



OPEN Evolution of characteristics of MASLD with and without diabetes: a meta-analysis of placebo arms

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Background We explored the changes in metabolic dysfunction-associated steatotic liver disease (MASLD) severity over time by analyzing data from the placebo arms of randomized controlled trials (RCTs), focusing on the presence of diabetes.

Methods RCTs on MASLD that included a placebo arm were identified using a systematic search of the literature. Primary outcomes were changes in hepatic steatosis and fibrosis.

Results The meta-analysis included 8 RCTs involving 386 patients without diabetes and 24 RCTs involving 637 patients with diabetes. The pooled estimate of mean change in steatosis grade was -0.1 in patients without diabetes, and -0.37 in patients with diabetes ($P = 0.066$). The mean change in fibrosis stage was 0.05 in patients without diabetes, and -0.03 in patients with diabetes ($P = 0.359$). The mean change in nonalcoholic fatty liver disease activity score was -0.55 in patients without diabetes, and -1.50 in patients with diabetes ($P = 0.100$). The mean change in ALT and AST were significantly larger in patients without diabetes compared to those with diabetes ($P < 0.05$).

Conclusion Placebo treatment had a greater effect in improving liver steatosis in patients with diabetes compared to those without. These findings highlight the importance of tailored treatment strategies in MASLD, particularly considering diabetes status.

Keywords Metabolic dysfunction-associated steatotic liver disease, Natural course, Randomized controlled trial, Steatosis, Lifestyle modification

Abbreviations

MASLD	metabolic dysfunction-associated steatotic liver disease
RCT	randomized controlled trials
NAS	nonalcoholic fatty liver disease activity score
MASH	metabolic dysfunction-associated steatohepatitis
T2DM	type 2 diabetes mellitus
MRI	magnetic resonance imaging
BMI	the body mass index
ALT	alanine aminotransferase
AST	aspartate aminotransferase
HOMA-IR	homeostatic model assessment for insulin resistance
SD	standard deviation
CI	confidence interval
LFC	liver fat content
HDL	high-density lipoprotein
LDL	low-density lipoprotein

The rise in the prevalence of obesity and metabolic syndrome has led to a significant increase in the prevalence of hepatic steatosis and steatohepatitis, emerging as widespread liver diseases affecting approximately 31.3% of the global population¹. Recent studies suggests that metabolic dysfunction-associated steatotic liver disease (MASLD) may either progress or regress over time². In addition, individuals affected by MASLD have an increased risk of liver-related events and cardiovascular disease leading to substantial mortality³. Therefore, understanding the natural history of MASLD is crucial for the effective management and development of targeted interventions.

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Unlike cross-sectional studies that have utilize data collected at a single time point, randomized controlled trials (RCTs) allow the observation of disease progression or improvement. In particular, the placebo arms in RCTs present a natural evolution of the disease, enabling the analysis of changes in disease severity without active intervention. Consequently, two previous meta-analyses analyzed changes in the severity of MASLD using the placebo arms of previous RCTs and showed an improvement in the nonalcoholic fatty liver disease activity score (NAS) or significant resolution of metabolic dysfunction-associated steatohepatitis (MASH)^{4,5}. However, a limitation of these studies lies in the analysis of mixed cohorts of patients with and without diabetes, which complicates the interpretation of the results.

Compelling evidence suggests that the disease burden of MASLD extends beyond liver-related issues because it increases the risk of developing cardiometabolic diseases including obesity, insulin resistance, hypertension, dyslipidemia, and diabetes³. MASLD and diabetes have strong interactions and complex bidirectional relationships. Several studies have suggested that MASLD patients with concomitant diabetes have an increased risk of hepatocellular carcinoma and mortality compared to those without^{6,7}. Furthermore, MASLD and diabetes frequently co-exist and act synergistically to increase the risk of liver and non-liver-related events^{6,7}. However, it remains unclear whether the evolution of the severity of MASLD differs depending on the presence or absence of diabetes.

Using data from placebo arms in existing RCTs on MASLD with or without type 2 diabetes mellitus (T2DM), we compared the changes in the severity of MASLD over time according to the presence of T2DM to investigate the natural history of patients with MASLD with and without concomitant diabetes.

Methods

Search strategy

This meta-analysis was conducted in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement⁸. A comprehensive search was conducted on PubMed, MEDLINE, Embase, and the Cochrane Central Register of Controlled Trials, covering the entire period from the inception of each database until December 12, 2021. The search strategy was formulated and conducted by an experienced librarian in collaboration with the principal investigator of the study. All references were imported into EndNote X9 to remove duplicates. The full search strategy is as follows: (1) (clinical adj3 trial).tw. OR (singl\$ OR doubl\$ OR trebl\$ OR tripl\$).tw. AND (mask\$ OR blind\$).tw. OR placebo\$.tw. OR random\$.tw. OR exp randomized controlled trials/ OR exp random allocation/ OR exp double-blind method/ OR exp single-blind method/ OR exp placebos/ OR research design/; (2) (nash OR ((nonalcoholic OR non*alcoholic OR non alcoholic) adj3 steatohepatit*)).tw.; (3) 1 AND 2.

Study selection and data extraction

Two authors (HAL and HAL) independently screened the titles and abstracts of the identified references and conducted a full-text evaluation for inclusion. Data were independently extracted from the included articles by the reviewers using Microsoft Excel. The extracted data included the inclusion criteria, study location, sample size, lifestyle modification details, frequency of follow-up visits, trial duration, and predetermined outcomes. Discrepancies were resolved through discussion, and when necessary, a third reviewer (HYK) was consulted.

Inclusion criteria

RCTs comparing outcomes between a control intervention and a pharmacological intervention in adults diagnosed with MASLD based on histology or magnetic resonance imaging (MRI) were included. To compare outcomes according to the presence or absence of diabetes, studies in which individuals with and without diabetes were mixed without providing subgroup analysis data based on diabetes status were excluded. Control interventions included both placebo and lifestyle interventions. Trials that compared different lifestyle interventions were also excluded. Studies reporting sufficient data on outcomes of interest, including liver steatosis and/or fibrosis, were included. Nonrandomized and noncomparative studies were excluded. Studies, commentaries, editorials, letters, systematic reviews, meta-analyses, case series, and studies reported only as abstracts, as well as pediatric and duplicate studies from the same database were excluded.

Outcomes

The primary outcomes were changes in liver steatosis and fibrosis measured using histology. Other outcomes included changes in the NAS and its other components (i.e., lobular inflammation and hepatocyte ballooning), anthropometric parameters including body weight and the body mass index (BMI), biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), lipid profiles, HbA1c, and homeostatic model assessment for insulin resistance (HOMA-IR).

The mean, mean difference, and standard deviation (SD) were calculated for each outcome. In trials that did not report any variability measures, the mean and SD were estimated from the sample size, median and range⁹. If the SD information on variability was insufficient, the SD was estimated using the standard error and confidence interval (CI), or the SD was imputed according to the method introduced in the Cochrane Handbook¹⁰.

Risk of bias assessment

Two reviewers (HAL and HAL) independently used the Cochrane risk of bias tool to evaluate risk of bias in individual trials¹⁰. Adequacy of the trials was examined in six domains: randomization, allocation concealment, blinding (i.e., patients, providers, data collectors, and outcome assessors), incomplete outcome data, funding sources, and other sources of bias. Each study was independently evaluated in these domains, and the results were integrated to assign an overall risk of bias. Specifically, studies were classified as “low risk” if the majority of domains indicated a low risk of bias, “moderate risk” if there was a mix of low, unclear, or high risks, and “high

risk” if several domains indicated a high risk of bias. Disagreements were resolved by consensus and consultation with the third author (HYK).

Statistical analysis

A random-effects model was used to estimate the pooled mean difference in primary and secondary outcomes before and after the placebo intervention¹¹. We evaluated heterogeneity using the I^2 and Cochran's Q test statistics, with heterogeneity defined as an I^2 value of 40% or a Cochran's Q test P value of ≤ 0.10 .¹² Subgroup analysis was performed according to region (multinational, Asian, North American or European), study center (multicenter or single center), duration of the intervention period (< 24 weeks, between 24 and 48 weeks, or > 48 weeks), risk of bias (low, moderate, or high), and method of lifestyle modification (i.e., dietitian with or without exercise instructions, diet and exercise counseling without dietitian, diet counseling without dietitian, or not provided or reported). The association between risk factors and outcomes was analyzed using a mixed-model meta-regression with the Hartung-Knapp estimator^{13,14}. All statistical analyses were performed using R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Included studies

A total of 1,685 articles were identified using the search strategy. Of these, the title and abstracts of 1,465 articles were screened after the removal of duplicates. A total of 172 full-text articles were retrieved for review, of which 140 were excluded. Finally, 8 RCTs involving 386 patients without diabetes and 24 RCTs involving 637 patients with diabetes were included in the systematic review and meta-analysis (Fig. 1). The characteristics of the included studies are shown in Supplementary Table 1.

Eleven trials were conducted in North America^{15–25}, 13 in Europe^{26–38}, five in Asia^{39–43}, and one in South America⁴⁴; two trials were multinational studies^{45,46}. Nineteen studies were single-center trials and 13 were multicenter trials. The median duration of the intervention period was 24.0 weeks (range 6–96 weeks). The intervention periods were between 6 and 24 weeks ($n=22$), 24 and 48 weeks ($n=5$), and > 48 weeks ($n=5$). In 46.9% of the trials, lifestyle modifications (either dietary or physical activity) were recommended. Recommendations to increase physical activity were provided in 40.6% of the trials. Overall, 21.9% of the trials reported that dietitians were involved in lifestyle modifications. All RCTs had a low ($n=15$) or moderate ($n=17$) risk of bias (Supplementary Fig. 1).

Primary outcomes

Changes in liver steatosis

The effects of the placebo intervention on primary and secondary outcomes in patients with and without diabetes are presented in Table 1; Fig. 2. The pooled mean change in steatosis measured by histology in 369 patients without diabetes was -0.1 (95% CI -0.29 to 0.10), and in 78 patients with diabetes was -0.37 (95% CI -0.52 to 0.23), respectively. In the meta-regression analysis, the presence of diabetes showed a tendency toward an increased reduction in steatosis grade ($\beta = -0.287$; 95% CI -0.717 to 0.143 ; $P = 0.066$).

Subgroup analyses were performed for the patients with and without diabetes (Table 2). In patients without diabetes, the intervention period affected the mean change in steatosis grade ($P = 0.001$). The pooled mean change in steatosis grade in studies with an intervention period of 24 weeks was 0.173 (95% CI -0.06 to 0.41), however, it increased in a study with 24–48-week intervention (-0.65 ; 95% CI -1.15 to -0.15) and in studies with interventions > 48 weeks (-0.27 ; 95% CI -0.44 to -0.09). Lifestyle modification affected the mean change in steatosis grade ($P = 0.001$) with increased pooled mean change in steatosis in a study that included a dietitian with exercise instruction (-0.65 , 95% CI -1.15 to -0.15), while -0.20 (95% CI -0.39 to 0.0) in studies with no provided or reported lifestyle modification. In patients with diabetes, several factors, including the intervention period, the risk of bias and lifestyle modifications, did not significantly have an effect on the grade of steatosis.

Changes in liver fibrosis stage

The pooled mean change in fibrosis stage measured using histology in 386 patients without diabetes was 0.05 (95% CI -0.08 to 0.18), and that in 129 patients with diabetes was -0.03 (95% CI -0.16 to 0.10). The presence of diabetes did not significantly affect the change in the stage of fibrosis stage ($\beta = -0.092$; 95% CI -0.303 to 0.119 ; $P = 0.359$). In the subgroup analysis of patients with and without diabetes, several factors, including intervention period, risk of bias, and lifestyle modifications, did not significantly affect the change in the stage of fibrosis.

Secondary outcomes

Changes in the NAS

The pooled mean changes in lobular inflammation of 386 patients without diabetes was -0.08 (95% CI -0.21 to 0.05), and that of 129 patients with diabetes was 0.04 (95% CI -0.34 to 0.42). The presence of diabetes did not significantly affect the change in lobular inflammation ($\beta = 0.136$; 95% CI -0.219 to 0.491 ; $P = 0.416$).

The subgroup analysis in patients without diabetes showed that the pooled mean change in lobular inflammation was significantly enhanced in studies with low bias risk, compared to those with high bias risk (-0.17 ; 95% CI -0.26 to -0.07 vs. 0.04 ; 95% CI -0.10 to 0.17) ($\beta = 0.256$; 95% CI 0.126 to 0.386 ; $P = 0.003$). The intervention period affected the mean change in lobular inflammation ($P = 0.041$). The pooled mean change in the degree of lobular inflammation in studies with an intervention period of ≤ 24 weeks was 0.02 , (95% CI -0.12 to 0.15), however, it was higher in a study with 24–48 weeks (-0.12 ; 95% CI -0.62 to 0.38) and in studies with > 48 weeks (-0.18 ; 95% CI -0.28 to -0.08) of intervention. In patients with diabetes, the subgroup analysis showed that several factors, including the intervention period and lifestyle modifications, did not significantly affect lobular inflammation (Supplementary Table 3).

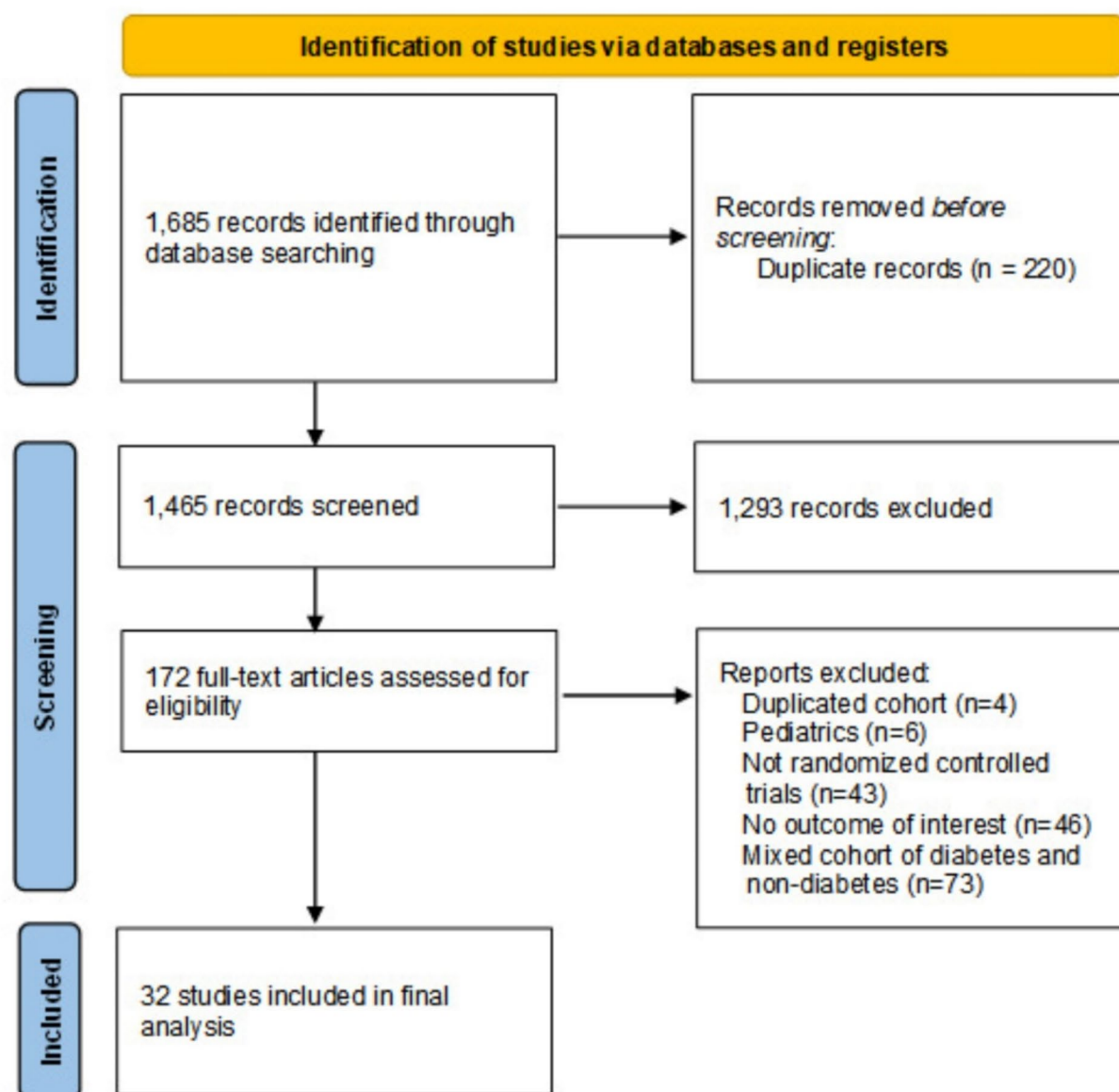


Fig. 1. Flow diagram for systematic reviews and meta-analyses.

The pooled mean change in the ballooning of hepatocytes of 310 patients without diabetes was -0.13 (95% CI -0.22 to -0.04), and that of 108 patients with diabetes was -0.21 (95% CI -0.33 to -0.09), respectively ($P=0.298$). The presence of diabetes did not significantly affect the change in hepatocyte ballooning ($\beta = -0.071$; 95% CI -0.260 to 0.119 ; $P=0.414$). The pooled mean change in the NAS of 376 patients without diabetes was -0.55 (95% CI -0.76 to -0.33), and that of 25 patients with diabetes was -1.50 (95% CI -2.14 to -0.87). The presence of diabetes did not significantly influence the change in NAS ($\beta = -0.939$; 95% CI -2.163 to 0.285 ; $P=0.100$). In the subgroup analysis of patients with and without diabetes, several factors, including the number of study centers included in the RCT, the intervention period, and the risk of bias, did not significantly affect changes in hepatocyte ballooning or the NAS.

Change in liver enzyme level

The pooled mean change in ALT level was significantly greater in 407 patients without diabetes than in 557 patients with diabetes (-20.22 IU/L, 95% CI -26.42 to -14.03 vs. -4.48 IU/L, 95% CI -8.54 to -0.42) (Supplementary Fig. 2). In the meta-regression analysis, the presence of diabetes was associated with a smaller reduction in ALT levels ($\beta = 16.554$; 95% CI 7.966 to 25.1442 ; $P=0.001$).

In the subgroup analysis of patients without diabetes, several factors, including the number of centers included in the RCT, the intervention period, and the risk of bias, did not significantly influence the change in ALT levels. In patients with diabetes, the intervention period affected the mean change in ALT levels ($P=0.001$).

	Patients without diabetes						Patients with diabetes					
	No of studies	Sample size	Effect size	95% CI	I ² (%)		No of studies	Sample size	Effect size	95% CI	I ² (%)	P value
<i>Primary outcomes</i>												
Change in statosis grade	7	369	-0.10	-0.29 to 0.10	69.3		4	78	-0.37	-0.52 to -0.23	0.0	0.066
Change in fibrosis stage	8	386	0.05	-0.08 to 0.18	70.1		5	129	-0.03	-0.16 to 0.10	0.0	0.359
<i>Secondary outcomes</i>												
Change in lobular inflammation	8	386	-0.08	-0.21 to 0.05	74.8		5	129	0.04	-0.34 to 0.42	71.8	0.416
Change in hepatocyte ballooning	6	310	-0.13	-0.22 to -0.04	56.5		4	108	-0.21	-0.33 to -0.09	10.8	0.414
Change in NAS	7	376	-0.55	-0.76 to -0.33	32.0		2	25	-1.50	-2.14 to -0.87	56.5	0.100
<i>Other outcomes</i>												
Change in ALT, IU/L	7	407	-20.22	-26.42 to -14.03	64.6		20	557	-4.48	-8.54 to -0.42	85.2	0.001
Change in AST, IU/L	7	407	-10.67	-14.86 to -6.49	68.9		17	452	-2.58	-5.02 to -0.14	85.7	0.002
Change in total cholesterol, mg/dL	4	281	-10.12	-13.28 to -7.12	7.3		14	374	-7.15	-13.15 to -1.14	80.9	0.454
Change in HDL-cholesterol, mg/dL	3	264	-0.28	-2.27 to 1.71	85.2		17	460	0.91	-0.15 to 1.96	82.8	0.332
Change in LDL-cholesterol, mg/dL	3	264	-10.09	-15.96 to -4.23	85.3		17	474	-8.09	-12.87 to -3.31	88.6	0.750
Change in triglyceride, mg/dL	5	304	-0.72	-24.20 to 22.76	93.5		19	508	-7.10	-16.30 to 2.10	84.2	0.531
Change in fasting glucose, mg/dL	4	281	-0.96	-4.39 to 2.47	96.8		19	494	-5.12	-12.07 to 1.82	94.0	0.588
Change in HOMA-IR	3	110	-0.02	-0.03 to -0.01	0.0		9	207	-0.04	-0.36 to 0.28	82.4	0.901
Change in HbA1c, %							22	600	-0.29	-0.53 to -0.06	97.8	NA
Change in body weight, kg	3	282	0.33	-0.28 to 0.94	0.0		20	520	-0.97	-1.62 to -0.33	93.5	0.004
Change in BMI, kg/m ²	6	348	-0.55	-1.24 to 0.15	84.9		16	393	-0.45	-0.78 to -0.12	93.8	0.845

Table 1. Analysis of primary and secondary outcomes. CI, confidence interval; NAS, nonalcoholic fatty liver disease activity score; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; BMI, body mass index.

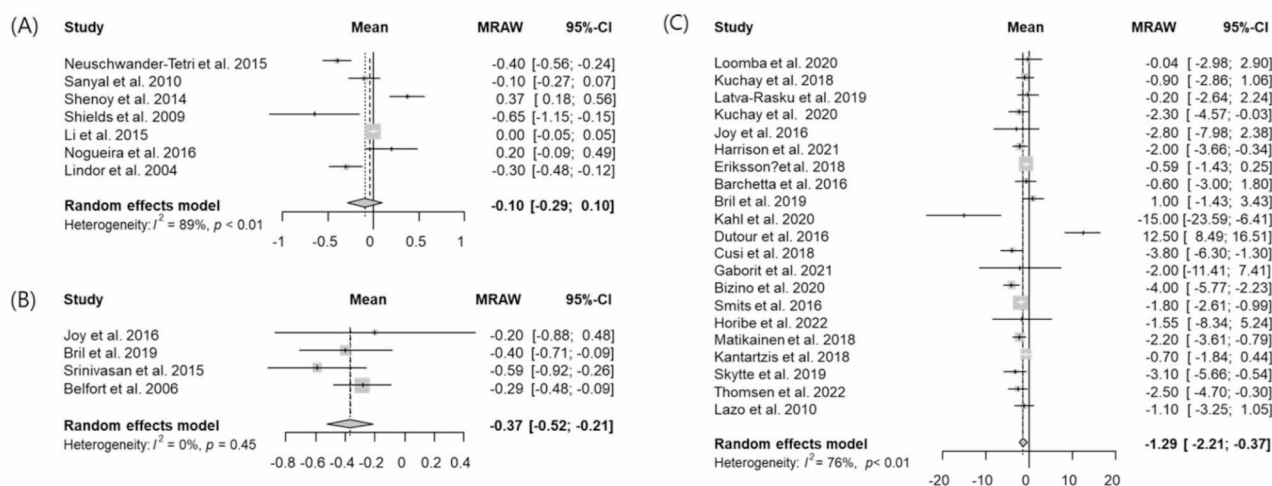


Fig. 2. Forest plots of meta-analysis showing change in hepatic steatosis. Change in steatosis grade assessed using histology of patients without diabetes (A) and with diabetes (B). Change in liver fat content using MRI of patients with diabetes (C).

The pooled mean change in ALT levels in studies with an intervention period of ≤ 24 weeks was -3.58 (95% CI -6.72 to -0.45), 2.12 (95% CI -7.32 to 11.57) in studies with $24-48$ weeks, and increased in studies with > 48 weeks (-20.34 ; 95% CI -22.95 to -17.73) intervention. Interestingly, lifestyle modifications affected the mean change in ALT levels ($P < 0.001$). The pooled mean change in the ALT level increased in studies that included a dietitian and exercise instruction (-9.14 , 95% CI -21.89 to 3.61) and a study that included a dietitian but with no exercise instruction (-23.0 , 95% CI -30.79 to -15.21), compared to those that included dietary modification and exercise counseling but without a dietitian (-5.61 , 95% CI -13.34 to 2.12), and a study without any suggested or reported lifestyle modification (-1.02 , 95% CI -6.14 to 4.11).

The pooled mean change in AST level was significantly greater in 407 patients without diabetes than in 452 patients with diabetes (-10.67 , 95% CI -14.86 to -6.49 vs. -2.58 , 95% CI -5.02 to -0.14 ; $P = 0.002$). In the meta-regression analysis, the presence of diabetes resulted in a decrease in the change in AST level ($\beta = 7.997$; 95% CI 3.245 to 12.750 ; $P = 0.002$).

In the subgroup analysis of patients without diabetes, several factors, including the intervention period and lifestyle modifications, did not significantly influence the change in AST levels. The subgroup analysis of patients with diabetes showed that the intervention period influenced the mean change in AST levels ($P = 0.006$). The pooled mean change of ALT level in studies with intervention period of ≤ 24 weeks was -1.57 (95% CI -3.90 to 0.75), -0.42 (95% CI -1.44 to 0.60) in studies with $24-48$ -week intervention, and increased in studies with interventions of > 48 weeks (-10.18 ; 95% CI -18.90 to -1.47).

Changes in lipid and glucose profiles

The pooled mean change in total cholesterol level of 281 patients without diabetes was -10.12 mg/dL (95% CI -13.28 to -7.12) and that of 374 patients with diabetes was -7.15 mg/dL (95% CI -13.15 to -1.14). The pooled mean change in the high-density lipoprotein (HDL)-cholesterol level of 264 patients without diabetes was -0.28 mg/dL (95% CI -2.27 to 1.71), and that of 460 patients with diabetes was 0.91 mg/dL (-0.15 to 1.96). The pooled mean change in low-density lipoprotein (LDL)-cholesterol level of 264 patients without diabetes was -10.09 mg/dL (95% CI -15.96 to -4.23), and that of 474 patients with diabetes was -8.09 mg/dL (95% CI -12.87 to -3.31). The pooled mean change in triglyceride level of 304 patients without diabetes was -0.72 mg/dL (95% CI -24.20 to 22.76), and that of 508 patients with diabetes was -7.10 mg/dL (95% CI -16.30 to 2.10). The presence of diabetes did not significantly influence changes in total cholesterol ($\beta = 4.636$; 95% CI -8.160 to 17.432 ; $P = 0.454$), HDL-cholesterol ($\beta = 1.173$; 95% CI -1.302 to 3.648 ; $P = 0.332$), LDL-cholesterol ($\beta = 1.860$; 95% CI -10.201 to 13.921 ; $P = 0.750$), and triglyceride ($\beta = -6.802$; 95% CI -28.982 to 15.379 ; $P = 0.531$) levels.

The pooled mean change in fasting glucose level of 281 patients without diabetes was -0.96 mg/dL (95% CI -4.39 to 2.47), and that of 494 patients with diabetes was -5.12 mg/dL (95% CI -12.07 to 1.82). The pooled mean change in HOMA-IR of 110 patients without diabetes was -0.02 (95% CI -0.03 to -0.01), and that of 207 patients with diabetes was -0.04 (95% CI -0.36 to 0.28). The presence of diabetes did not significantly affect the change in fasting glucose level ($\beta = -4.406$; 95% CI -21.047 to 12.234 ; $P = 0.588$) and HOMA-IR ($\beta = -0.045$; 95% CI -0.838 to 0.748 ; $P = 0.901$).

Changes in body weight and the BMI

The pooled mean change in the body weight of 282 patients without diabetes was 0.33 kg (95% CI -0.28 to -0.97), and that of 520 patients with diabetes was -0.97 kg (95% CI -1.62 to -0.33). In the meta-regression analysis, the presence of diabetes was significantly associated with a decrease in body weight ($\beta = -1.251$; 95% CI -2.968 to 0.466 ; $P = 0.004$).

	No of studies	Sample size	Effect size	95% CI	I ² (%)	P value
Patients without diabetes						
<i>Change in steatosis grade</i>						
Center						
Multicenter	2	181	-0.25	-0.55 to 0.04	84.2	0.491
Single center	5	188	-0.04	-0.36 to 0.28	87.9	
Intervention period						0.001
≤24 weeks	3	121	0.173	-0.06 to 0.41	86.1	
24–48 weeks	1	10	-0.65	-1.15 to -0.15	NA	
>48 weeks	3	238	-0.27	-0.44 to -0.09	68.9	
Risk of bias						0.232
Low	3	238	-0.27	-0.44 to -0.09	68.9	
Moderate	4	131	0.02	-0.35 to 0.40	86.1	
Lifestyle modification						<0.001
Dietitian with exercise instruction	1	10	-0.65	-1.15 to -0.15	NA	
Dietitian without exercise instruction	1	23	0.20	-0.09 to 0.49	NA	
Diet and exercise counseling without dietitian	2	137	-0.19	-0.58 to 0.20	95.4	
Diet counseling without dietitian	1	59	0.37	0.18 to 0.56	NA	
Not provided or reported	2	140	-0.20	-0.39 to 0.0	59.2	
<i>Change in fibrosis stage</i>						
Center						0.599
Multicenter	2	181	0.0	-0.20 to 0.19	61.1	
Single center	6	205	0.07	-0.10 to 0.24	75.6	
Intervention period						0.733
≤24 weeks	4	138	0.09	-0.15 to 0.32	84.6	
24–48 weeks	1	10	0.20	-0.33 to 0.73	NA	
>48 weeks	3	238	-0.01	-0.13 to 0.12	22.3	
Risk of bias						0.403
Low	4	255	0.0	-0.11 to 0.11	0.0	
Moderate	4	131	0.12	-0.13 to 0.37	85.2	
Lifestyle modification						0.204
Dietitian with exercise instruction	1	10	0.20	-0.33 to 0.73	NA	
Dietitian without exercise instruction	2	40	0.05	-0.21 to 0.31	0.0	
Diet and exercise counseling without dietitian	2	137	-0.02	-0.21 to 0.17	76.8	
Diet counseling without dietitian	1	59	0.36	0.16 to 0.56	NA	
Not provided or reported	2	140	-0.06	-0.19 to 0.07	0.0	
Patients with diabetes						
<i>Change in steatosis grade</i>						
Center						NA
Multicenter	4	78	-0.37	-0.52 to -0.21	0.0	
Intervention period						0.146
≤24 weeks	2	27	-0.28	-0.47 to -0.10	0.0	
24–48 weeks	1	19	-0.59	-0.92 to -0.26	NA	
>48 weeks	1	32	-0.40	-0.71 TO -0.09	NA	
Continued						

	No of studies	Sample size	Effect size	95% CI	I ² (%)	P value
Risk of bias						
Low	2	53	-0.32	-0.48 to -0.15	0.0	0.309
Moderate	2	25	-0.51	-0.82 to -0.21	1.9	
Lifestyle modification						0.891
Dietitian with exercise instruction	2	40	-0.41	-0.70 to -0.12	58.0	
Diet and exercise counseling without dietitian	1	32	-0.40	-0.71 to -0.09	NA	
Not provided or reported	1	6	-0.20	-0.88 to 0.48	NA	
Change in fibrosis stage						
Center						0.857
Multicenter	4	78	-0.04	-0.17 to 0.10	0.0	
Single center	1	51	0.0	-0.33 to 0.33	NA	
Intervention period						0.628
≤24 weeks	2	27	-0.02	-0.21 to 0.16	0.0	
24–48 weeks	1	19	0.06	-0.19 to 0.31	NA	
>48 weeks	2	83	-0.13	-0.43 to 0.16	26.6	
Risk of bias						0.603
Low	2	53	-0.10	-0.38 to 0.18	46.8	
Moderate	3	76	0.01	-0.18 to 0.20	0.0	
Lifestyle modification						0.271
Dietitian with exercise instruction	2	40	0.02	-0.13 to 0.18	0.0	
Dietitian without exercise instruction	1	51	0.0	-0.33 to 0.33	NA	
Diet and exercise counseling without dietitian	1	32	-0.30	-0.68 to 0.07	NA	
Not provided or reported	1	6	-0.20	-0.73 to 0.33	NA	

Table 2. Subgroup analysis of primary outcomes. CI, confidence interval, P value <0.05 denotes statistical significance.

Discussion

This meta-analysis presents a thorough and current review of the literature on histological and clinical outcomes in the placebo groups of RCTs on MASLD, particularly comparing outcomes based on the presence or absence of diabetes. Our study revealed a trend toward greater improvement in the grade of steatosis in patients with diabetes than in those without diabetes. However, patients without diabetes showed a more significant decrease in ALT and AST levels than those with diabetes. In addition, our meta-analysis found that the intervention period and lifestyle modification method could influence outcomes in the placebo arms of MASLD RCTs.

Our analysis included studies that specifically enrolled individuals with or without diabetes as distinct subgroups. Thus, this study provides valuable information on the influence of diabetes on the natural history of MASLD in response to lifestyle modifications. A recent meta-analysis of 39 RCTs for MASH including studies with mixed populations of patients with and without diabetes showed that the improvement in steatosis grade was 0.35 in the placebo arms⁵. In our study, patients with diabetes showed a tendency toward greater reduction in hepatic steatosis (-0.37) than in those without diabetes (-0.1). Hepatic lipid accumulation is associated with impaired insulin sensitivity, which leads to pathological increases in hepatic glucose production⁴⁷. Although the mechanism of the treatment response has not been fully elucidated, because insulin resistance is the key pathophysiology of both MASLD and diabetes. Therefore, the effects of lifestyle modifications can be more pronounced in patients with MASLD and diabetes, as previous studies have shown a positive relationship between improvements in insulin sensitivity and histological improvements in MASLD. In the present study, diabetes was significantly associated with a decrease in body weight, which is another factor that should be considered when analyzing and interpreting the results of the MASLD trials. This may contribute to a more notable improvement in hepatic steatosis in patients with diabetes than in those without diabetes.

Given the close link between diabetes and MASLD, numerous clinical trials have been conducted evaluating antidiabetic drugs as MASH therapeutics⁴⁸. Most clinical trials included mixed populations of patients with and without diabetes or only patients with diabetes. However, considering the difference between the clinical courses of patients with and without MASLD, the response to treatment should be individually assessed in patients with MASLD according to the absence or presence of diabetes. In addition, efforts are required to identify subpopulations that are likely to benefit from aggressive lifestyle interventions or pharmacological therapies, including antidiabetics.

We performed a subgroup analysis with multiple variables to elucidate the heterogeneity among the studies. Although the reduction in steatosis was not significant in patients without diabetes, studies with an intervention period of > 24 weeks showed a greater reduction in liver steatosis. A similar effect of the intervention period was also observed in the reduction of lobular inflammation. Taking into account these findings, it would be desirable to prolong the duration of the MASLD trials by more than 6 months to more clearly capture the effects of lifestyle modifications. This information should be considered when designing clinical trials for lifestyle modifications in patients with MASLD who do not have diabetes.

Presence or content of lifestyle modification was another contributing factor to the effect of placebo treatment. Studies involving dietitians showed the greatest effect of lifestyle modification compared with those without dietitians. The differences in intervention methods within MASLD trials highlight a significant limitation in the comparative analysis, as specific intervention details were often not well documented in the literature. Consequently, when interpreting these results, it is essential to consider the heterogeneity in intervention methods. Furthermore, for an accurate comparison between the placebo arm and active treatment in clinical trial designs, the standardization of lifestyle modifications across all trials should be considered when designing future studies.

In our study, the fibrosis stage did not significantly improve or progress in either placebo group, suggesting that more intensive lifestyle modifications (e.g., a longer intervention period and intermittent fasting) are needed to improve hepatic fibrosis. In contrast, the change in the NAS was significant in both patients with and without diabetes; however, the improvement in each component of the NAS had mixed results. Diabetes was associated with decreased ALT and AST levels. A previous study reported a high prevalence of MASH (58%) in patients with type 2 diabetes with normal or near-normal liver enzyme levels⁴⁹. Therefore, the response of liver enzymes to placebo treatment may not be significant in patients with diabetes.

Overall, this study found that patients with diabetes experienced significant improvement in liver steatosis through lifestyle modifications, likely due to enhanced insulin resistance. Patients without diabetes showed smaller improvements in steatosis but exhibited substantial improvements in markers such as AST and ALT, indicative of MASH, suggesting that that extended lifestyle modification could lead to greater steatosis improvements in non-diabetic patients. The minimal improvements in fibrosis and lobular inflammation observed in this study suggest that prolonged and intensive lifestyle modifications may be necessary to effectively address these issues.

This meta-analysis had several limitations. First, because the studies included various populations of patients with different severities of MASLD, an analysis was performed on patients with and without MASH. Second, many studies reported results for mixed populations of patients with and without diabetes, without subgroup analysis, and were excluded from our study. This exclusion may have omitted important data, potentially affecting the reliability of the effect sizes. Additionally, the small sample size and limited number of studies in certain subgroups, sometimes only one study, reduce the interpretability and strength of the conclusions. These findings should be considered preliminary, and further research with larger sample sizes is necessary to validate the results. Third, the representative objectives of MASH trials including MASH resolution without worsening of fibrosis or improvement in ≥ 1 stage of fibrosis without worsening of NAS, and clinical endpoints including decompensation or mortality could not be analyzed, because those outcomes were not assessed in most of the included studies. Fourth, the Hawthorne effect might have led to a positive behavioral change in the included patients⁵⁰. In addition, although we attempted to investigate the specific methods of lifestyle modification in detail, but some documents lacked comprehensive recording of these details. Fifth, although

the extent of changes in steatosis and transaminases differed between the groups with and without diabetes, it remains unclear whether inflammation should improve before steatosis decreases or if reductions in steatosis should precede a decrease in inflammation, highlighting the complexity of liver pathology in metabolic diseases. Sixth, the relatively short intervention periods of 6 to 24 weeks in most studies may have been insufficient to adequately observe the effects of lifestyle interventions, particularly in terms of histological improvement. This limitation underscores the need for future studies to be designed with sufficiently long intervention periods. Seventh, the study included trials with varying durations, interventions, and populations, leading to high heterogeneity. To address this limitation, we performed subgroup analyses based on various factors. However, further subgroup analyses were limited by the small number of available studies and insufficient detail in the original articles, which may reduce the statistical power and reliability of the findings. Additionally, for certain outcomes, the wide confidence intervals reflect low precision in the pooled estimates, likely due to small sample sizes and between-study variability. This limitation highlights the necessity for future trials with more robust sample sizes and standardized methodologies to improve the precision and reliability of meta-analyses in this field. Eighth, one significant limitation of our study is that the small number of included studies (fewer than 10) may reduce the reliability of funnel plots and Egger's test, precluding their application and thereby constraining our ability to evaluate publication bias. This limitation should be considered when interpreting the findings, and future meta-analyses with larger datasets will be necessary to properly evaluate publication bias. Ninth, while histologic assessment remains the gold standard, it is inherently invasive, and data on non-invasive markers such as elastography and serum biomarkers were not consistently reported across the included studies. Given the invasive nature of liver biopsies, future meta-analyses should consider focusing exclusively on studies that utilize non-invasive tests. Tenth, several non-significant trends were observed in our study, which could potentially represent true differences. But due to the limited sample size and the broad scope of our analysis, they may also reflect statistical noise. Finally, although we included only RCTs to reduce heterogeneity, variability in histological evaluation may exist between included studies. In fact, detailed methods of liver biopsy and the quality of biopsy specimens have been overlooked in some studies.

Conclusions

The findings from this meta-analysis underscore the significant impact of diabetes status on the natural history and treatment response in patients with MASLD. Patients with diabetes showed greater improvements in liver steatosis, likely due to enhanced insulin resistance, consistent with prior studies. Our study highlights the need for individualized treatment approaches based on diabetes status, affecting the evaluation of active treatments in clinical trials. Stratifying patient groups by diabetes status is necessary to properly assess the placebo effect, given the differential effects of lifestyle modifications. Future research should explore mechanisms behind these differential responses and identify optimal duration and method of intervention for both groups, informing clinical trial designs and developing tailored therapeutic strategies. To reduce reporting bias and enhance clinical relevance, future MASLD trials should standardize outcome reporting and incorporate hard clinical outcomes, such as mortality and liver-related events, alongside surrogate markers.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

Conflict of interest

The authors declare that they have no conflicts of interest relevant to this work.

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Trial registration number

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Additional information

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