

SUPPORTING INFORMATION

Systematic review with meta-analysis: the effects of probiotics in non-alcoholic fatty liver disease

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Supplemental table 1: Checklist of items in reporting a systematic review or meta-analysis (PRISMA statement)

Section/topic	Item	Checklist item	Reported on page
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both	1
Abstract			
Structured summary	2	Provide a structured summary including, as applicable, background, objectives, data sources, study eligibility criteria, participants, interventions, study appraisal and synthesis methods, results, limitations, conclusions and implications of key findings, systematic review registration number	1-2
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known	2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS)	2
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (such as web address), and, if available, provide registration information including registration number	NA
Eligibility criteria	6	Specify study characteristics (such as PICOS, length of follow-up) and report characteristics (such as years considered, language, publication status) used as criteria for eligibility, giving rationale	3
Information sources	7	Describe all information sources (such as databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched	3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated	Supplementary data
Study selection	9	State the process for selecting studies (that is, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis)	3
Data collection process	10	Describe method of data extraction from reports (such as piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators	4
Data items	11	List and define all variables for which data were sought (such as PICOS, funding sources) and any assumptions and simplifications made	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis	4
Summary measures	13	State the principal summary measures (such as risk ratio, difference in means	4-5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (such as I2 statistic) for each meta-analysis	4-5
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (such as publication bias, selective reporting within studies)	4-5
Additional analyses	16	Describe methods of additional analyses (such as sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified	4-5
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram	5-6, Fig 1
Study characteristics	18	For each study, present characteristics for which data were extracted (such as study size, PICOS, follow-up period) and provide the citations	Table1

Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see item 12).	Supplementary data
Results of individual studies	20	For all outcomes considered (benefits or harms), present for each study (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot	Fig 3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency	5-21
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see item 15)	Supplementary data
Additional analysis	23	Give results of additional analyses, if done (such as sensitivity or subgroup analyses, meta-regression) (see item 16)	5-21
Discussion			
Summary of evidence	24	Summarise the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (such as health care providers, users, and policy makers)	22-25
Limitations	25	Discuss limitations at study and outcome level (such as risk of bias), and at review level (such as incomplete retrieval of identified research, reporting bias)	25
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research	26
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (such as supply of data) and role of funders for the systematic review	26

SEARCH STRATEGY (till to 2019.5.6)

PubMed

Query:

(((((((((probiotic*[Title/Abstract]) OR prebiotic*[Title/Abstract]) OR synbiocit*[Title/Abstract]) OR bifidobacter*[Title/Abstract]) OR Lactobacill*[Title/Abstract]) OR flora[Title/Abstract])) OR "Probiotics"[Mesh])) AND (((((((((((Non alcoholic Fatty Liver Disease[Title/Abstract]) OR NAFLD[Title/Abstract]) OR Nonalcoholic Fatty Liver Disease[Title/Abstract]) OR Fatty Liver, Nonalcoholic[Title/Abstract]) OR Fatty Livers, Nonalcoholic[Title/Abstract]) OR Liver, Nonalcoholic Fatty[Title/Abstract]) OR Livers, Nonalcoholic Fatty[Title/Abstract]) OR Nonalcoholic Fatty Liver[Title/Abstract]) OR Nonalcoholic Fatty Livers[Title/Abstract]) OR Nonalcoholic Steatohepatitis[Title/Abstract]) OR Nonalcoholic Steatohepatitides[Title/Abstract]) OR Steatohepatitides, Nonalcoholic[Title/Abstract]) OR Steatohepatitis, Nonalcoholic[Title/Abstract]) OR NASH[Title/Abstract] OR "Non-alcoholic Fatty Liver Disease"[Mesh])

Result: 358

Embase

Query:

('nonalcoholic fatty liver'/exp OR 'non alcoholic fatty liver disease':ab,ti OR 'nafld':ab,ti OR 'nonalcoholic fatty liver disease':ab,ti OR 'fatty liver, nonalcoholic':ab,ti OR 'fatty livers, nonalcoholic':ab,ti OR 'liver, nonalcoholic fatty':ab,ti OR 'livers, nonalcoholic fatty':ab,ti OR 'nonalcoholic fatty liver':ab,ti OR 'nonalcoholic fatty livers':ab,ti OR 'nonalcoholic steatohepatitis':ab,ti OR 'nonalcoholic steatohepatitides':ab,ti OR 'steatohepatitides, nonalcoholic':ab,ti OR 'steatohepatitis, nonalcoholic':ab,ti OR 'nash':ab,ti) AND ('probiotic agent'/exp OR 'probiotic*':ab,ti OR 'prebiotic*':ab,ti OR 'synbiocit*':ab,ti OR 'bifidobacter*':ab,ti OR 'lactobacill*':ab,ti OR 'flora':ab,ti)

Result: 842

Query:

D Search Hits

- #1 MeSH descriptor: [Non-alcoholic Fatty Liver Disease] explode all trees 659
- #2 Non alcoholic Fatty Liver Disease 1560
- #3 NAFLD 1418
- #4 Nonalcoholic Fatty Liver Disease 2014
- #5 Fatty Liver, Nonalcoholic 2225
- #6 Fatty Livers, Nonalcoholic 16
- #7 Liver, Nonalcoholic Fatty 2225
- #8 Livers, Nonalcoholic Fatty 16
- #9 Nonalcoholic Fatty Liver 2225
- #10 Nonalcoholic Fatty Livers 16
- #11 Nonalcoholic Steatohepatitis 892
- #12 Nonalcoholic Steatohepatitides 0
- #13 Steatohepatitides, Nonalcoholic 0
- #14 Steatohepatitis, Nonalcoholic 892
- #15 NASH 1545
- #16 MeSH descriptor: [Probiotics] explode all trees 1776
- #17 probiotic* 5727
- #18 prebiotic* 1312
- #19 synbiocit* 0
- #20 bifidobacter* 2469
- #21 Lactobacill* 4461
- #22 flora 3838
- #23 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 3248
- #24 #16 or #17 or #18 or #19 or #20 or #21 or #22 10806
- #25 #23 and #24 128

Result: 128

Web of Science

Query:

TS= ((probiotic* OR prebiotic* OR synbiocit* OR bifidobacter* OR Lactobacill* OR flora) AND (Non alcoholic Fatty Liver Disease OR NAFLD OR Nonalcoholic Fatty Liver Disease OR Fatty Liver, Nonalcoholic OR Fatty Livers, Nonalcoholic OR Liver, Nonalcoholic Fatty OR Livers, Nonalcoholic Fatty OR Nonalcoholic Fatty Liver OR Nonalcoholic Fatty Livers OR Nonalcoholic Steatohepatitis OR Nonalcoholic Steatohepatitides OR Steatohepatitides, Nonalcoholic OR Steatohepatitis, Nonalcoholic OR NASH)) OR TI= ((probiotic* OR prebiotic* OR synbiocit* OR bifidobacter* OR Lactobacill* OR flora) AND (Non alcoholic Fatty Liver Disease OR NAFLD OR Nonalcoholic Fatty Liver Disease OR Fatty Liver, Nonalcoholic OR Fatty Livers, Nonalcoholic OR Liver, Nonalcoholic Fatty OR Livers, Nonalcoholic Fatty OR Nonalcoholic Fatty Liver OR Nonalcoholic Fatty Livers OR Nonalcoholic Steatohepatitis OR Nonalcoholic Steatohepatitides OR Steatohepatitides, Nonalcoholic OR Steatohepatitis, Nonalcoholic OR NASH))

Result: 752

OVID

Query:

(probiotic*.ab. or probiotic*.ti. or prebiotic*.ab. or prebiotic*.ti. or synbiocit*.ab. or synbiocit*.ti. or bifidobacter*.ab. or bifidobacter*.ti. or Lactobacill*.ab. or Lactobacill*.ti. or flora.ab. or flora.ti. or Probiotics.ab. or Probiotics.ti.) and (((((((((((((((((((((((((Non alcoholic Fatty Liver Disease.ab.) or Non alcoholic Fatty Liver Disease.ti.) or NAFLD.ab.) or NAFLD.ti.) or Nonalcoholic Fatty Liver Disease.ab.) or Nonalcoholic Fatty Liver Disease.ti.) or Fatty Liver, Nonalcoholic.ab.) or Fatty Liver, Nonalcoholic.ti.) or Fatty Livers, Nonalcoholic.ab.) or Fatty Livers, Nonalcoholic.ti.) or Liver, Nonalcoholic Fatty.ab.) or Liver, Nonalcoholic Fatty.ti.) or Livers, Nonalcoholic Fatty.ab.) or Livers, Nonalcoholic Fatty.ti.) or Nonalcoholic Fatty Liver.ab.) or Nonalcoholic Fatty Liver.ti.) or Nonalcoholic Fatty Livers.ab.) or Nonalcoholic Fatty Livers.ti.) or Nonalcoholic Steatohepatitis.ab.) or Nonalcoholic Steatohepatitis.ti.) or Nonalcoholic Steatohepatitides.ab.) or Nonalcoholic Steatohepatitides.ti.) or Steatohepatitides, Nonalcoholic.ab.) or Steatohepatitides, Nonalcoholic.ti.) or Steatohepatitis, Nonalcoholic.ab.) or Steatohepatitis, Nonalcoholic.ti.) or NASH.ab.) or NASH.ti.))

Result: 1026

SUPPLEMENTARY FIGURES

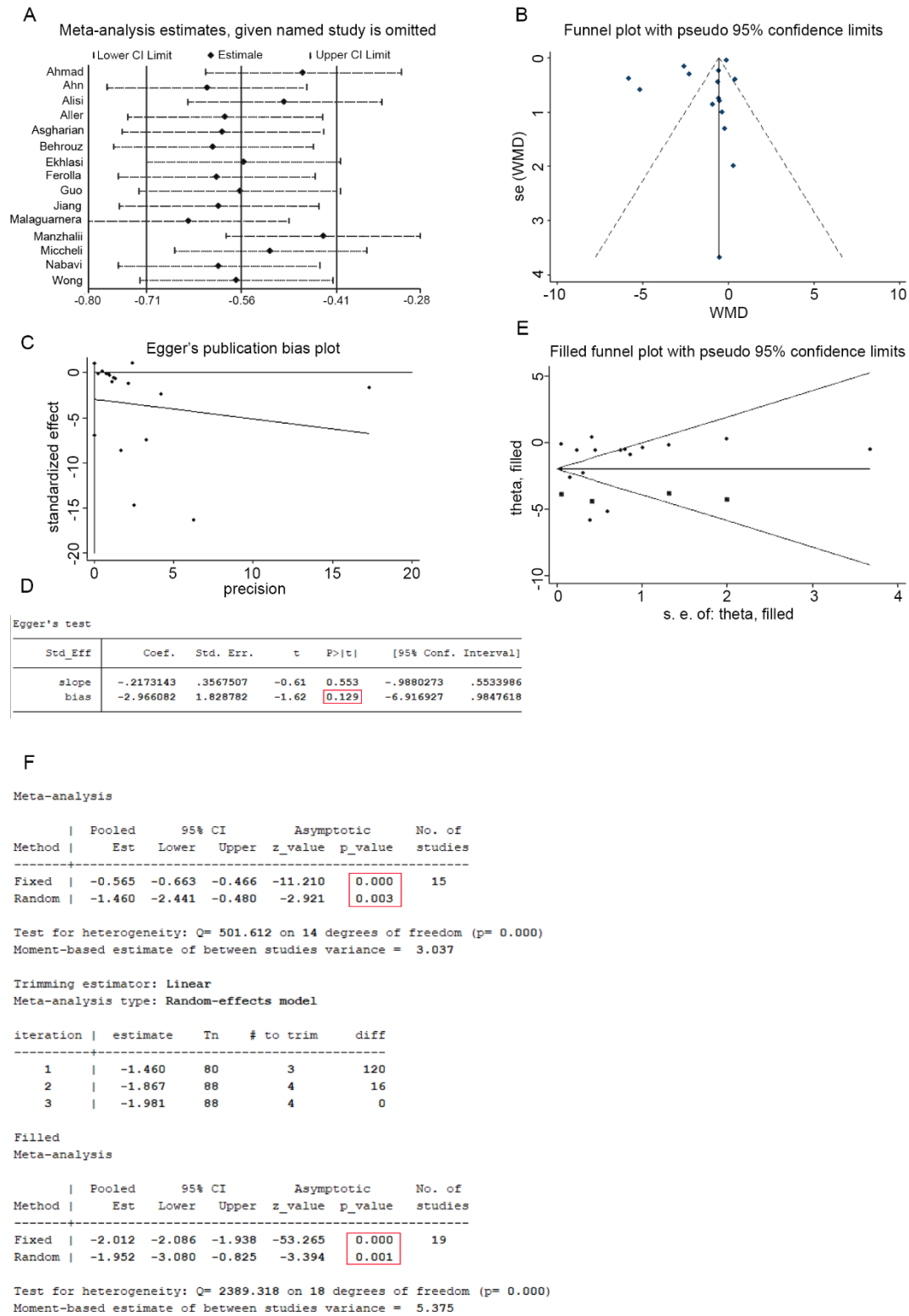


Figure S1: Supplementary data of body mass index (BMI), showing the interpretation of single study (A), funnel plot (B), Egger's tests (C, D), trim-and-fill computation (E, F).

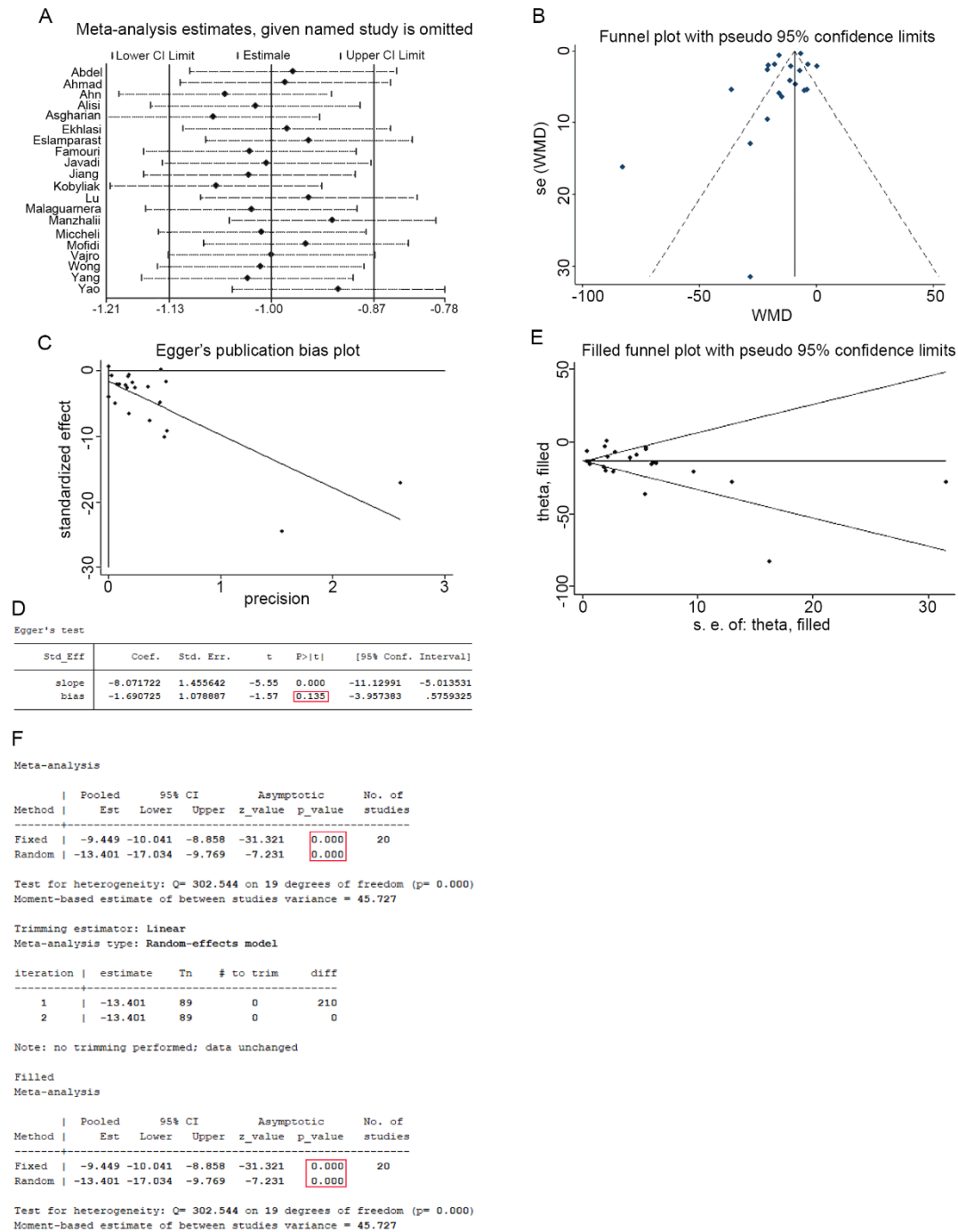


Figure S2: Supplementary data of alanine aminotransferase (ALT), showing the interpretation of single study (A), funnel plot (B), Egger's tests (C, D), trim-and-fill computation (E, F).

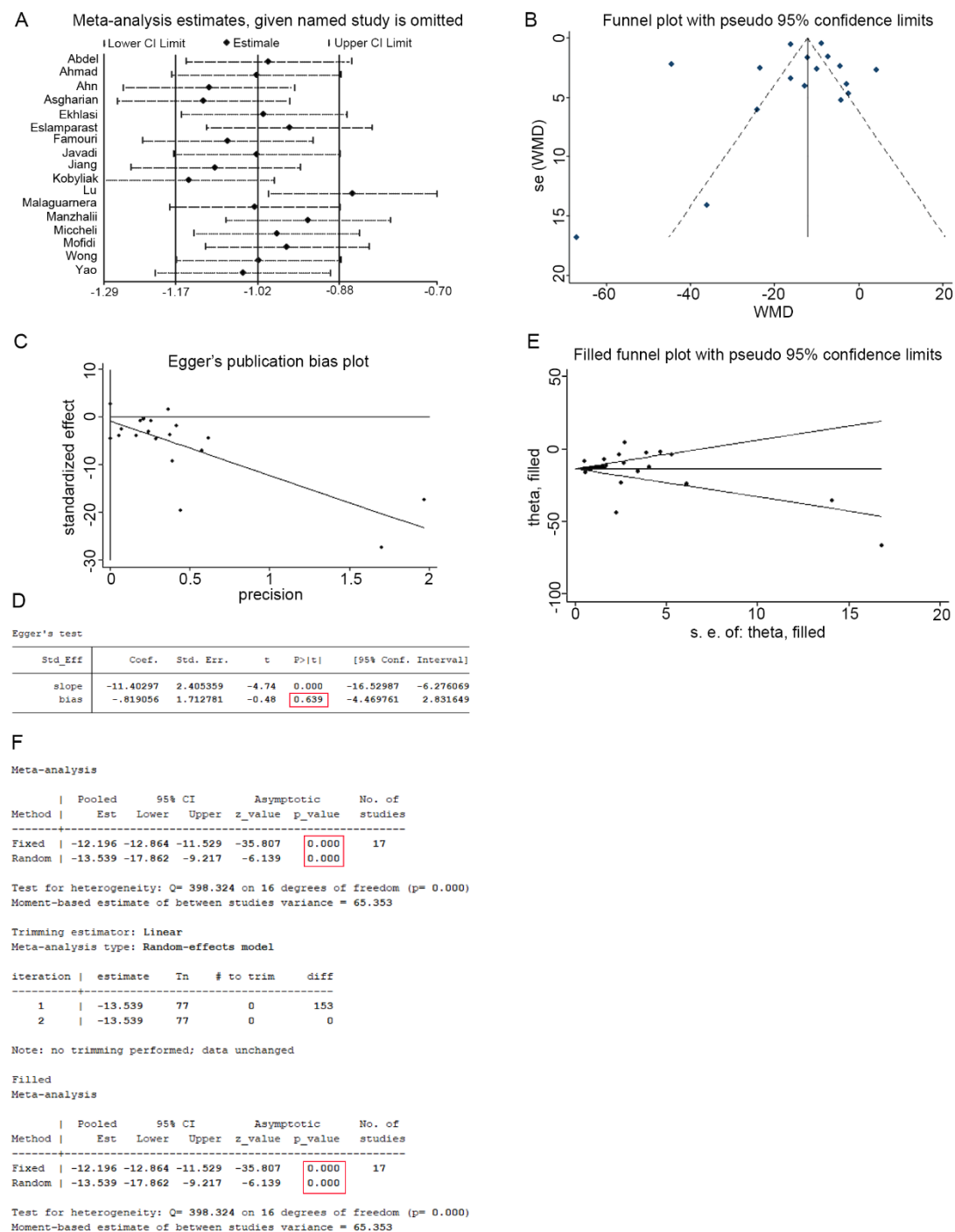


Figure S3: Supplementary data of aspartate transaminase (AST), showing the interpretation of single study (A), funnel plot (B), Egger's tests (C, D), trim-and-fill computation (E, F).

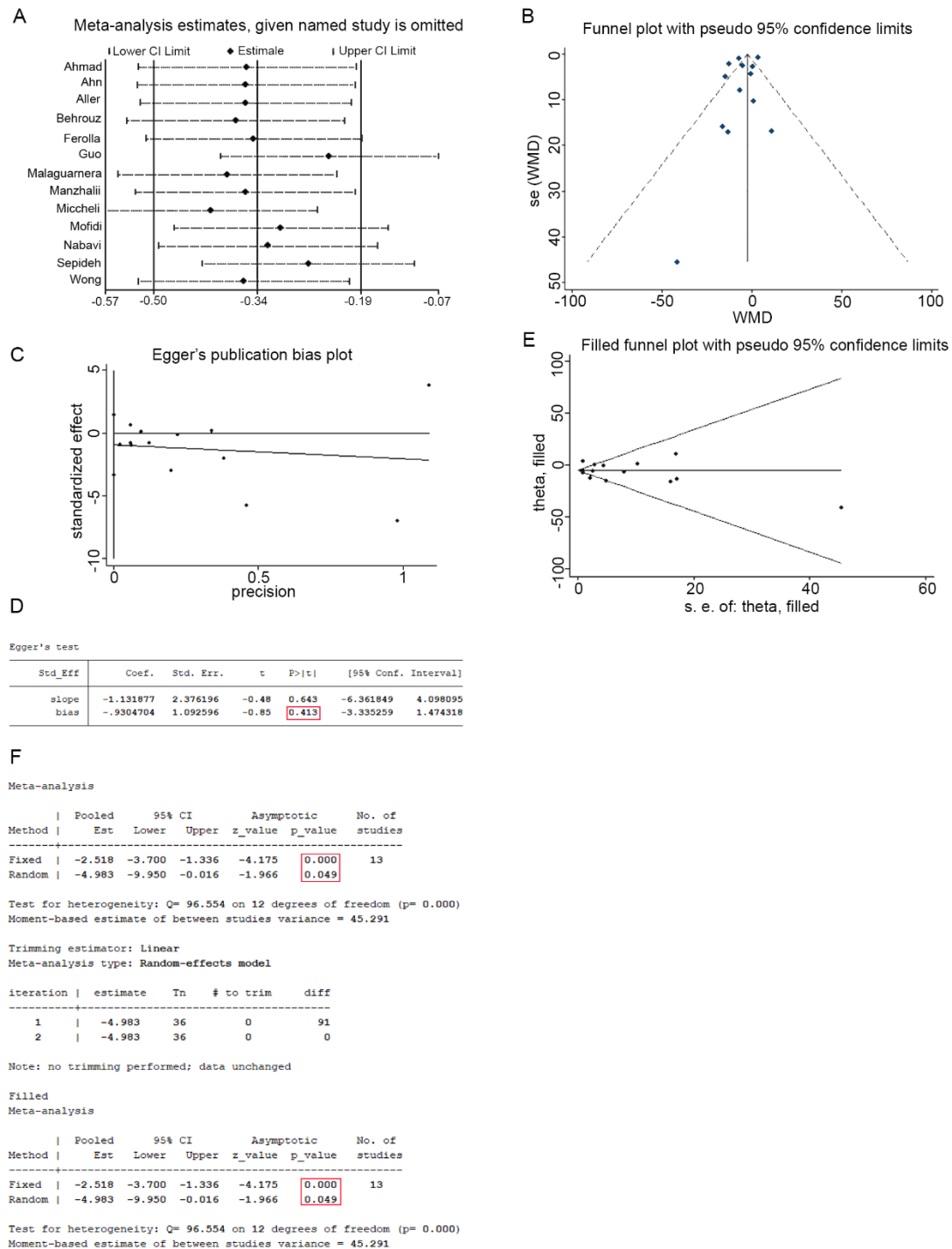


Figure S4: Supplementary data of fasting blood sugar (FBS), showing the interpretation of single study (A), funnel plot (B), Egger's tests (C, D), trim-and-fill computation (E, F).

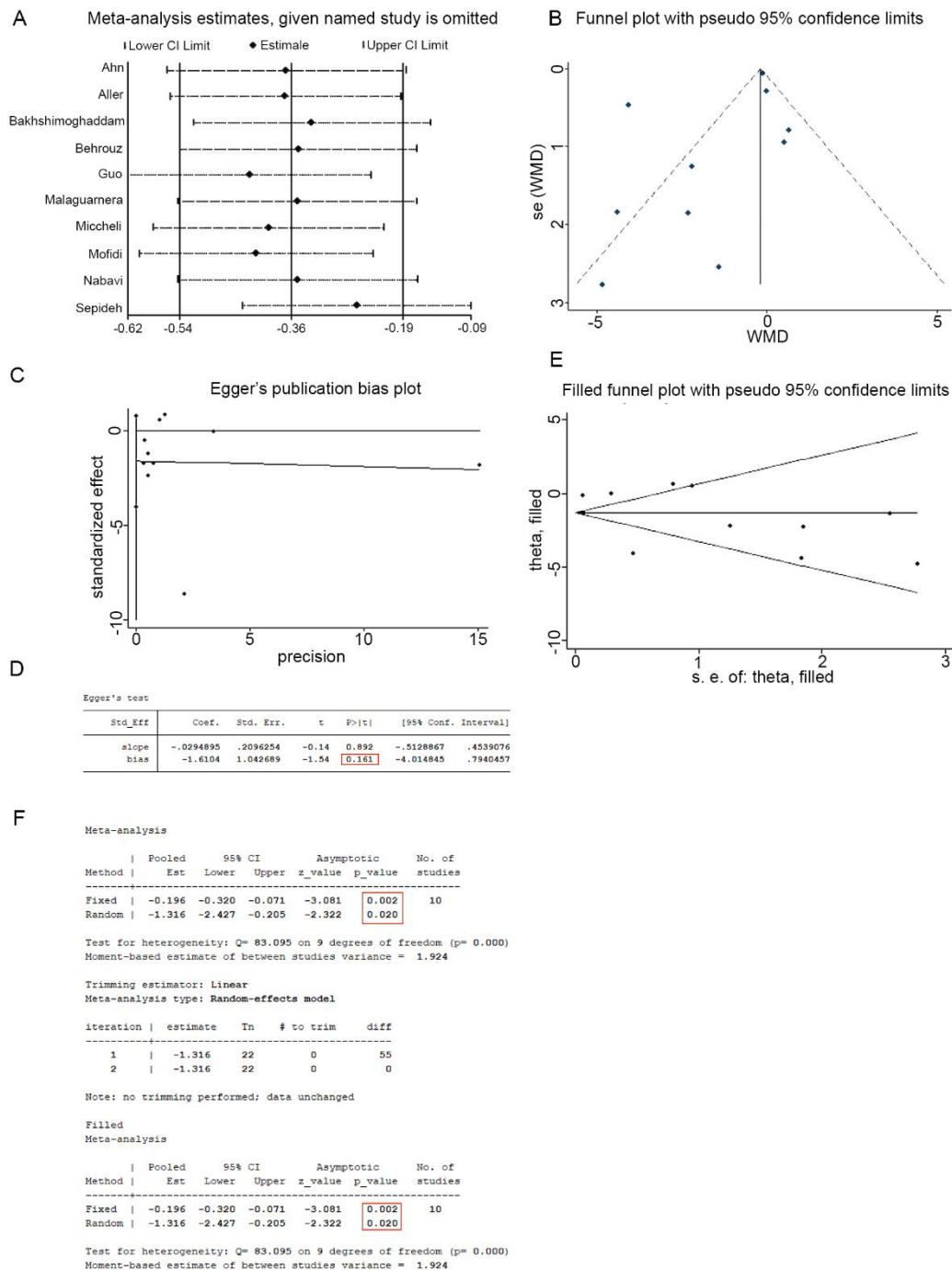


Figure S5: Supplementary data of insulin, showing the interpretation of single study (A), funnel plot (B), Egger's tests (C, D), trim-and-fill computation (E, F).

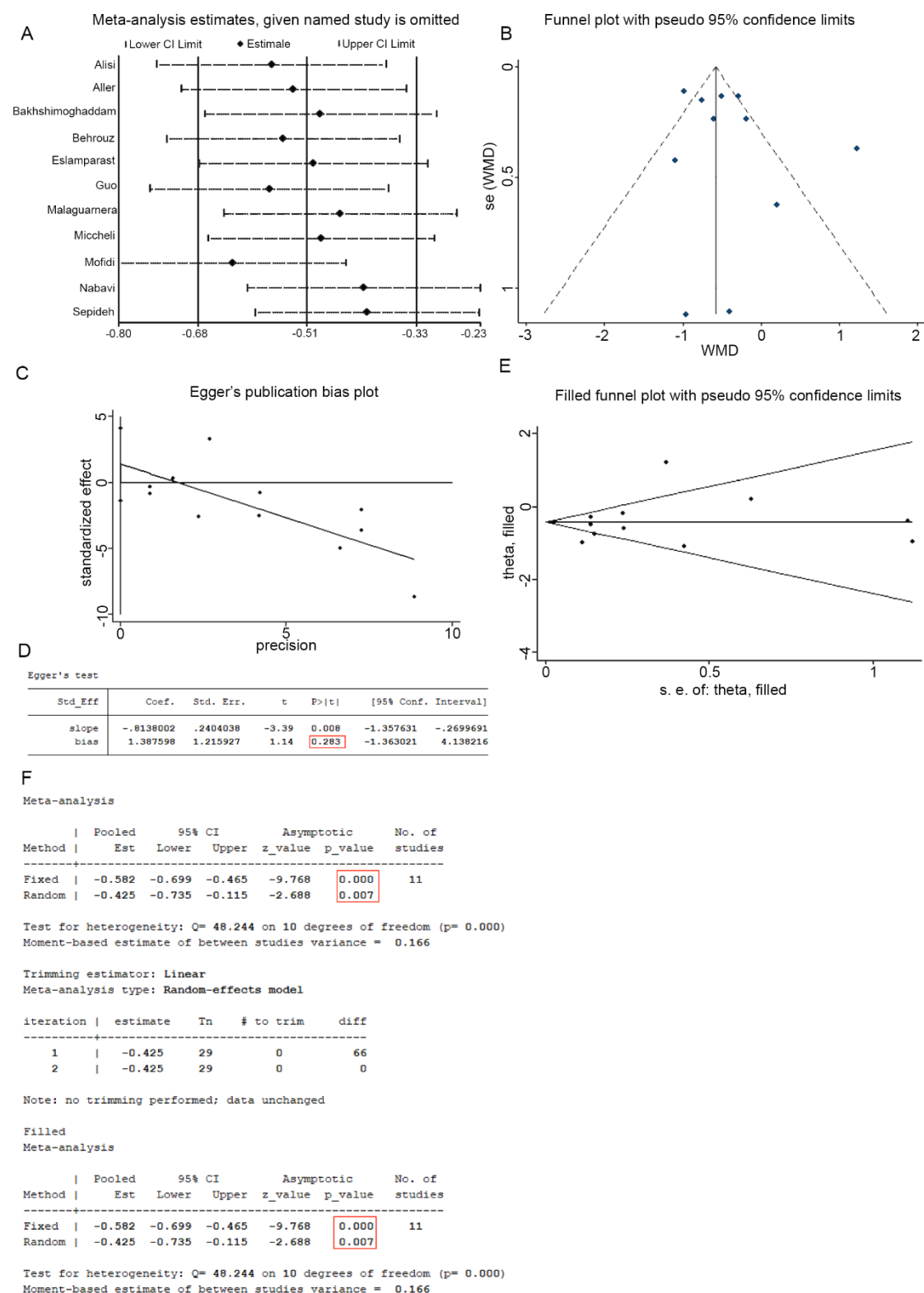


Figure S6: Supplementary data of homeostasis model assessment- insulin resistance (HOMA-IR), showing the interpretation of single study (A), funnel plot (B), Egger's tests (C, D), trim-and-fill computation (E, F).

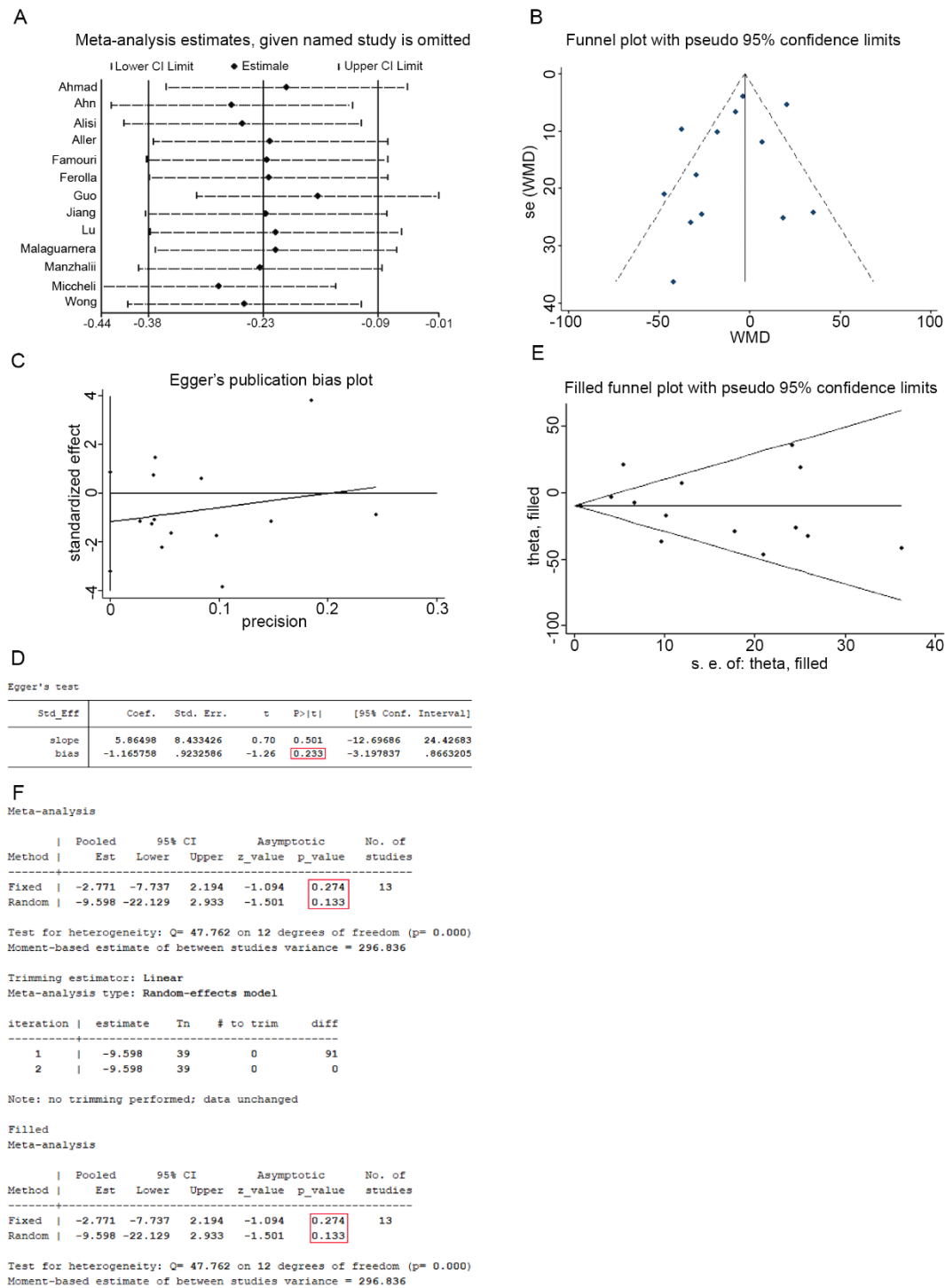


Figure S7: Supplementary data of triglycerides (TG), showing the interpretation of single study (A), funnel plot (B), Egger's tests (C, D), trim-and-fill computation (E, F).

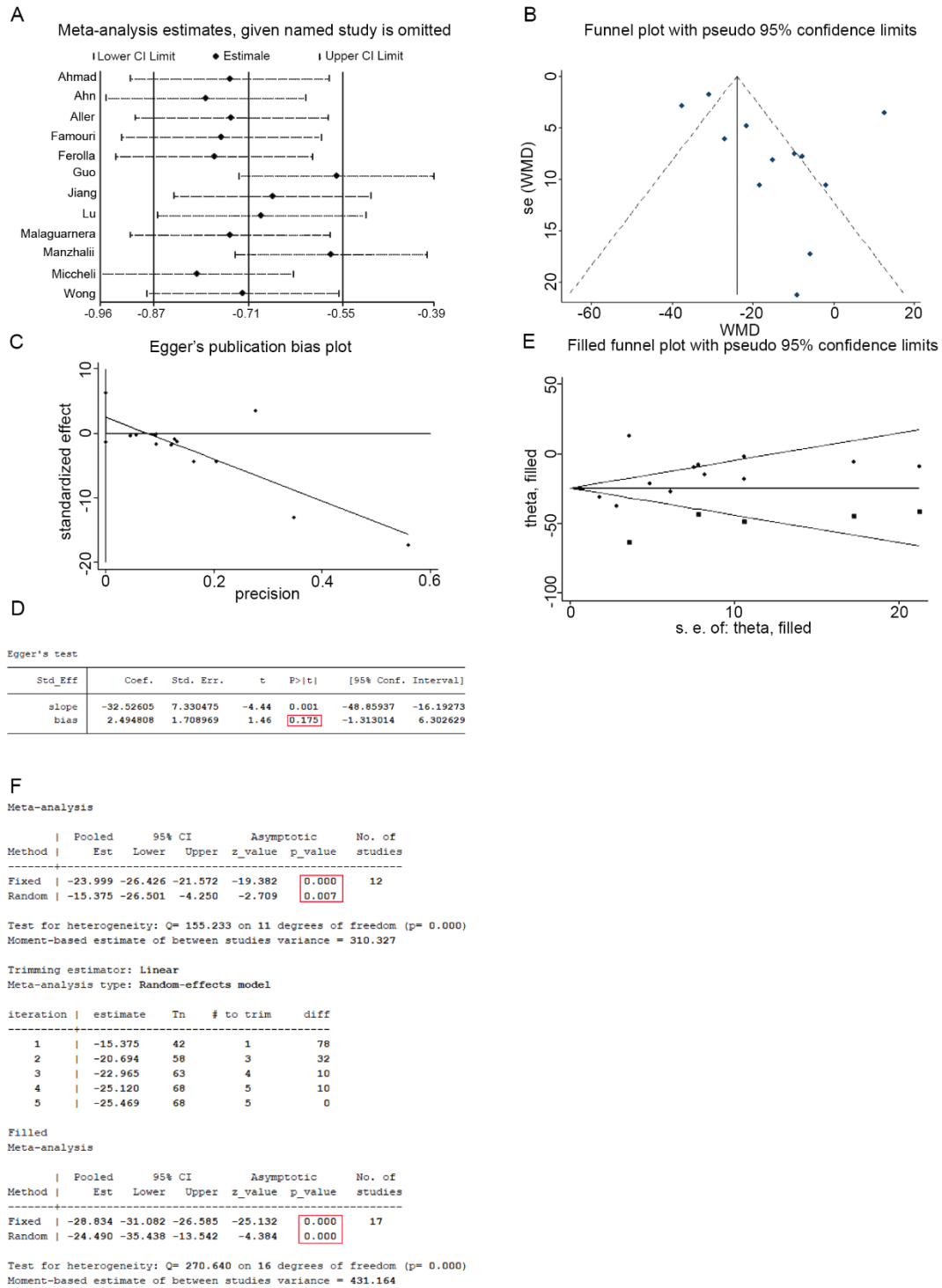


Figure S8: Supplementary data of total cholesterol (TC), showing the interpretation of single study (A), funnel plot (B), Egger's tests (C, D), trim-and-fill computation (E, F).

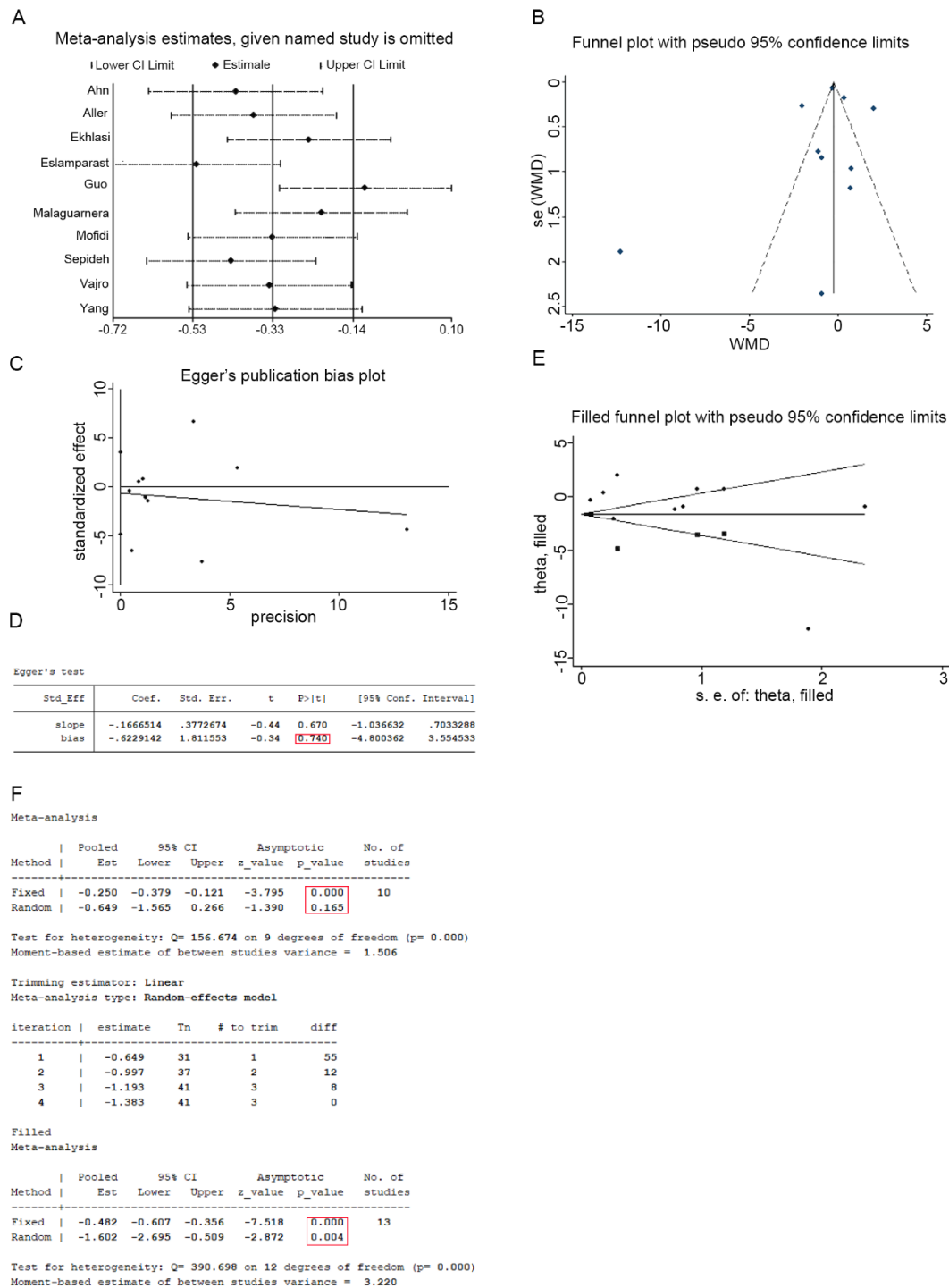


Figure S9: Supplementary data of tumor necrosis factor – alpha (*Tnf- α*), showing the interpretation of single study (A), funnel plot (B), Egger's tests (C, D), trim-and-fill computation (E, F).

Supplemental table 2: Evidence quality assessment

Question: Should probiotics be used for NAFLD?

Item: BMI

Quality assessment							No of patients		Effect		Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Probiotics	Control	Relative (95% CI)	Absolute		
BMI (Better indicated by lower values)												
15	randomised trials	serious ¹	serious ²	no serious indirectness	no serious imprecision	strong association ³	410	408	-	MD 0.56 lower (0.66 to 0.47 lower)	⊕⊕⊕⊕ MODERATE	IMPORTANT

¹ Studies included possess several bias

² Studies included reported inconsistency results

³ Studies included are more than 10

Item: Liver function

Quality assessment							No of patients		Effect		Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Probiotics	Control	Relative (95% CI)	Absolute		
ALT (Better indicated by lower values)												
20	randomised trials	serious ¹	no serious inconsistency	no serious indirectness	no serious imprecision	very strong association ²	561	555	-	MD 13.4 lower (17.03 to 9.77 lower)	⊕⊕⊕⊕ HIGH	CRITICAL
AST (Better indicated by lower values)												
17	randomised trials	serious ¹	serious ³	no serious indirectness	no serious imprecision	strong association ⁴	499	493	-	MD 12.2 lower (12.86	⊕⊕⊕⊕ MODERATE	CRITICAL

										to 11.53 lower)		
GGT (Better indicated by lower values)												
7	randomi sed trials	serio us ⁵	serious ³	no serious indirectne ss	no serious imprecisi on	none	248	240	-	MD 6.44 lower (7.46 to 5.41 lower)	⊕⊕⊕⊕ LOW	CRITICA L
LSM (Better indicated by lower values)												
4	randomi sed trials	serio us ⁵	no serious inconsisten cy	no serious indirectne ss	Serious ⁵	none	99	103	-	MD 0.65 lower (0.76 to 0.55 lower)	⊕⊕⊕⊕ LOW	CRITICA L
Hepatic steatosis (Better indicated by lower values)												
4	randomi sed trials	serio us ⁵	no serious inconsisten cy	no serious indirectne ss	Serious ⁵	none	131	128	-	not pooled	⊕⊕⊕⊕ LOW	CRITICA L

¹ Studies included possess several bias

² Studies included are more than 20

³ Studies included reported inconsistency results

⁴ Studies included are more than 10

⁵ Studies included are less than 5

Item: Glycemic indices

Quality assessment							No of patients		Effect		Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Probiotics	Control	Relative (95% CI)	Absolute		
FBS (Better indicated by lower values)												
13	randomised trials	serious ¹	serious ²	no serious indirectness	no serious imprecision	strong association ³	346	347	-	MD 2.52 lower (3.7 to 1.34 lower)	⊕⊕⊕⊕ MODERATE	IMPORTANT

Insulin (Better indicated by lower values)												
10	randomised trials	serious ¹	no serious inconsistency	no serious indirectness	no serious imprecision	strong association ³	271	273	-	MD 0.2 lower (0.32 to 0.07 lower)	⊕⊕⊕⊕	HIGH
GLP-1 (Better indicated by lower values)												
2	randomised trials	serious ¹	no serious inconsistency	no serious indirectness	serious ⁴	none	37	38	-	MD 1.37 higher (1.24 to 1.5 higher)	⊕⊕○○	IMPORTANT
HOMA-IR (Better indicated by lower values)												
12	randomised trials	serious ¹	serious ²	no serious indirectness	no serious imprecision	strong association ³	317	317	-	MD 0.58 lower (0.7 to 0.47 lower)	⊕⊕⊕○	IMPORTANT

Item: Lipid profiles

8	randomised trials	serious ¹	serious ²	no serious indirectness	no serious imprecision	none	212	208	-	MD 0.31 higher (3.52 lower to 4.15 higher)	⊕⊕○○ LOW	IMPORTANT
Triglyceride (Better indicated by lower values)												
13	randomised trials	serious ¹	serious ²	no serious indirectness	no serious imprecision	strong association ³	383	383	-	MD 2.77 lower (7.74 lower to 2.19 higher)	⊕⊕⊕○ MODERATE	IMPORTANT
Cholesterol (Better indicated by lower values)												
12	randomised trials	serious ¹	serious ²	no serious indirectness	no serious imprecision	strong association ³	361	361	-	MD 24 lower (26.43 to 21.57 lower)	⊕⊕⊕○ MODERATE	IMPORTANT

¹ Studies included possess several bias

² Studies included reported inconsistency results

³ Studies included are more than 10

Item: Inflammation factors

Quality assessment							No of patients		Effect		Quali	Importan
No of studi es	Design	Risk of bias	Inconsiste ncy	Indirectn ess	Imprecis ion	Other considerati ons	Probioti cs	Contr ol	Relati ve (95% CI)	Absol ute		
TNF-a (Better indicated by lower values)												
10	randomi sed trials	seriou s ¹	serious ²	no serious indirectne ss	no serious imprecisi on	reporting bias strong association ⁴	239	240	-	MD 0.25 lower (0.38 to 0.12 lower)	⊕⊕○○ LOW	IMPORTA NT
IL-6 (Better indicated by lower values)												
4	randomi	seriou	serious ²	no serious	Serious ⁴	none	95	100	-	MD	⊕○○○	IMPORTA

	sed trials	s ¹		indirectne ss						0.08 lower (0.37 lower to 0.21 higher)	O VER Y LOW	NT
CRP (Better indicated by lower values)												
4	randomi sed trials	seriou s ¹	no serious inconsisten cy	no serious indirectne ss	Serious ⁴	none	117	111	-	MD 1.27 lower (2.1 to 0.44 lower)	⊕⊕O O LOW	IMPORTA NT

¹ Studies included possess several bias

² Studies included reported inconsistency results

³ Studies included are more than 10

⁴ Studies included are less than 5

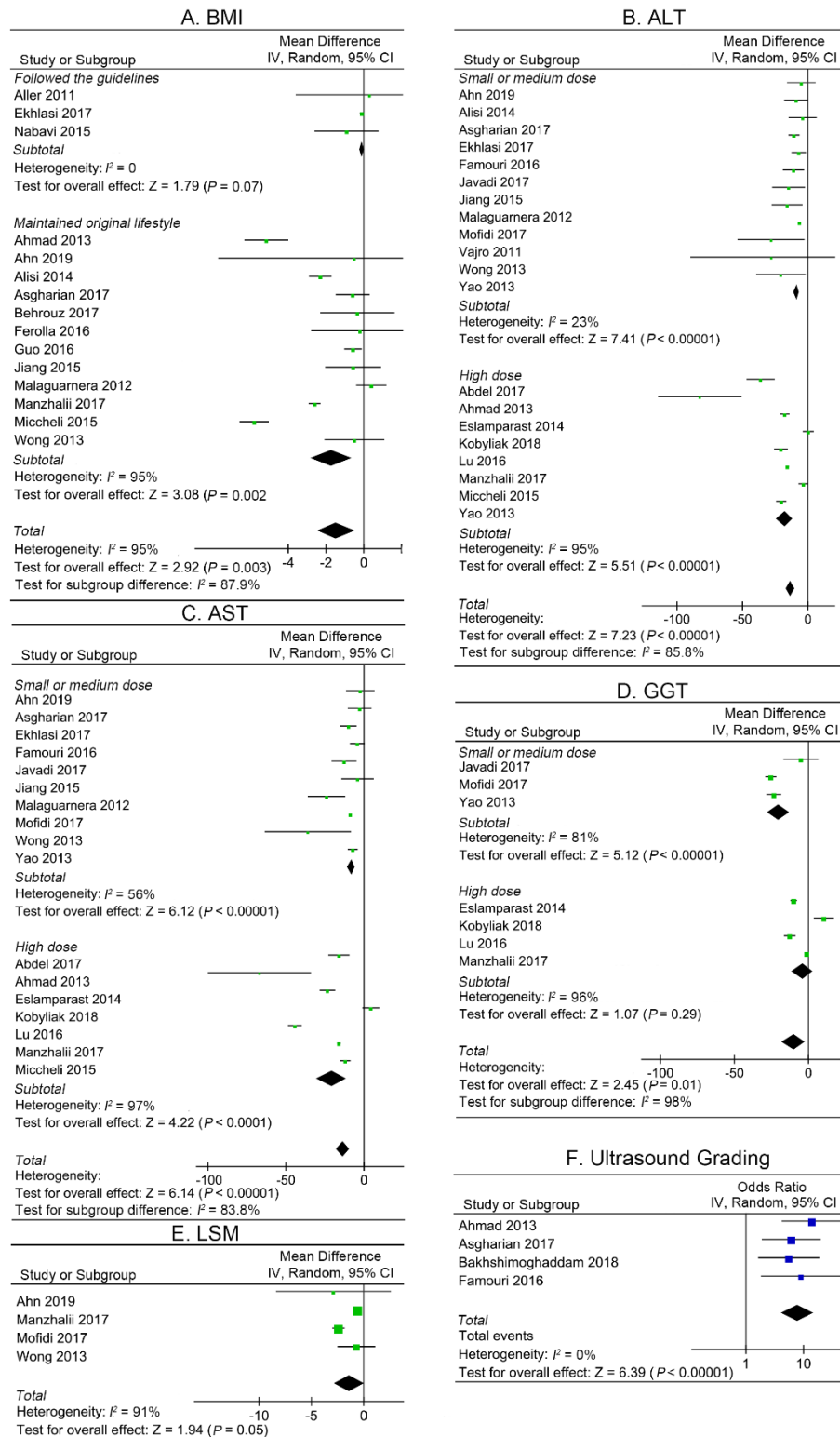


Figure S10: Forest plots of comparison for the effects of probiotics in NAFLD patients, showing (A) body mass index (BMI), (B) alanine aminotransferase (ALT), (C) aspartate transaminase (AST), (D) gamma -glutamyl transpeptidase (GGT), and subgroup analyses by probiotic strains of each index.