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

Mark Yu Zheng Wong, Jonathan Jiunn Liang Yap, Rehena Sultana, Mark Cheah, George Boon Bee Goh, Khung Keong Yeo

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 I². 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. NAFLD has increasingly been recognised as the hepatic manifestation of the metabolic syndrome (MetS), and is one facet of a multi-system disease, with close relations to abdominal obesity, type 2 diabetes mellitus (T2DM), insulin resistance and hyperlipidaemia.2–7

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.3–5,9–11 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.3,5,8,12 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.3,5,12,13

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, Asian and Hispanic populations,15,16 and have been attributed to factors including lifestyle, environment, insulin resistance, body composition (adipose distribution and muscle bulk) and genetics.14,15,17–19 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 statement36 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 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. 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.

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 I². A value of I² 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 104981 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 41189 individuals, assessed the effect of NAFLD on CIMT. Thirty-nine studies investigated the mean differences in CIMT between NAFLD and controls, while 13 studies used logistic regression to quantify the associations.
between NAFLD and an ‘increased CIMT’. 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, $P_{	ext{heterogeneity}}<0.001$, $\Gamma^2=81.8\%$, 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, $P_{\text{heterogeneity}}=0.06$, $\Gamma^2=50\%$, n=7 studies) in Asian populations vs (OR: 2.70, 95% CI: 1.58 to 4.60, $P_{\text{heterogeneity}}<0.001$, $\Gamma^2=93.6\%$, n=3 studies) in Western populations ($P_{\text{difference}}=0.15$). 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 ($P_{\text{difference}}=0.83$) (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 136294 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, five studies investigated the cross-sectional associations between NAFLD and the presence of CAC score >100, and four studies investigated the longitudinal influence of NAFLD on CAC score progression/development. Fifty studies investigated the cross-sectional associations between NAFLD and the presence of CAC score >100, and four studies investigated the longitudinal influence of NAFLD on CAC score progression/development. 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, $P_{\text{heterogeneity}}=0.018$, $\Gamma^2=47.7\%$), and CAC score >100 (pooled OR: 1.28, 95% CI 1.01 to 1.63, $P_{\text{heterogeneity}}=0.015$, $\Gamma^2=67.8\%$) (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, $P_{\text{heterogeneity}}=0.34$, $\Gamma^2=10.6\%$) (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

<table>
<thead>
<tr>
<th>Name, year</th>
<th>Study region</th>
<th>Study population</th>
<th>Study size n (%)</th>
<th>NAFLD</th>
<th>Age (NAFLD+ vs NAFLD−)</th>
<th>% male (NAFLD+ vs NAFLD−)</th>
<th>NAFLD assessment</th>
<th>Outcome assessment</th>
<th>Confounder adjustment</th>
<th>NOS (max=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oni et al* 2019</td>
<td>North America</td>
<td>Population based</td>
<td>4123</td>
<td>729 (17.7)</td>
<td>61 vs 63</td>
<td>47.0 vs 44.0</td>
<td>CT, LS ratio &lt;1</td>
<td>Ultrasound, mean IMT (L&amp;R)</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>Mohammadzadeh et al* 2019</td>
<td>Iran</td>
<td>Hospital based</td>
<td>300</td>
<td>150 (50.0)</td>
<td>49.9 vs 52.5</td>
<td>65.3 vs 57.3</td>
<td>Ultrasound</td>
<td>Ultrasound, mean IMT (L&amp;R)</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>Yi et al* 2018</td>
<td>Asia</td>
<td>Outpatient clinic</td>
<td>1981</td>
<td>1888 (95.3)</td>
<td>45.9 vs 44.8</td>
<td>63.4 vs 40.1</td>
<td>Ultrasound</td>
<td>Ultrasound, mean of max IMT (L&amp;R)</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>Kim et al* 2018</td>
<td>Asia</td>
<td>Population (health screen)</td>
<td>819</td>
<td>330 (40.3)</td>
<td>53.4 vs 53.1</td>
<td>64.2 vs 41.5</td>
<td>Ultrasound</td>
<td>Ultrasound, mean IMT (L&amp;R)</td>
<td>–</td>
<td>6</td>
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<tr>
<td>Venjiappan et al* 2018</td>
<td>Asia</td>
<td>Hospital based, patients with T2DM</td>
<td>124</td>
<td>73 (58.9)</td>
<td>Overall=53.8</td>
<td>Overall=54.0</td>
<td>Ultrasound</td>
<td>Ultrasound, mean of max IMT (L&amp;R)</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>Gummesson et al* 2018</td>
<td>Europe</td>
<td>Population based</td>
<td>1015</td>
<td>106 (10.4)</td>
<td>58.3 vs 57.5</td>
<td>71.7 vs 52.5</td>
<td>CT, liver HU &lt;40</td>
<td>Ultrasound, mean IMT</td>
<td>–</td>
<td>7</td>
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<tr>
<td>Cetindağ et al* 2017</td>
<td>Turkey</td>
<td>Outpatient clinic</td>
<td>120</td>
<td>93 (77.5)</td>
<td>34.5 vs 33.8</td>
<td>100 vs 100</td>
<td>Ultrasound and biopsy</td>
<td>Ultrasound, mean IMT (6 measurements)</td>
<td>Age/sex-matched controls</td>
<td>7</td>
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<tr>
<td>Guo et al* 2017</td>
<td>Asia</td>
<td>Hospital based, patients with T2DM</td>
<td>8571</td>
<td>4340 (50.6)</td>
<td>57.4 vs 61.9</td>
<td>54.6 vs 55.9</td>
<td>Ultrasound</td>
<td>Ultrasound, mean IMT (6 measurements)</td>
<td>Age</td>
<td>7</td>
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<tr>
<td>Hong et al* 2016</td>
<td>Asia</td>
<td>Population (health screen)</td>
<td>955</td>
<td>342 (35.8)</td>
<td>53 vs 51 (median)</td>
<td>48.8 vs 42.1</td>
<td>Ultrasound</td>
<td>Ultrasound, mean IMT (99 computer points)</td>
<td>–</td>
<td>7</td>
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<tr>
<td>Zhang et al* 2016</td>
<td>Asia</td>
<td>Outpatient clinic, patients with T1DM</td>
<td>722</td>
<td>123 (17.0)</td>
<td>47.4 vs 46.0</td>
<td>52.8 vs 51.1</td>
<td>Ultrasound</td>
<td>Ultrasound, mean IMT (6 measurements)</td>
<td>Age, sex, BMI, WC, SBP, DBP, total cholesterol, TAG, LDL, HDL, MetS, ALT, AST, GGT, hsCRP, medications</td>
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<td>Ozturk et al* 2015</td>
<td>Turkey</td>
<td>Outpatient clinic, MetS(−)</td>
<td>82</td>
<td>41 (50.0)</td>
<td>32.8 vs 31.8</td>
<td>100 vs 100</td>
<td>Biopsy</td>
<td>Ultrasound, mean IMT (L&amp;R)</td>
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<tr>
<td>Asakawa et al* 2014</td>
<td>Asia</td>
<td>Population (health screen)</td>
<td>76</td>
<td>24 (31.6)</td>
<td>61.5 vs 61.0 (median)</td>
<td>91.7 vs 75.0</td>
<td>Ultrasound</td>
<td>Ultrasound, max IMT</td>
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<td>Ayaz et al* 2014</td>
<td>Turkey</td>
<td>Outpatient clinic</td>
<td>90</td>
<td>60 (66.7)</td>
<td>44.5 vs 39.5 (median)</td>
<td>36.7 vs 26.7</td>
<td>Ultrasound</td>
<td>Ultrasound, mean IMT (8 measurements)</td>
<td>–</td>
<td>6</td>
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<tr>
<td>Kim et al* 2014</td>
<td>Asia</td>
<td>Population (health screen), MetS(−)</td>
<td>1285</td>
<td>180 (14.0)</td>
<td>55.7 vs 55.7</td>
<td>58.0 vs 36.0</td>
<td>CT, liver minus spleen &lt;5</td>
<td>Ultrasound, mean IMT (4 measurements)</td>
<td>–</td>
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<tr>
<th>Name, year</th>
<th>Study region</th>
<th>Study population</th>
<th>Study size n (%)</th>
<th>Age (NAFLD+ vs NAFLD−)</th>
<th>% male (NAFLD+ vs NAFLD−)</th>
<th>NAFLD assessment</th>
<th>Outcome assessment</th>
<th>Confounder adjustment</th>
<th>NOS (max=9)</th>
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<tr>
<td>Kim et al 2014</td>
<td>Asia</td>
<td>Hospital based, patients with T2DM</td>
<td>1211</td>
<td>74 (61.7)</td>
<td>56.7 vs 55.6</td>
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<td>Nahandi et al 2014</td>
<td>Iran</td>
<td>Hospital based, patients without diabetes</td>
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<td>43.3 vs 43.1</td>
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<td>HLP, sex, Smk, HT, obesity, walking, liver enzymes</td>
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<td>Dogru et al 2013</td>
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<td>Outpatient clinic</td>
<td>189</td>
<td>115 (60.8)</td>
<td>31 vs 28 (median)</td>
<td>100 vs 100</td>
<td>Liver biopsy</td>
<td>Ultrasound, mean IMT (L&amp;R)</td>
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<td>161</td>
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<td>44 vs 32</td>
<td>Ultrasound</td>
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<td>Mishra et al 2013</td>
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<td>645</td>
<td>101 (15.7)</td>
<td>31.6 vs 27.1</td>
<td>100 vs 100</td>
<td>Ultrasound</td>
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<td>Huang et al 2012</td>
<td>Asia</td>
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<td>8632</td>
<td>250 (30.0)</td>
<td>58.5 vs 58.5</td>
<td>31.4 vs 30.9</td>
<td>Ultrasound</td>
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<td>Kang et al 2012</td>
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<td>Thakur et al 2012</td>
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<td>80</td>
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<td>67.5 vs 67.5</td>
<td>Ultrasound</td>
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<td>Colak et al 2012</td>
<td>Turkey</td>
<td>Outpatient clinic</td>
<td>87</td>
<td>57 (65.5)</td>
<td>44.2 vs 42.7</td>
<td>45.6 vs 46.7</td>
<td>Liver biopsy</td>
<td>Ultrasound, mean IMT (6 measurements)</td>
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<td>Agarwal et al 2011</td>
<td>Asia</td>
<td>Hospital based, patients with T2DM</td>
<td>124</td>
<td>71 (57.3)</td>
<td>57 vs 61</td>
<td>52.5 vs 58.5</td>
<td>Ultrasound</td>
<td>–</td>
<td>6</td>
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<td>Mohammadi et al 2011</td>
<td>Iran</td>
<td>Hospital based</td>
<td>335</td>
<td>250 (74.6)</td>
<td>46.6 vs 44.9</td>
<td>55.6 vs 54.1</td>
<td>Ultrasound</td>
<td>HT, DM, HLP, hyperglycaemia</td>
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<td>Poanta et al 2011</td>
<td>Europe</td>
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<td>56</td>
<td>38 (67.9)</td>
<td>59.4 vs 61.5</td>
<td>50.0 vs 83.3</td>
<td>Ultrasound</td>
<td>–</td>
<td>5</td>
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<td>Kilicler et al 2010</td>
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<td>114</td>
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<td>100 vs 100</td>
<td>Biopsy</td>
<td>Ultrasound, mean IMT (L&amp;R) Age matched controls</td>
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<tr>
<td>Salvi et al 2010</td>
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<td>220</td>
<td>92 (41.8)</td>
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<td>Ultrasound</td>
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<td>Vachopoulos et al 2010</td>
<td>Europe</td>
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<td>51</td>
<td>28 (54.9)</td>
<td>55.4 vs 51.5</td>
<td>52.3 vs 64.3</td>
<td>Biopsy</td>
<td>Ultrasound, mean IMT (L&amp;R) Age sex matched controls</td>
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Table 1 Continued

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<tr>
<th>Study Region</th>
<th>Study Population</th>
<th>Study Size</th>
<th>n (%) NAFLD</th>
<th>Age (NAFLD+ vs NAFLD-)</th>
<th>% male (NAFLD+ vs NAFLD-)</th>
<th>NAFLD Assessment</th>
<th>Outcome Assessment</th>
<th>Confounder Adjustment</th>
<th>NOS (max=9)</th>
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<td>69.7 vs 24.0</td>
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<td>Turkey</td>
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<td>61 (60.4)</td>
<td>60.3 vs 60.1</td>
<td>44.2 vs 50.0</td>
<td>MR spectroscopy, liver fat content &gt;5.5%</td>
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<tr>
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<td>87.2 vs 87.2</td>
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<td>Age, sex, BMI, Smk, HT, dyslipidaemia, DM</td>
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<td>47.5 vs 50.0</td>
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<td>Age/sex-matched controls</td>
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<td>Europe</td>
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<td>45 vs 45</td>
<td>58.8 vs 59.4</td>
<td>Biopsy</td>
<td>Ultrasound, mean IMT (6 measurements)</td>
<td>Age, sex, BMI, Smk, LDL, HOMA-IR, MetS</td>
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<td>30 (50.0)</td>
<td>53.2 vs 51.6</td>
<td>50.0 vs 50.0</td>
<td>Ultrasound</td>
<td>Ultrasound, mean IMT (6 measurements)</td>
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<td>7</td>
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<tr>
<td>Europe</td>
<td>Outpatient clinic</td>
<td>90</td>
<td>50 (55.5)</td>
<td>46 vs 46</td>
<td>60.0 vs 65</td>
<td>Biopsy</td>
<td>Ultrasound, mean IMT (6 measurements)</td>
<td>Age, Sex, HOMA-IR, MetS</td>
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</table>

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

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>
<thead>
<tr>
<th>Name, year</th>
<th>Study region</th>
<th>Study population</th>
<th>Study size</th>
<th>n (%) NAFLD</th>
<th>Age (NAFLD+ vs NAFLD−)</th>
<th>% male (NAFLD+ vs NAFLD−)</th>
<th>NAFLD assessment</th>
<th>Outcome assessment</th>
<th>Outcome definition</th>
<th>Confounder adjustment</th>
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<td>43.7 vs 43.0</td>
<td>CT, Liver Spleen ratio ≤1.1</td>
<td>MDCT, Agatston method</td>
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<td>Overall=52.0</td>
<td>CT, spleen minus liver &gt;10</td>
<td>MDCT, Agatston method</td>
<td>CAC &gt;100</td>
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<td>73.0 vs 52.5</td>
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<td>MDCT, Agatston method</td>
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<td>Overall=65.8</td>
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<td>72.8 vs 49.5</td>
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<td>MDCT, Agatston method</td>
<td>CAC &gt;100</td>
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<td>Overall=59.5</td>
<td>Ultrasound</td>
<td>MDCT, Agatston method</td>
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<td>47 vs 44</td>
<td>CT, LS ratio &lt;1</td>
<td>EBCT or MDCT, Agatston method</td>
<td>CAC &gt;0</td>
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<td>89.1 vs 64.7</td>
<td>Ultrasound</td>
<td>MDCT, Agatston method</td>
<td>CAC &gt;0</td>
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<td>CT, liver HU &lt;40</td>
<td>MDCT, Agatston method</td>
<td>CAC &gt;0</td>
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<td>Cho et al 17 2018</td>
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<td>91.2 vs 72.2</td>
<td>Ultrasound</td>
<td>MDCT, Agatston method</td>
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<td>Lee et al 18 2018</td>
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<td>5121</td>
<td>1979 (38.6)</td>
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<td>77.6 vs 62.1</td>
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<td>MDCT, Agatston method</td>
<td>CAC &gt;10</td>
<td>Age, sex, obesity, DM, HT, HLP, Smk, family history of CAD, hsCRP</td>
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<table>
<thead>
<tr>
<th>Name, year</th>
<th>Study region</th>
<th>Study population</th>
<th>Study size</th>
<th>n (%) NAFLD</th>
<th>Age (NAFLD+ vs NAFLD−)</th>
<th>% male (NAFLD+ vs NAFLD−)</th>
<th>NAFLD assessment</th>
<th>Outcome assessment</th>
<th>Outcome definition</th>
<th>Confounder adjustment</th>
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<td>Wu et al(^2) 2017</td>
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<td>Population based</td>
<td>2345</td>
<td>1272 (54.2)</td>
<td>Overall=55.7</td>
<td>Overall=44.1</td>
<td>Ultrasound</td>
<td>64 slice MDCT, Agatston method</td>
<td>CAC &gt;0</td>
<td>Age, sex, Smk, HT, DM, HC, LDL, physical activity, education, income</td>
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<tr>
<td>Kim et al(^3) 2016</td>
<td>Asia</td>
<td>Population (health screen)</td>
<td>1473</td>
<td>677 (46.0)</td>
<td>–</td>
<td>68.4 vs 47.1</td>
<td>Ultrasound</td>
<td>64 slice MDCT, Agatston method</td>
<td>CAC &gt;0</td>
<td>Age, sex, Smk, alcohol, exercise, BMI, WC, SBP, total cholesterol, TAG, HDL, LDL, blood urea nitrogen, creatinine, glucose, hsCRP</td>
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<td>Kung et al(^4) 2015</td>
<td>Asia</td>
<td>Population (health screen)</td>
<td>3014</td>
<td>512 (17.0)</td>
<td>Overall=51.1</td>
<td>Overall=49.5</td>
<td>Ultrasound</td>
<td>Presence of calcified coronary plaques</td>
<td>Presence of calcified plaques</td>
<td>Age, Smk, physical activity, BMI</td>
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<tr>
<td>Mellinger et al(^5) 2015</td>
<td>North America</td>
<td>Population (health screen)</td>
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<td>346 (44.8)</td>
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<td>83.5 vs 55.4</td>
<td>Ultrasound</td>
<td>CT, liver phantom ratio &lt;0.33</td>
<td>MDCT, Agatston method</td>
<td>CAC &gt;0</td>
<td>Age, sex, alcohol, BMI, menopause, HRT, BMI</td>
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<td>Kim et al(^6) 2015</td>
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<td>Population (health screen), postmenopausal women</td>
<td>754</td>
<td>129 (17.1)</td>
<td>59.5 vs 57.1</td>
<td>0.0 vs 0.0</td>
<td>Ultrasound</td>
<td>CAC &gt;0</td>
<td>Age, race, sex, study centre, income, education, alcohol, Smk, physical activity, BMI</td>
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<td>VanWagner et al(^7) 2014</td>
<td>North America</td>
<td>Population based</td>
<td>2424</td>
<td>232 (9.57)</td>
<td>50.5 vs 49.9</td>
<td>58.2 vs 41.1</td>
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<td>CAC &gt;0</td>
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<td>Population (health screen)</td>
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<td>3784 (37.3)</td>
<td>Overall=49.1</td>
<td>Overall=76.3</td>
<td>Ultrasound</td>
<td>CAC &gt;0</td>
<td>Age, sex, TAG, HDL, WC, SBP, alcohol, Smk, physical activity, Rx CHD, Rx HTN, Hx DM, HOMA-IR</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Santos et al(^9) 2007</td>
<td>South America</td>
<td>Population (health screen)</td>
<td>505</td>
<td>204 (40.4)</td>
<td>48 vs 46</td>
<td>100 vs 100</td>
<td>Ultrasound</td>
<td>CAC &gt;0</td>
<td>Age, pulse pressure, BMI, Smk, alcohol, Mets, LDL, TG/HDL ratio, fasting glucose, BP medication, lipid medication, ALT/AST ratio, GGT</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

**CAC development/progression**

| Cho et al\(^10\) 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 |
| King et al\(^11\) 2017 | Asia | Population (health screen), non-obese participants | 447 | 105 (23.5) | Overall=54.1 | Overall=70.9 | Ultrasound | CAC >0 | Age, sex, WC, alcohol, Smk, exercise, baseline CAC, LDL, hsCRP, follow-up interval | 8 |

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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, $P_{\text{heterogeneity}}=0.15, I^2=31.7\%$, n=10 studies) in Asian populations vs (OR: 1.20 95% CI 1.03 to 1.38, $P_{\text{heterogeneity}}=0.004, I^2=73\%$, n=5 studies) in Western populations ($P_{\text{difference}}=0.98$). 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, 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 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
literature, further reinforcing the findings of previous studies and meta-analyses. 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. 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 2 Forest plots showing relationship between NAFLD and presence of increased CIMT. CIMT, carotid intima-media thickness; NFLD, non-alcoholic fatty liver disease.

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.
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 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.

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.
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 atherosclerosis.104

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.

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, drafting 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 drafting of the manuscript. guarantor. JULY—conception of idea, drafting of research question, design of inclusion/exclusion criteria, collection of data (literature search), drafting of the manuscript and editing of the manuscript. KKY—conception of idea, drafting of research question, design of inclusion/exclusion criteria and editing of the manuscript. BBMB—conception of idea, drafting of research question, design of inclusion/exclusion criteria and drafting of the manuscript. KKK—conception of idea, drafting of research question, design of inclusion/exclusion criteria, editing of the manuscript and guarantor.
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Funding KKY has received research funding from Medtronic, Boston Scientific, Amgen, AstraZeneca, Shockwave Medical (all significant, via institution); consulting or honoraria fees (all modest) from Medtronic, Boston Scientific, Abbott Vascular, Amgen, Bayer, Novartis; speaker or proctor fees from Abbott Vascular, Boston Scientific, Medtronic, Philips, Shockwave Medical, Alvimedica, Menarini, AstraZeneca, Amgen, Bayer.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Ethical approval is not applicable for this systematic review/meta-analysis.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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