver enzymes	8
Thakur *et al*[59] 2012	Asia	Hospital based	80	40 (50.0)	42.1 vs 41.9	67.5 vs 67.5	Ultrasound	Ultrasound, mean IMT (6 measurements)	CIMT >0.556	Generalised and abdominal obesity, MetS, fasting insulin, dyslipidaemia, SBP, DBP, hsCRP	8
Kim *et al*[83] 2009	Asia	Population (health screen)	1021	507 (49.7)	–	62.5 vs 46.5	Ultrasound	Ultrasound, mean of max IMT (L&R)	CIMT >0.8	Age, sex, WC, SBP, fasting glucose, total/HDL cholesterol ratio, Smk, alcohol	9
Fracanzani *et al*[74] 2008	Europe	Hospital based	375	125 (33.3)	50.5 vs 52.0	87.2 vs 87.2	Ultrasound+biopsy	Ultrasound, mean IMT (6 measurements)	CIMT >0.64	Sex, Smk, HDL, LDL, TAG, fasting glucose, MetS, DM, BMI, AAT	8
Brea *et al*[79] 2005	Europe	Hospital based	80	40 (50.0)	53.2 vs 51.6	50.0 vs 50.0	Ultrasound	Ultrasound, mean IMT	CIMT top quartile	Sex, age, BMI, SBP, DBP, DM, lab serum values	8

BMI, body mass index; CIMT, carotid intima-media thickness; Cr, creatinine; DM, diabetes mellitus; L&R, left and right; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NOS, Newcastle–Ottawa Scale.

Table 3 Characteristics of included studies which investigated the association between NAFLD and CAC presence, development or progression

Name, year	Study region	Study population	Study size	n (%) NAFLD	Age (NAFLD+ vs NAFLD–)	% male (NAFLD+ vs NAFLD–)	NAFLD assessment	Outcome assessment	Outcome definition	Confounder adjustment	NOS (max=9)
CAC presence (CAC >0 and CAC >100)											
Jacobs *et al*[85] 2016	North America	Population based	250	71 (28.4)	66.8 vs 67.8	43.7 vs 43.0	CT, Liver Spleen ratio ≤1.1	MDCT, Agatston method	CAC >100 & CAC >0	Age, sex, HR, Smk, creatinine, BMI, alcohol, total cholesterol, HDL, TAG, VAT/SAT/WC	9
Chhabra *et al*[92] 2013	North America	Population (health screen)	377	43 (11.4)	Overall=57.1	Overall=52.0	CT, spleen minus liver >10	MDCT, Agatston method	CAC >100	Age, sex, Smk, LDL, HT, DM, MetS	9
Kim *et al*[60] 2012	Asia	Population (health screen)	4023	1617 (40.2)	57.5 vs 56.4	73.0 vs 52.5	Ultrasound	16 & 64 slice MDCT, Agatston method	CAC >100 & CAC >0	Age, sex, BMI, WC, alcohol, Smk, physical activity, DM, HT, total cholesterol, TAG, HDL, CRP	9
Chen *et al*[94] 2010	Asia	Population (health screen)	295	121 (41.0)	Overall=52.6	Overall=65.8	Ultrasound and CT	64 slice MDCT, Agatston method	CAC >100	Age, sex, BMI, Smk, HT, DM, fasting glucose, total cholesterol, TAG, HDL, LDL, ALT, AST, serum uric acid, gallbladder stones	9
Jung *et al*[93] 2010	Asia	Population (health screen)	928	219 (34.4)	54.0 vs 51.7	72.8 vs 49.5	Ultrasound	64 slice MDCT, Agatston method	CAC >100	Age, Sex, BMI, WHR, uric acid, SBP, DBP, GGT, TAG, HDL, fasting glucose, Smk, DM, HT, statins	9
Kim *et al*[96] 2020	Asia	Population (health screen)	7259	3328 (45.8)	Overall=54	Overall=59.5	Ultrasound	64 slice MDCT, Agatston method	CAC >0	Age, sex, HT, DM, obesity, abdominal obesity, eGFR, CRP, Smk, alcohol, AST, ALT, GGT	9
Oni *et al*[22] 2019	North America	Population based	4123	729 (17.7)	61 vs 63	47 vs 44	CT, LS ratio <1	EBCT or MDCT, Agatston method	CAC >0	Age, gender, ethnicity, SBP, fasting glucose, lipid-lowering meds, HT meds, LDL, Smk, BMI, logCRP	9
Chang *et al*[27] 2019	Asia	Population (health screen)	86 911	34 382 (39.6)	41.1 vs 40.3	89.1 vs 64.7	Ultrasound	64 slice MDCT, Agatston method	CAC >0	Age, sex, BMI, Smk, physical activity, education, total caloric intake, family history of CVD, DM, HT, LDL, meds, dyslipidaemia, hsCRP, HOMA-IR	9
Gummesson *et al*[47] 2018	Europe	Population based	1015	106 (10.4)	58.3 vs 57.5	71.7 vs 52.5	CT, liver HU <40	MDCT, Agatston method	CAC >0	Sex, age, education, BMI, alcohol, Smk, sedentary time, waist, VAT, physical activity, DM, HT, LDL, HDL, TG, CRP, insulin, hsCRP	9
Cho *et al*[31] 2018	Asia	Population (health screen)	798	272 (34.1)	53.4 vs 54.1	91.2 vs 72.2	Ultrasound	64 slice MDCT, Agatston method	CAC >0	Age, sex, BMI, Smk, alcohol, exercise, LDL-cholesterol, hsCRP	9
Lee *et al*[29] 2018	Asia	Population (health screen)	5121	1979 (38.6)	54.0 vs 53.7	77.6 vs 62.1	Ultrasound	64 slice MDCT, Agatston method	CAC >10	Age, sex, obesity, DM, HT, HLP, Smk, family history of CAD, hsCRP	9

Continued

Table 3 Continued

Name, year	Study region	Study population	Study size	n (%) NAFLD	Age (NAFLD+ vs NAFLD–)	% male (NAFLD+ vs NAFLD–)	NAFLD assessment	Outcome assessment	Outcome definition	Confounder adjustment	NOS (max=9)
Wu *et al*[82] 2017	Asia	Population based	2345	1272 (54.2)	Overall=55.7	Overall=44.1	Ultrasound	64 slice MDCT, Agatston method	CAC >0	Age, sex, Smk, HT, DM, HC, LDL, physical activity, education, income	9
Kim *et al*[84] 2016	Asia	Population (health screen)	1473	677 (46.0)	–	68.4 vs 47.1	Ultrasound	64 slice MDCT, Agatston method	CAC >0	Age, sex, Smk, alcohol, exercise, BMI, WC, SBP, total cholesterol, TAG, HDL, LDL, blood urea nitrogen, creatinine, glucose, hsCRP	9
Kang *et al*[88] 2015	Asia	Population (health screen)	772	346 (44.8)	50.0 vs 48.6	83.5 vs 55.4	Ultrasound	Presence of calcified coronary plaques	Presence of calcified plaques	Age, Smk, HT, DM, LDL, HDL, MetS	8
Mellinger *et al*[86] 2015	North America	Population (health screen)	3014	512 (17.0)	Overall=51.1	Overall=49.5	CT, liver phantom ratio <0.33	MDCT, Agatston method	CAC >0	Age, sex, alcohol, Smk, menopause, HRT, BMI	9
Kim *et al*[87] 2015	Asia	Population (health screen), postmenopausal women	754	129 (17.1)	59.5 vs 57.1	0.0 vs 0.0	Ultrasound	64 slice MDCT, Agatston method	CAC >0	Age, BMI, SBP, DBP, fasting glucose, total cholesterol, LDL, TAG, HDL, CRP, HOMA-IR	8
VanWagner *et al*[89] 2014	North America	Population based	2424	232 (9.57)	50.5 vs 49.9	58.2 vs 41.1	CT, liver HU ≤40	ECG-gated CT, Agatston method	CAC >0	Age, race, sex, study centre, income, education, alcohol, Smk, physical activity, BMI	9
Sung *et al*[90] 2012	Asia	Population (health screen)	10153	3784 (37.3)	Overall=49.1	Overall=76.3	Ultrasound	64 slice MDCT, Agatston method	CAC >0	Age, sex, TAG, HDL, LDL, WC, SBP, alcohol, Smk, activity, Hx CHD, Hx HTN, Hx DM, HOMA-IR	9
Santos *et al*[91] 2007	South America	Population (health screen)	505	204 (40.4)	48 vs 46	100 vs 100	Ultrasound	EBCT, Agatston method	CAC >0	Age, pulse pressure, BMI, Smk, alcohol, MetS, LDL, TG/HDL ratio, fasting glucose, BP medication, lipid medication, ALT/AST ratio, GGT	9
CAC development/progression											
Cho *et al*[81] 2018	Asia	Population (health screen), MetS(–) participants	798	272 (34.1)	53.4 vs 54.1	91.2 vs 72.2	Ultrasound	64 slice MDCT, Agatston method	Incident CAC or increase by >2.5 units between baseline & final square root of CAC score	Age, sex, BMI, Smk, alcohol, exercise, LDL-C, hsCRP, follow-up interval, baseline CAC score	9
Kang *et al*[96] 2017	Asia	Population (health screen), non-obese participants	447	105 (23.5)	Overall=54.1	Overall=70.9	Ultrasound	64 slice MDCT, Agatston method	Incident CAC or increase by >2.5 units between baseline & final square root of CAC score	Age, sex, WC, alcohol, Smk, exercise, baseline CAC, LDL, hsCRP, follow-up interval	8

Continued

Table 3 Continued

Name, year	Study region	Study population	Study size	n (%) NAFLD	Age (NAFLD+ vs NAFLD−)	% male (NAFLD+ vs NAFLD−)	NAFLD assessment	Outcome assessment	Outcome definition	Confounder adjustment	NOS (max=9)
Kim *et al*[85] 2017	Asia	Population (health screen)	1575	734 (46.6)	40.0 vs 398	94.8 vs 85.0	Ultrasound	64 slice MDCT, Agatston method	Any development (incidence)	Age, sex, ALT, Smk, FBS, LDL, BMI	9
Park *et al*[84] 2016	Asia	Population (health screen)	1732	846 (48.8)	57.1 vs 57.4	81.3 vs 67.7	Ultrasound	256 slice MDCT, Agatston method	Development & progression (>10 CAC from baseline)	Age, sex, BMI, HT, DM, hypercholesterolaemia, TAG, HDL, GFR, Smk, WC, incident DM, lipid- lowering agents	9

BMI, body mass index; CAC, coronary artery calcification; CAD, coronary artery disease; CRP, C reactive protein; CVD, cardiovascular disease; DM, diabetes mellitus; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NOS, Newcastle–Ottawa Scale.

Subgroup analyses

As with the CIMT analysis, we further stratified the associations of NAFLD with CAC score >0 based on ethnicity (figure 6). The pooled associations between NAFLD and CAC score >0 were (OR: 1.21 95% CI 1.10 to 1.33, $P_{heterogeneity}$=0.15, I^2=31.7%, n=10 studies) in Asian populations vs (OR: 1.20 95% CI 1.03 to 1.38, $P_{heterogeneity}$=0.004, I^2=73%, n=5 studies) in Western populations ($P_{difference}$=0.98). There were too few studies to conclusively compare ethnic differences for the associations with CAC score >100, or for the progression/development of CAC.

Evaluation of publication bias

When assessing the studies that investigated the relationships between NAFLD and CIMT, the funnel plot showed asymmetry (online supplemental figures 2 and 3), with studies favouring increased Std Diff in means CIMT (Egger's, p<0.05) and positive ORs for increased CIMT (Egger's, p=0.002). For studies investigating the relationships between NAFLD and CAC outcomes (online supplemental figures 4 and 5), the funnel plots excluded bias with symmetrical distribution of studies on both sides of the mean, while the Egger's test was non-significant (p=0.07 for CAC presence, and p=0.15 for CAC progression/development).

DISCUSSION

In this meta-analysis, we evaluated the associations of NAFLD with two established markers of subclinical atherosclerosis, synthesising the results of 64 published studies with a total of 172 385 patients. In line with existing literature, we have demonstrated that subjects with NAFLD have an increased risk of prevalent subclinical atherosclerosis than those without, even after adjustment for common cardiometabolic risk factors. Our subgroup analyses also revealed these associations to be consistent across both Western and Asian populations. This is also the first meta-analysis to demonstrate that subjects with NAFLD are at increased risk of development and progression of subclinical atherosclerosis. This may provide additional insights into screening and surveillance strategies for patients with NAFLD,[2] potentially identifying higher-risk NAFLD populations, and may also provide further insight into the role of NAFLD in the development of CVD.

Our meta-analysis serves as a timely update to build on the previous work of Zhou *et al*, Kapuria *et al* and Jaruvongvanich *et al*,[23–25] incorporating the results of over 21 new studies published from 2016 and 2020, comprising over 100 000 participants (~50 000 of which have NAFLD). The inclusion of these new studies enables us to conduct a more robust analysis of the differences between ethnic populations, with a larger number of studies conducted in both Western and Asian populations. Our overall findings of the associations between NAFLD and an increased risk of subclinical atherosclerosis (as measured by CIMT and/or CAC score) are in agreement with existing

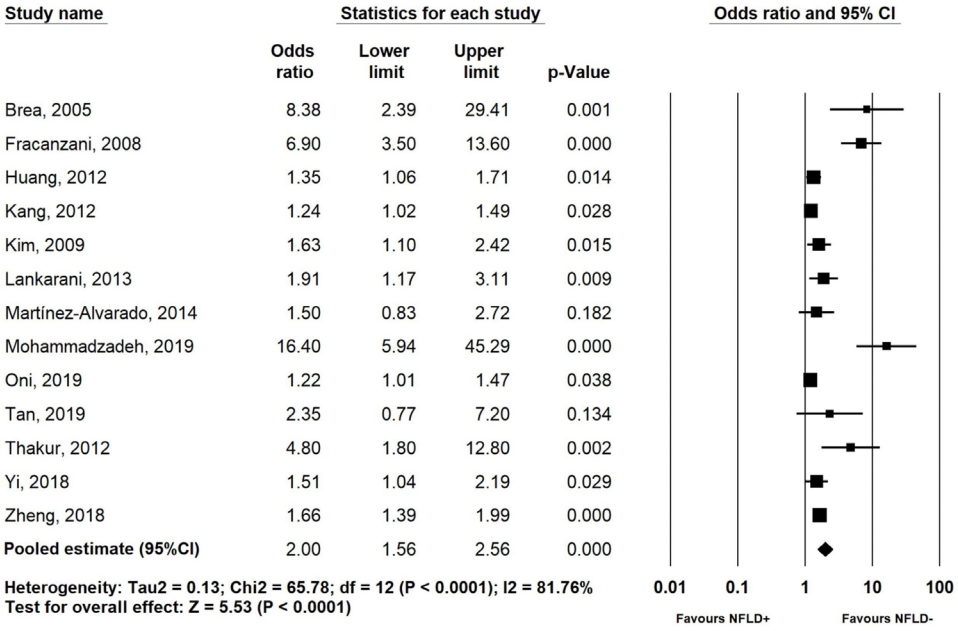

Figure 2 Forest plots showing relationship between NAFLD and presence of increased CIMT. CIMT, carotid intima-media thickness; NFLD, non-alcoholic fatty liver disease.

literature, further reinforcing the findings of previous studies and meta-analyses.[11 12 23–25 97] In addition to these associations with subclinical atherosclerosis, other meta-analyses have also found NAFLD to be significantly associated with increased cardiovascular mortality, coronary artery disease (CAD), incident CVD events, and other subclinical manifestations of CVD including abnormalities in myocardial metabolism, ventricular structure and function.[98–100] Our findings reiterate how the increased risk of CVD in patients with NAFLD can be attributed to an increased underlying subclinical atherosclerotic burden, and suggest that patients with NAFLD should be considered at high risk of atherosclerotic CVD.

Interestingly, we did not observe differential associations between NAFLD and both CAC or CIMT across Asian and Western populations. Our subgroup analyses found similar associations between NAFLD and CAC in both Asian (OR: 1.21 (1.10 to 1.33)) and Western regions (OR: 1.20 (1.03 to 1.38)), with a $P_{difference}$=0.98. Likewise, similar associations between NAFLD and increased CIMT were found across both regions. Despite literature suggesting ethnic differences in the pathogenesis, severity

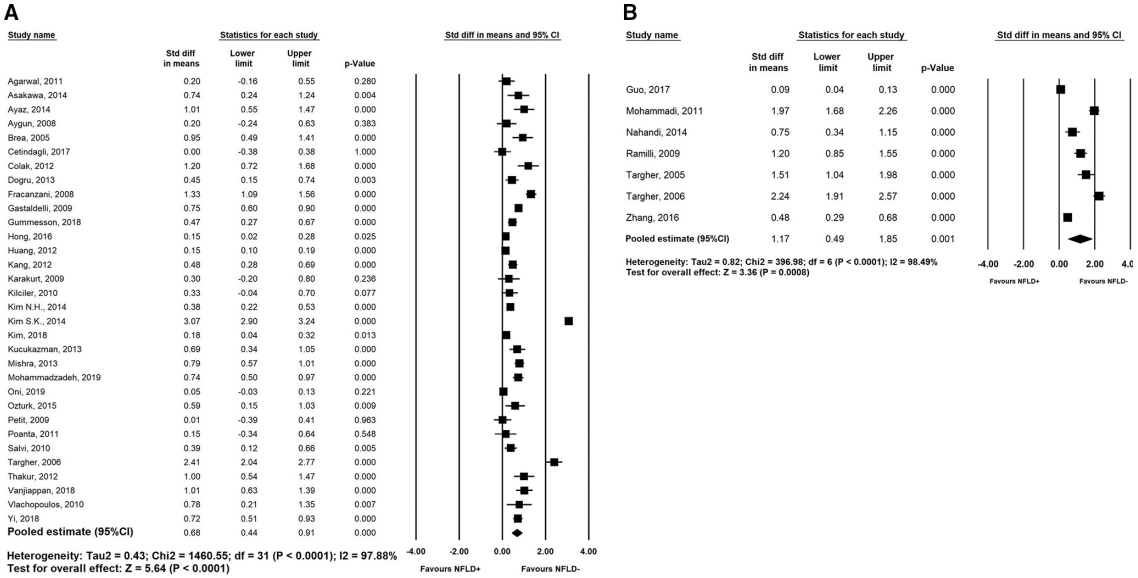

Figure 3 (A) Forest plots showing pooled standard differences in unadjusted CIMT means between NAFLD(+) and NAFLD(–) groups. (B) Forest plots showing pooled standard differences in adjusted CIMT means between NAFLD(+) and NAFLD(–) groups. CIMT, carotid intima-media thickness; NFLD, non-alcoholic fatty liver disease.

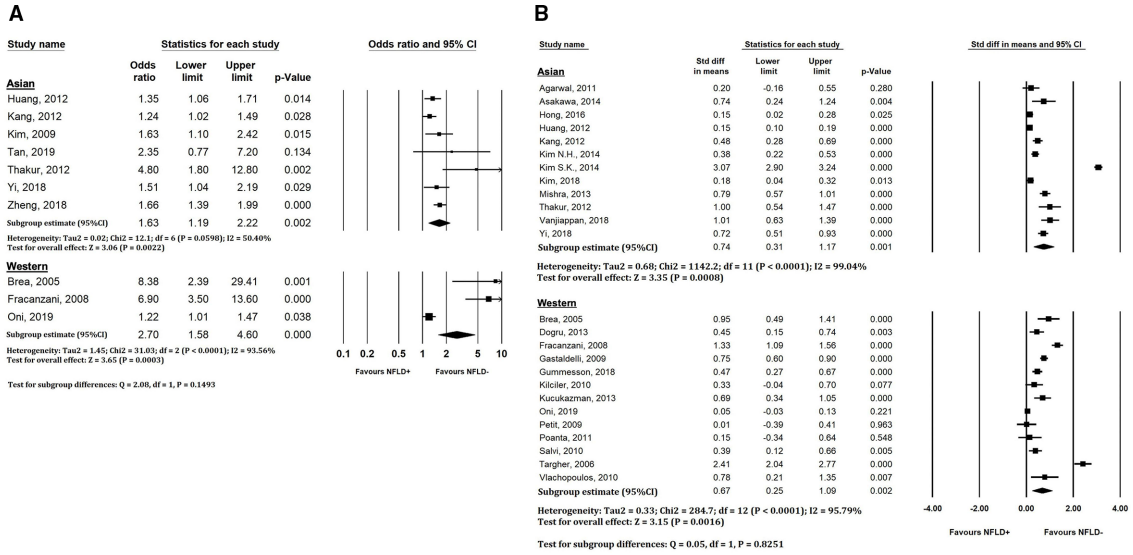

Figure 4 (A) Forest plots showing relationship between NAFLD and presence of increased CIMT, stratified by region of study. (B) Forest plots showing pooled standard differences in CIMT means between NAFLD(+) and NAFLD(–) groups, stratified by region of study. CIMT, carotid intima-media thickness; NFLD, non-alcoholic fatty liver disease.

and outcomes of NAFLD,[14 17 101] remarkably few studies have specifically investigated these ethnic differences in the context of associations with subclinical atherosclerosis. The Multi-Ethnic Study of Atherosclerosis found a positive association between NAFLD and both CAC and increased CIMT in white and Hispanic individuals, but not in Chinese individuals.[21 22] While we did not specifically look at ethnic differences, our results show that NAFLD serves as an important atherogenic risk factor in both Western and Asian populations.

The associations between NAFLD and atherosclerotic CVD were originally considered epiphenomena due to a shared confluence of metabolic risk factors.[102] However, increasingly, evidence has now recognised that NAFLD is an independent risk factor for CVD, with NAFLD thought to play an active role in the systemic release of proatherogenic and proinflammatory mediators, with additional contributions to insulin resistance and abnormal atherogenic lipid profiles, all of which increase the risk of atherogenesis.[3 5 8] These potential pathways and mechanisms are covered in detail in other reviews.[2 3 103] Nonetheless, the interplay between NAFLD, MetS, diabetes and CVD remains complex. Evidence on the effect of NAFLD on subclinical atherosclerosis within subjects with T2DM, for example, remains equivocal.[33 52 71 77] In our subgroup analysis of studies conducted within populations with T2DM, our forest plots did not show significant Std Diff in mean CIMT between those with NAFLD and those

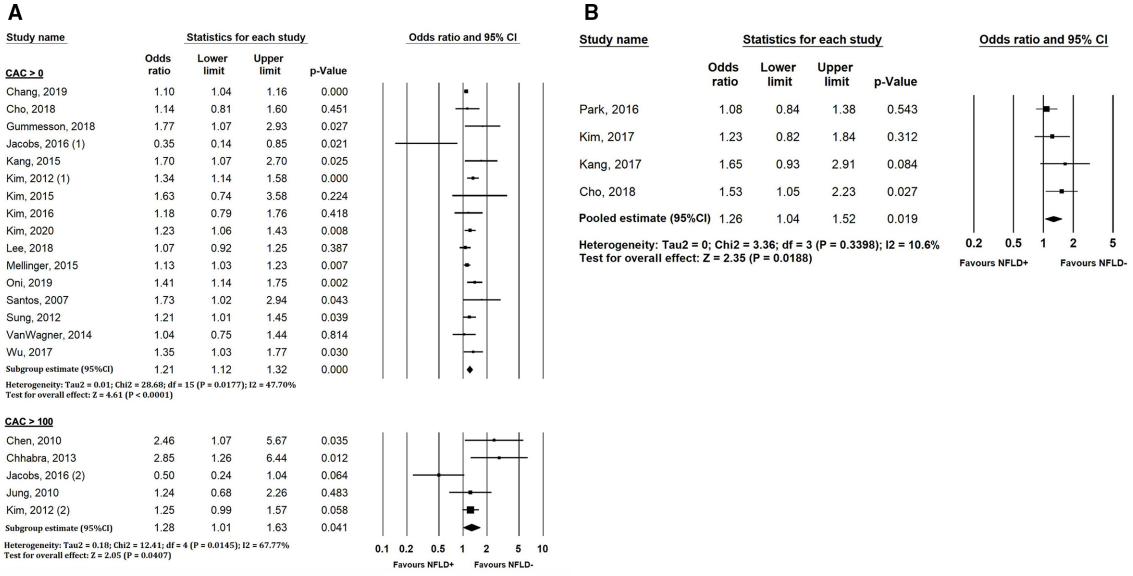

Figure 5 (A) Forest plots showing relationship between NAFLD and CAC scores >0 and >100. (B) Forest plots showing relationship between NAFLD and the development/progression of CAC. CAC, coronary artery calcification; NFLD, non-alcoholic fatty liver disease.

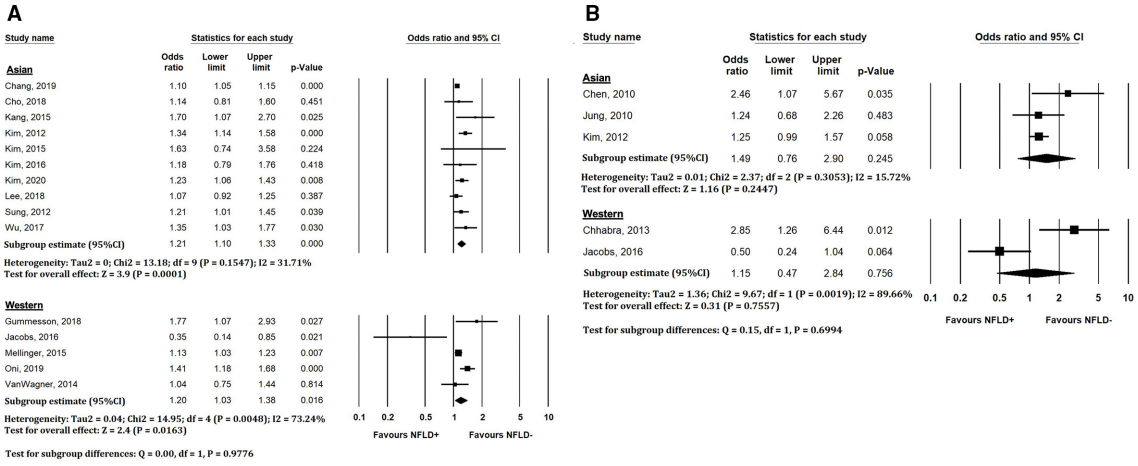

Figure 6 (A) Forest plots showing relationship between NAFLD and CAC score >0, stratified by region of study. (B) Forest plots showing relationship between NAFLD and CAC score >100, stratified by region of study. CAC, coronary artery calcification; NFLD, non-alcoholic fatty liver disease.

without (p=0.107). Diabetes is a potent risk factor for both CAD and CVD, and may have thus masked subtler associations between NAFLD and subclinical atherosclerosis. Alternatively, this may also highlight the role of insulin resistance in mediating the relationship between NAFLD and atherosclerosis.[104]

Only recently have studies begun to investigate the longitudinal associations between NAFLD and CAC progression/development, with this paper being the first meta-analysis to synthesise the results of four studies published from 2016 onwards.[31 34 95 96] We demonstrated that patients with NAFLD are at greater risk of development/progression of CAC, even after adjustment for known confounders. While our results do not elucidate the exact pathophysiological mechanisms by which NAFLD may affect CAC development/progression, they do provide insight into the causal relationship between NAFLD and subclinical atherosclerosis. It should be noted that Park *et al* found differential associations between NAFLD and CAC development and progression, reporting that NAFLD might play a role in the early development of atherosclerosis, but not in the progression to more severe degrees of atherosclerosis.[34] Future studies may be warranted to confirm such observations.

Strengths of our study include the large participant numbers, the assessment of various modalities of subclinical atherosclerosis including CIMT and CAC, the large number of studies from both Western and Asian populations enabling robust analysis of regional differences, and our analysis of not just cross-sectional, but longitudinal outcomes (CAC development and progression). Nonetheless, our results should be interpreted with caution, taking into consideration certain limitations. Heterogeneity was consistently present across the different subclinical atherosclerotic outcomes. This can be attributed to differences in study design, population characteristics, the use of different cut-off definitions for both CAC and increased CIMT, the adjustment for different cardiometabolic confounders and the different modalities of NAFLD diagnosis. In addition, even though liver biopsy remains the gold standard for NAFLD evaluation, US was the most common modality used in the NAFLD assessment in the included studies, and is cited to have diminished accuracy when it comes to the diagnosis of milder hepatic steatosis.[105 106] While we did not find regional differences in the results, we could not perform actual ethnic comparisons as these data were not available. Whether these regional data accurately reflect ethnic data is uncertain and also the influence of cultural and socioeconomic factors cannot be quantified. Nevertheless, this provides one of the first combined regional comparison of such results. Finally, potential publication bias exists with regard to the studies investigating CIMT-related outcomes.

CONCLUSION

In conclusion, this meta-analysis reports a significant positive association between NAFLD and subclinical atherosclerosis, as defined by increased CIMT and CAC scores. These observed associations are not just cross-sectional, but also longitudinal, and are seen across both Western and Asian populations. These results re-emphasise the importance of early risk evaluation and prophylactic intervention measures to preclude progression to clinical CVD in NAFLD.

Contributors MYZW—conception of idea, crafting of research question, design of inclusion/exclusion criteria, collection of data (literature search), statistical analysis, quality (risk of bias) evaluation, figure creation, writing of the manuscript, and writing and drafting of the manuscript, guarantor. JJLY—conception of idea, crafting of research question, design of inclusion/exclusion criteria, collection of data (literature search), drafting of the manuscript and editing of the manuscript. RS—design of inclusion/exclusion criteria, collection of data (literature search), statistical analysis, quality (risk of bias) evaluation, figure creation and editing of the manuscript, guarantor. MC—conception of idea, crafting of research question, design of inclusion/exclusion criteria and editing of the manuscript. GBBG—conception of idea, crafting of research question, design of inclusion/exclusion criteria and editing of the manuscript. KKY—conception of idea, crafting of research question, design of inclusion/exclusion criteria, editing of the manuscript and guarantor.

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REFERENCES

1 Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002;346:1221–31.
2 Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013;10:330–44.
3 Stahl EP, Dhindsa DS, Lee SK, *et al*. Nonalcoholic fatty liver disease and the Heart: JACC state-of-the-art review. *J Am Coll Cardiol* 2019;73:948–63.
4 Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013;10:686–90.
5 Adams LA, Anstee QM, Tilg H, *et al*. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut* 2017;66:1138–53.
6 Marchesini G, Brizi M, Bianchi G, *et al*. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001;50:1844–50.
7 Musso G, Gambino R, Bo S, *et al*. Should nonalcoholic fatty liver disease be included in the definition of metabolic syndrome?: a cross-sectional comparison with adult treatment panel III criteria in nonobese nondiabetic subjects. *Diabetes Care* 2008;31:562–8.
8 Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* 2010;363:1341–50.
9 Fuchs M, Sanyal AJ. Lipotoxicity in NASH. *J Hepatol* 2012;56:291–3.
10 Targher G, Bertolini L, Rodella S, *et al*. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. *Diabetes Care* 2007;30:2119–21.
11 Oni ET, Agatston AS, Blaha MJ, *et al*. A systematic review: burden and severity of subclinical cardiovascular disease among those with nonalcoholic fatty liver; should we care? *Atherosclerosis* 2013;230:258–67.
12 Sookoian S, Pirola CJ. Non-Alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. *J Hepatol* 2008;49:600–7.
13 Cai J, Zhang S, Huang W. Association between nonalcoholic fatty liver disease and carotid atherosclerosis: a meta-analysis. *Int J Clin Exp Med* 2015;8:7673–8.
14 Farrell GC, Wong VW-S, Chitturi S. Nafld in Asia—as common and important as in the West. *Nat Rev Gastroenterol Hepatol* 2013;10:307–18.
15 Mohanty SR, Troy TN, Huo D, *et al*. Influence of ethnicity on histological differences in non-alcoholic fatty liver disease. *J Hepatol* 2009;50:797–804.
16 Rich NE, Oji S, Mufti AR, *et al*. Racial and ethnic disparities in nonalcoholic fatty liver disease prevalence, severity, and outcomes in the United States: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2018;16:198–210.
17 Pan J-J, Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. *World J Hepatol* 2014;6:274–83.
18 Wong RJ, Ahmed A. Obesity and non-alcoholic fatty liver disease: disparate associations among Asian populations. *World J Hepatol* 2014;6:263–73.
19 Yoon K-H, Lee J-H, Kim J-W, *et al*. Epidemic obesity and type 2 diabetes in Asia. *The Lancet* 2006;368:1681–8.
20 Remigio-Baker RA, Allison MA, Forbang NI, *et al*. Race/ethnic and sex disparities in the non-alcoholic fatty liver disease-abdominal aortic calcification association: the multi-ethnic study of atherosclerosis. *Atherosclerosis* 2017;258:89–96.
21 Al Rifai M, Silverman MG, Nasir K, *et al*. The association of nonalcoholic fatty liver disease, obesity, and metabolic syndrome, with systemic inflammation and subclinical atherosclerosis: the multi-ethnic study of atherosclerosis (MESA). *Atherosclerosis* 2015;239:629–33.
22 Oni E, Budoff MJ, Zeb I, *et al*. Nonalcoholic fatty liver disease is associated with arterial distensibility and carotid intima-media thickness: (from the multi-ethnic study of atherosclerosis). *Am J Cardiol* 2019;124:534–8.
23 Jaruvongvanich V, Wirunsawanya K, Sanguankeo A, *et al*. Nonalcoholic fatty liver disease is associated with coronary artery calcification: a systematic review and meta-analysis. *Digestive and Liver Disease* 2016;48:1410–7.
24 Kapuria D, Takyar VK, Etzion O, *et al*. Association of hepatic steatosis with subclinical atherosclerosis: systematic review and Meta-Analysis. *Hepatology Communications* 2018;2:877–87.
25 Zhou Y-Y, Zhou X-D, Wu S-J, *et al*. Nonalcoholic fatty liver disease contributes to subclinical atherosclerosis: a systematic review and meta-analysis. *Hepatol Commun* 2018;2:376–92.
26 Kim S-H, Park HY, Lee HS, *et al*. Association between non-alcoholic fatty liver disease and coronary calcification depending on sex and obesity. *Sci Rep* 2020;10:1025.
27 Chang Y, Ryu S, Sung K-C, *et al*. Alcoholic and non-alcoholic fatty liver disease and associations with coronary artery calcification: evidence from the Kangbuk Samsung health study. *Gut* 2019;68:1667–75.
28 Zheng J, Zhou Y, Zhang K, *et al*. Association between nonalcoholic fatty liver disease and subclinical atherosclerosis: a cross-sectional study on population over 40 years old. *BMC Cardiovasc Disord* 2018;18:147.
29 Lee SB, Park G-M, Lee J-Y, *et al*. Association between non-alcoholic fatty liver disease and subclinical coronary atherosclerosis: an observational cohort study. *J Hepatol* 2018;68:1018–24.
30 Kim H-J, Park H-B, Suh Y, *et al*. Comparison of carotid intima-media thickness and coronary artery calcium score for estimating subclinical atherosclerosis in patients with fatty liver disease. *Cardiovasc J Afr* 2018;29:93–8.
31 Cho YK, Kang YM, Yoo JH, *et al*. The impact of non-alcoholic fatty liver disease and metabolic syndrome on the progression of coronary artery calcification. *Sci Rep* 2018;8:12004.
32 Wu R, Hou F, Wang X, *et al*. Nonalcoholic fatty liver disease and coronary artery calcification in a northern Chinese population: a cross sectional study. *Sci Rep* 2017;7:9933.
33 Guo K, Zhang L, Lu J, *et al*. Non-Alcoholic fatty liver disease is associated with late but not early atherosclerotic lesions in Chinese inpatients with type 2 diabetes. *J Diabetes Complications* 2017;31:80–5.
34 Park HE, Kwak M-S, Kim D, *et al*. Nonalcoholic fatty liver disease is associated with coronary artery calcification development: a longitudinal study. *J Clin Endocrinol Metab* 2016;101:3134–43.
35 Hong HC, Hwang SY, Ryu JY, *et al*. The synergistic impact of nonalcoholic fatty liver disease and metabolic syndrome on subclinical atherosclerosis. *Clin Endocrinol* 2016;84:203–9.
36 Stroup DF, Berlin JA, Morton SC, *et al*. Meta-analysis of observational studies in epidemiology: a proposal for reporting. meta-analysis of observational studies in epidemiology (moose) group. *JAMA* 2000;283:2008–12.
37 Liberati A, Altman DG, Tetzlaff J, *et al*. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med* 2009;6:e1000100.
38 Deeks JJ, Dinnes J, D'Amico R. Evaluating non-randomised intervention studies. In: *Health technology assessment*. Winchester, England, 2003: 7. 1–173.
39 Herzog R, Álvarez-Pasquin MJ, Díaz C, *et al*. Are healthcare workers' intentions to vaccinate related to their knowledge, beliefs and attitudes? A systematic review. *BMC Public Health* 2013;13:154.
40 Wells GSB, O'Connell J, Robertson J. The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analysis, 2011. Available: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

41 McPheeters ML, Kripalani S, Peterson NB, *et al*. Closing the quality gap: revisiting the state of the science (Vol. 3: quality improvement interventions to address health disparities). *Evid Rep Technol Assess* 2012:1–475.
42 Chmielowska M, Fuhr DC. Intimate partner violence and mental ill health among global populations of Indigenous women: a systematic review. *Soc Psychiatry Psychiatr Epidemiol* 2017;52:689–704.
43 Lo CK-L, Mertz D, Loeb M. Newcastle-Ottawa scale: comparing reviewers' to authors' assessments. *BMC Med Res Methodol* 2014;14:45.
44 Mohammadzadeh A, Shahkarami V, Shakiba M, *et al*. Association of non-alcoholic fatty liver disease with increased carotid intima-media thickness considering other cardiovascular risk factors. *Iranian Journal of Radiology* 2019;16.
45 Yi X, Liu Y-H, Zhou X-F, *et al*. The influence of abdominal and ectopic fat accumulation on carotid intima-media thickness: a Chongqing study. *J Stroke Cerebrovasc Dis* 2018;27:1992–7.
46 Vanjiappan S, Hamide A, Ananthakrishnan R, *et al*. Nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and its association with cardiovascular disease. *Diabetes Metab Syndr* 2018;12:479–82.
47 Gummesson A, Strömberg U, Schmidt C, *et al*. Non-alcoholic fatty liver disease is a strong predictor of coronary artery calcification in metabolically healthy subjects: a cross-sectional, population-based study in middle-aged subjects. *PLoS One* 2018;13:e0202666.
48 Cetindağlı I, Kara M, Tanoglu A, *et al*. Evaluation of endothelial dysfunction in patients with nonalcoholic fatty liver disease: association of selenoprotein P with carotid intima-media thickness and endothelium-dependent vasodilation. *Clin Res Hepatol Gastroenterol* 2017;41:516–24.
49 Zhang L, Guo K, Lu J, *et al*. Nonalcoholic fatty liver disease is associated with increased carotid intima-media thickness in type 1 diabetic patients. *Sci Rep* 2016;6:26805.
50 Ozturk K, Uygun A, Guler AK, *et al*. Nonalcoholic fatty liver disease is an independent risk factor for atherosclerosis in young adult men. *Atherosclerosis* 2015;240:380–6.
51 Nahandi MZ, Khoshbaten M, Ramazanzadeh E, *et al*. Effect of non-alcoholic fatty liver disease on carotid artery intima-media thickness as a risk factor for atherosclerosis. *Gastroenterol Hepatol Bed Bench* 2014;7:55–62.
52 Kim S-K, Choi YJ, Huh BW, *et al*. Nonalcoholic fatty liver disease is associated with increased carotid intima-media thickness only in type 2 diabetic subjects with insulin resistance. *J Clin Endocrinol Metab* 2014;99:1879–84.
53 Kim NH, Park J, Kim SH, *et al*. Non-alcoholic fatty liver disease, metabolic syndrome and subclinical cardiovascular changes in the general population. *Heart* 2014;100:938–43.
54 Ayaz T, Kirbas A, Durakoglugil T, *et al*. The relation between carotid intima media thickness and serum osteoprotegerin levels in nonalcoholic fatty liver disease. *Metab Syndr Relat Disord* 2014;12:283–9.
55 Asakawa Y, Takekawa H, Suzuki K, *et al*. Relationship between fatty liver disease and intracranial main artery stenosis. *Intern Med* 2014;53:1259–64.
56 Mishra S, Yadav D, Gupta M, *et al*. A study of carotid atherosclerosis in patients with non-alcoholic fatty liver disease. *Ind J Clin Biochem* 2013;28:79–83.
57 Kucukazman M, Ata N, Yavuz B, *et al*. Evaluation of early atherosclerosis markers in patients with nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 2013;25:147–51.
58 Dogru T, Genc H, Tapan S, *et al*. Plasma fetuin-A is associated with endothelial dysfunction and subclinical atherosclerosis in subjects with nonalcoholic fatty liver disease. *Clin Endocrinol* 2013;78:712–7.
59 Thakur ML, Sharma S, Kumar A, *et al*. Nonalcoholic fatty liver disease is associated with subclinical atherosclerosis independent of obesity and metabolic syndrome in Asian Indians. *Atherosclerosis* 2012;223:507–11.
60 Kim D, Choi S-Y, Park EH, *et al*. Nonalcoholic fatty liver disease is associated with coronary artery calcification. *Hepatology* 2012;56:605–13.
61 Kang JH, Cho KI, Kim SM, *et al*. Relationship between nonalcoholic fatty liver disease and carotid artery atherosclerosis beyond metabolic disorders in non-diabetic patients. *J Cardiovasc Ultrasound* 2012;20:126–33.
62 Huang Y, Bi Y, Xu M, *et al*. Nonalcoholic fatty liver disease is associated with atherosclerosis in middle-aged and elderly Chinese. *Arterioscler Thromb Vasc Biol* 2012;32:2321–6.
63 Colak Y, Karabay CY, Tuncer I, *et al*. Relation of epicardial adipose tissue and carotid intima-media thickness in patients with nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 2012;24:613–8.
64 Poanta LI, Albu A, Fodor D. Association between fatty liver disease and carotid atherosclerosis in patients with uncomplicated type 2 diabetes mellitus. *Med Ultrason* 2011;13:215–9.
65 Mohammadi A, Bazazi A, Ghasemi-Rad M. Evaluation of atherosclerotic findings in patients with nonalcoholic fatty liver disease. *Int J Gen Med* 2011;4:717–22.
66 Agarwal AK, Jain V, Singla S, *et al*. Prevalence of non-alcoholic fatty liver disease and its correlation with coronary risk factors in patients with type 2 diabetes. *J Assoc Physicians India* 2011;59:351–4.
67 Vlachopoulos C, Manesis E, Baou K, *et al*. Increased arterial stiffness and impaired endothelial function in nonalcoholic fatty liver disease: a pilot study. *Am J Hypertens* 2010;23:1183–9.
68 Salvi P, Ruffini R, Agnoletti D, *et al*. Increased arterial stiffness in nonalcoholic fatty liver disease: the Cardio-GOOSE study. *J Hypertens* 2010;28:1699–707.
69 Kilciler G, Genc H, Tapan S, *et al*. Mean platelet volume and its relationship with carotid atherosclerosis in subjects with non-alcoholic fatty liver disease. *Ups J Med Sci* 2010;115:253–9.
70 Ramilli S, Pretolani S, Muscari A, *et al*. Carotid lesions in outpatients with nonalcoholic fatty liver disease. *World J Gastroenterol* 2009;15:4770–4.
71 Petit JM, Guiu B, Terriat B, *et al*. Nonalcoholic fatty liver is not associated with carotid intima-media thickness in type 2 diabetic patients. *J Clin Endocrinol Metab* 2009;94:4103–6.
72 Karakurt F, Carlioglu A, Koktener A, *et al*. Relationship between cerebral arterial Pulsatility and carotid intima media thickness in diabetic and non-diabetic patients with non-alcoholic fatty liver disease. *J Endocrinol Invest* 2009;32:63–8.
73 Gastaldelli A, Kozakova M, Højlund K, *et al*. Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early atherosclerosis in a large European population. *Hepatology* 2009;49:1537–44.
74 Fracanzani AL, Burdick L, Raselli S, *et al*. Carotid artery intima-media thickness in nonalcoholic fatty liver disease. *Am J Med* 2008;121:72–8.
75 Aygun C, Kocaman O, Sahin T, *et al*. Evaluation of metabolic syndrome frequency and carotid artery intima-media thickness as risk factors for atherosclerosis in patients with nonalcoholic fatty liver disease. *Dig Dis Sci* 2008;53:1352–7.
76 Targher G, Bertolini L, Padovani R, *et al*. Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. *Diabetes Care* 2006;29:1325–30.
77 Targher G, Bertolini L, Padovani R, *et al*. Non-Alcoholic fatty liver disease is associated with carotid artery wall thickness in diet-controlled type 2 diabetic patients. *J Endocrinol Invest* 2006;29:55–60.
78 Targher G, Bertolini L, Padovani R, *et al*. Associations between liver histology and carotid intima-media thickness in patients with nonalcoholic fatty liver disease. *Arterioscler Thromb Vasc Biol* 2005;25:2687–8.
79 Brea A, Mosquera D, Martín E, *et al*. Nonalcoholic fatty liver disease is associated with carotid atherosclerosis: a case-control study. *Arterioscler Thromb Vasc Biol* 2005;25:1045–50.
80 Tan EC-L, Tai M-LS, Chan W-K, *et al*. Association between non-alcoholic fatty liver disease evaluated by transient elastography with extracranial carotid atherosclerosis in a multiethnic Asian community. *JGH Open* 2019;3:117–25.
81 Martínez-Alvarado MDR, Juárez-Rojas JG, Medina-Urrutia AX, *et al*. Association of fatty liver with cardiovascular risk factors and subclinical atherosclerosis in a Mexican population. *Rev Invest Clin* 2014;66:407–14.
82 Lankarani KB, Mahmoodi M, Lotfi M, *et al*. Common carotid intima-media thickness in patients with non-alcoholic fatty liver disease: a population-based case-control study. *Korean J Gastroenterol* 2013;62:344–51.
83 Kim HC, Kim DJ, Huh KB. Association between nonalcoholic fatty liver disease and carotid intima-media thickness according to the presence of metabolic syndrome. *Atherosclerosis* 2009;204:521–5.
84 Kim BJ, Cheong ES, Kang JG, *et al*. Relationship of epicardial fat thickness and nonalcoholic fatty liver disease to coronary artery calcification: from the Caesar study. *J Clin Lipidol* 2016;10:619–26.
85 Jacobs K, Brouha S, Bettencourt R, *et al*. Association of nonalcoholic fatty liver disease with visceral adiposity but not coronary artery calcification in the elderly. *Clinical Gastroenterology and Hepatology* 2016;14:1337–44.
86 Mellinger JL, Pencina KM, Massaro JM, *et al*. Hepatic steatosis and cardiovascular disease outcomes: an analysis of the Framingham heart study. *J Hepatol* 2015;63:470–6.

87 Kim MK, Ahn CW, Nam JS, *et al*. Association between nonalcoholic fatty liver disease and coronary artery calcification in postmenopausal women. *Menopause* 2015;22:1323–7.
88 Kang MK, Kang BH, Kim JH. Nonalcoholic fatty liver disease is associated with the presence and morphology of subclinical coronary atherosclerosis. *Yonsei Med J* 2015;56:1288–95.
89 VanWagner LB, Ning H, Lewis CE, *et al*. Associations between nonalcoholic fatty liver disease and subclinical atherosclerosis in middle-aged adults: the coronary artery risk development in young adults study. *Atherosclerosis* 2014;235:599–605.
90 Sung K-C, Wild SH, Kwag HJ, *et al*. Fatty liver, insulin resistance, and features of metabolic syndrome: relationships with coronary artery calcium in 10,153 people. *Diabetes Care* 2012;35:2359–64.
91 Santos RD, Nasir K, Conceição RD, *et al*. Hepatic steatosis is associated with a greater prevalence of coronary artery calcification in asymptomatic men. *Atherosclerosis* 2007;194:517–9.
92 Chhabra R, O'Keefe JH, Patil H, *et al*. Association of coronary artery calcification with hepatic steatosis in asymptomatic individuals. *Mayo Clin Proc* 2013;88:1259–65.
93 Jung D-H, Lee Y-J, Ahn H-Y, *et al*. Relationship of hepatic steatosis and alanine aminotransferase with coronary calcification. *Clin Chem Lab Med* 2010;48:1829–34.
94 Chen C-H, Nien C-K, Yang C-C, *et al*. Association between nonalcoholic fatty liver disease and coronary artery calcification. *Dig Dis Sci* 2010;55:1752–60.
95 Kim J, Lee DY, Park SE, *et al*. Increased risk for development of coronary artery calcification in subjects with non-alcoholic fatty liver disease and systemic inflammation. *PLoS One* 2017;12:e0180118.
96 Kang YM, Jung CH, Cho YK, *et al*. Fatty liver disease determines the progression of coronary artery calcification in a metabolically healthy obese population. *PLoS One* 2017;12:e0175762.
97 Ampuero J, Gallego-Durán R, Romero-Gómez M. Association of NAFLD with subclinical atherosclerosis and coronary-artery disease: meta-analysis. *Rev Esp Enferm Dig* 2015;107:10–16.
98 Bonci E, Chiesa C, Versacci P, *et al*. Association of nonalcoholic fatty liver disease with subclinical cardiovascular changes: a systematic review and meta-analysis. *Biomed Res Int* 2015;2015:1–11.
99 Musso G, Gambino R, Cassader M, *et al*. 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;43:617–49.
100 Targher G, Byrne CD, Lonardo A, *et al*. Non-Alcoholic fatty liver disease and risk of incident cardiovascular disease: a meta-analysis. *J Hepatol* 2016;65:589–600.
101 Szanto KB, Li J, Cordero P, *et al*. Ethnic differences and heterogeneity in genetic and metabolic makeup contributing to nonalcoholic fatty liver disease. *Diabetes Metab Syndr Obes* 2019;12:357–67.
102 McKimmie RL, Daniel KR, Carr JJ, *et al*. Hepatic steatosis and subclinical cardiovascular disease in a cohort enriched for type 2 diabetes: the diabetes heart study. *Am J Gastroenterol* 2008;103:3029–35.
103 Francque SM, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: pathophysiological mechanisms and implications. *J Hepatol* 2016;65:425–43.
104 Gaggini M, Morelli M, Buzzigoli E, *et al*. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. *Nutrients* 2013;5:1544–60.
105 Hernaez R, Lazo M, Bonekamp S, *et al*. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011;54:1082–90.
106 Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. *WJG* 2014;20:7392–402.