# Efficacy of L-Ornithine L-Aspartate for the Treatment of Hepatic Encephalopathy and Hyperammonemia in Cirrhosis: Systematic Review and Meta-Analysis of Randomized Controlled Trials

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***Background/objectives:*** **L-Ornithine L-Aspartate (LOLA) is a mixture of two endogenous amino acids with the capacity to fix ammonia in the form of urea and/or glutamine. Its' efficacy for the treatment of Hepatic Encephalopathy (HE), a known hyperammonemic disorder, remains the subject of debate. This study quantitatively analyzed the efficacy of LOLA in patients with cirrhosis and HE.** ***Methods:*** **Efficacy was defined as the extent of lowering of blood ammonia and improvement of mental state assessed in clinically overt HE (OHE) by Westhaven criteria or psychometric testing for assessment of Minimal HE (MHE). Appropriate keywords were used for electronic and/or manual searches of databases to identify RCTs for inclusion. Study quality and risk of bias were assessed using the Jadad Composite Scale together with The Cochrane Scoring Tool. Random Effects Models were used to express pooled Risk Ratio (RR) or Mean Difference (MD) with associated 95% Confidence Intervals (CI).** ***Results:*** **10 RCTs (884 patients) were included. Regression analysis showed no evidence of publication bias or other small study effects. Eight RCTs had low risk of bias by Jadad/Cochrane criteria. Comparison with placebo/no intervention controls revealed that LOLA was significantly more effective for improvement of mental state in all types of HE (RR 1.36 (95% CI 1.10–1.69), $p = 0.005$), OHE (RR: 1.19, 95% CI of 1.01–1.39, test for overall effect: $Z = 2.14$, $p = 0.03$), MHE (RR: 2.15 (1.48–3.14), $p < 0.0001$) and for lowering of blood ammonia (MD: −17.50 μmol/l (−27.73 to (−7.26)), $p = 0.0008$). Improvement of mental state was greater in trials with low risk of bias. Heterogeneity was reduced in trials from Europe or with >100 participants. Oral LOLA appeared particularly effective for the treatment of MHE.** ***Conclusion:*** **LOLA appears to improve mental state and lower ammonia in patients with HE or MHE. Further studies are required in some subgroups of HE and in the era of HE reclassification. (J Clin Exp Hepatol 2018;8:301–313)**

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Hepatic Encephalopathy (HE) is a serious neuropsychiatric complication of cirrhosis characterized by a spectrum of neurological and psychiatric symptoms. Deficits of attention and visuo-spatial construction in addition to impaired motor speed and accuracy are symptoms of early HE in patients with cirrhosis.[1] Overt Hepatic Encephalopathy (OHE) progressing through asterixis to stupor and coma is associated with poor prognosis and high mortality. Minimal Hepatic Encephalopathy (MHE) is a term used to define common low-grade alterations of mental status generally diagnosed by psychometric testing. This has recently been modified to include those patients with grade 1 overt HE and MHE now grouped as a single entity known as "covert HE".[2]

Hyperammonemia is consistently reported in patients with cirrhosis and OHE or MHE where treatment strategies remain principally focused on the lowering of circulating ammonia. The current mainstays of pharmacological therapy are the non-absorbable disaccharide lactulose[3] or the antibiotic rifaximin.[4]

L-Ornithine L-Aspartate (LOLA), a mixture of two endogenous amino acids, has established ammonia-lowering properties and mechanism of action.[5] Beneficial

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*Abbreviations:* HE: Hepatic Encephalopathy; LOLA: L-Ornithine L-Aspartate; OHE: Overt Hepatic Encephalopathy
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effects of LOLA for the management and treatment of HE have been reported in over 20 Randomized Clinical Trials (RCTs) in patients with cirrhosis. However, while recent reviews provide a consensus of opinion that LOLA is of benefit for the treatment of OHE,[6,7] evidence regarding its use in MHE has been questioned.[8,9] In the current AASLD-EASL Guidelines, recommendations relating to the use of LOLA for the treatment of HE in cirrhosis were based upon the results of a single RCT of intravenous LOLA while the oral formulation was deemed to be ineffective.[2]

Results of three meta-analyses have been published in the last decade.[10–12] However, in all cases, these studies involved small numbers of patients and/or were published in Abstract form only. There is a need to reassess the ability of oral or intravenous LOLA to treat OHE, MHE and to lower blood ammonia.

The objectives of the present systematic review with meta-analysis, therefore, were 2-fold namely (1) to provide an up-to-date evidence base for the efficacy of LOLA for the treatment of OHE and MHE in cirrhosis and (2) to conduct an objective assessment of the agent's efficacy for the lowering of blood ammonia.



## METHODS

### Search Criteria

This involved electronic and manual searches using appropriate keywords as follows: Search strings: (1) Hepatic Encephalopathy (HE), (2) Overt Hepatic Encephalopathy (OHE), (3) Minimal Hepatic Encephalopathy (MHE), (4) L-Ornithine L-Aspartate (LOLA), (5) Intravenous Formulation (iv), (6) oral formulation (oral), (7) cirrhosis, (8) Randomized Controlled Trial (RCT). Search string: (#1 or #2 or #3) and (#4 or #5) and #7 and #8. Medline, PubMed, the Cochrane Controlled Trials Register (2008), Google search and Clinicaltrials.gov were interrogated. Trials published in English, French, German or other languages with available translations were included in the searches.

### Inclusion and Exclusion Criteria

Trials of LOLA in male or female patients over the age of 18 years with MHE or OHE as defined by the classification system established by the Working Party of the 11th World Congress of Gastroenterology, Vienna[13] were included. The study was required to compare LOLA to placebo or no intervention control as part of a randomized controlled clinical trial (RCT) with adequate description of patient characteristics, patient numbers, trial design, blinding of personnel, patients and investigators, dropouts, dose and route of administration of test substances and control in sufficient detail to assess trial quality and risk of biases.

Uncontrolled trials, open-label trials, observational studies, non-cirrhotic patients, patients with acute liver failure/fulminant hepatic failure, absence of HE, studies published in abridged form (abstract, review, editorial, conference proceedings) or with inadequate details for assessment of trial outcomes/design/risks of bias were excluded. The decision to include or exclude trials was made independently by authors followed by discussions to arrive at a consensus. Final trial selections were made prior to assessment of trial quality or risk of bias in all cases. Based upon the above inclusion/exclusion criteria, a total of 9 published RCTs were retained for inclusion in the systematic review and meta-analysis.

The RCTs included in the current systematic review and meta-analysis were conducted (and the results published) prior to use of the term "Covert HE". Consequently, the term MHE has been employed throughout the present analysis since that was the most widely used term to describe HE that was not clinically overtly apparent being assessed using psychometric testing. Characteristics of the 10 eligible full-text articles are presented in Table 1.[14–23]

### Outcome Measures

The primary outcome measure was defined as improvement in mental state of patients with cirrhosis and at least one episode of OHE or MHE. Mental state improvement was determined by improvement of OHE grade using Westhaven criteria or improvement of MHE assessed by Number connection tests NCT-A, NCT-B or PHES.[24]

A second primary outcome measure was defined as reduction of hyperammonemia based upon measurement of blood ammonia using standard biochemical laboratory testing or commercially available ammonia test kits.

### Quality, Bias and Heterogeneity Assessment

Several of the trials included in this systematic review and meta-analysis were performed and/or published prior to the publication of widely-accepted guidelines for the conduction of systematic reviews and meta-analyses such as PRISMA.[25] Consequently, a custom-designed assessment paradigm was established whereby elements of the earlier Jadad composite scale for the assessment of trial quality[26] was performed in conjunction with essentials of the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement.[25]

The Jadad composite scale uses assessments of randomization methods, double blinding and adequate description of patient withdrawals and drop-outs with a maximum score of 5; trials with a score of 3 or above are of high quality.[26]

Assessments of the risk of bias were made using the Cochrane Collaboration's tool for assessment of risk of bias relating to selection, performance, detection, attention as well as reporting bias for each main outcome as described in the assessment tool.[27] Bias related to publication of trial results was assessed by regression analysis. In the present meta-analysis the overall trial quality was

 

**Table 1 Characteristics of included studies.**

| Author | Year | Full reference | No. of patients randomized | Dose/duration | Type of HE | Efficacy parameter measured |
|---|---|---|---|---|---|---|
| *Intravenous* | | | | | | |
| Sidhu et al.[14] | 2018 | Hepatology 2018 Feb; 67(2):700–10 | 162 $n = 83$ LOLA \| $n = 79$ PLA | 30 g/d, 5 d | HE II-IV | Reduction of hyperammonemia Metal State Improvement |
| Abid et al.[15] | 2011 | J Coll Physicians Surg Pak. 2011 Nov;21 (11):666–71. | 120 $n = 60$ LOLA \| $n = 60$ PLA | 20 g/d, 3 d | MHE HE I-IV | Reduction of hyperammonemia Mental State Improvement |
| Schmid et al.[16] | 2010 | Liver Int. 2010 Apr; 30(4):574–82. | 40 $n = 20$ LOLA \| $n = 20$ PLA | 20 g/d, 8 d | MHE HE I-II | Reduction of hyperammonemia Mental State Improvement |
| Ahmad et al.[17] | 2008 | J Coll Physicians Surg Pak. 2008 Nov; 18(11):684–7. | 80 $n = 40$ LOLA \| $n = 40$ PLA | 20 g/d, 5 d | HE I-III | Reduction of hyperammonemia Mental State Improvement |
| Chen et al.[18] | 2005 | J First Mil Med. Univ. 2005; 25:718–722. | 85 $n = 45$ LOLA \| $n = 40$ CON | 20 g/d, 7 d/BCAA i.v. | HE I-IV | Reduction of hyperammonemia |
| Kircheis et al.[19] | 1997 | Hepatology. 1997 Jun;25(6):1351–60. | 126 $n = 63$ LOLA \| $n = 63$ PLA | 20 g/d, 7 d | MHE HE I-II | Reduction of hyperammonemia Mental State Improvement |
| *Oral* | | | | | | |
| Alvares-da-Silva et al.[20] | 2014 | Hepatol Res. 2014 Sep;44(9):956–63. | 64 $n = 28$ LOLA \| $n = 35$ PLA | 15 g/d, 60 d | MHE | Reduction of hyperammonemia Mental State Improvement Quality of life |
| Sharma et al.[21] | 2014 | Saudi J Gastroenterol. 2014 Jul-Aug; 20(4):225–32. | 124 $n = 31$ LOLA \| $n = 31$ RIF \| $n = 32$ PRO \|$n = 30$ PLA | 18 g/d, 60 d | MHE | Mental State Improvement |
| Mittal et al.[22] | 2011 | Eur J Gastroenterol Hepatol. 2011 Aug; 23(8):725–32. | 160 $n = 40$ LAC \| $n = 40$ LOLA \| $n = 40$ PRO \| $n = 40$ PLA | 18 g/d, 3 mo | MHE | Reduction of hyperammonemia Mental State Improvement |
| Stauch et al.[23] | 1998 | J Hepatol. 1998 May;28(5):856–64. | 66 $n = 34$ LOLA \| $n = 32$ PLA | 18 g/d, 14 d | MHE HE I-II | Reduction of hyperammonemia Mental State Improvement |

considered to be high for trials with Jadad score 3 or above in addition to low risk of bias by the Cochrane tool.

*Statistical analyses* were performed according to standard published procedures. In the case of continuous outcome variables, groups were compared by mean differences with 95% confidence intervals; for dichotomous variables, the Relative Risk (RR) was considered with 95% confidence intervals. Since RR results in similarly consistent results as the Odds Ratio (OR),[28] RR was used for dichotomous variables to facilitate comparisons with the results of previous meta-analyses.[11,12] Heterogeneity was explored using the $\chi^2$ test with significance set at a $p$ value of 0.10 or less and also using the $I^2$ statistic.[29] Aggregation of the primary studies made use of the Random Effects Model rather than the Fixed Effects Model[30] in all cases. The primary subgroup analyses were carried out relating to intravenous versus oral formulations of LOLA as well as the type of HE (OHE, MHE). Further subgroups to assess heterogeneity were pre-specified and included study size, location, quality and era and was performed using the $I^2$ statistic where $I^2 > 50\%$ indicated significant heterogeneity.

All analyses were carried out using RevMan version 5 (Cochrane collaboration). The problem of reporting bias was addressed by Funnel Plot analyses and subsequent correction techniques.[31]

## RESULTS

Electronic searches of the databases identified 43 trials with a further 16 from manual searches. Five full-text articles were excluded for reasons of incompatibility of data presentation required for pooling. Following removal of duplicate citations and elimination of published studies in line with eligibility and inclusion/exclusion criteria, a total of 10 trials were included in the final meta-analysis (Figure 1) where sufficient data were available for pooling. Included trials are summarized in Table 1.

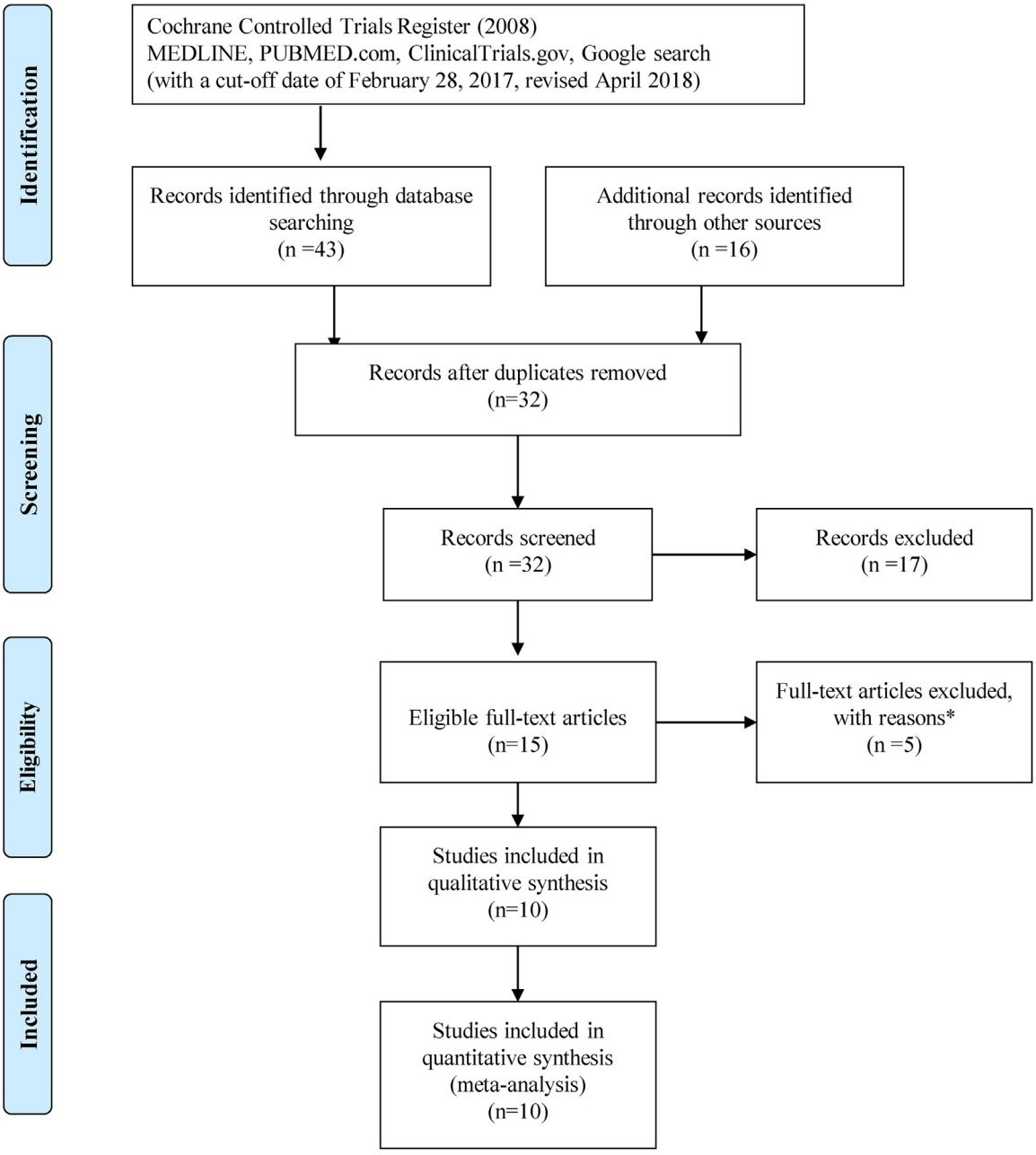


**Figure 1** Flowchart indicating key steps (identification, screening, eligibility and final inclusion) of trials included in qualitative and quantitative synthesis (meta-analysis). Of 32 records screened, 17 were excluded since they were published as abstracts, reviews, editorials or book chapters with insufficient data for analysis. Of the 15 eligible full-text articles, an additional 5 were excluded since they included patients with post-TIPS HE considered to be a distinct condition ($n$ = 2) or trials in which the data presentation was incompatible ($n$ = 1) or incomplete ($n$ = 1) for full assessment of trial design, quality or risk of bias.

## Risk of Bias in the Selected Trials

Individual assessments of trial quality and risk of bias are shown for all selected trials in Table 2 and Figure 2. Six of the 10 eligible full-text articles were assessed as high quality (by Jadad score) with a low risk of bias due to trial selection, performance, detection and attrition (using the Cochrane tool for risk of bias assessment) described in Methods. There was no evidence of reporting bias in these trials.

## Subgroup Analysis

Preplanned subgroup analysis was performed on the HE and ammonia lowering outcomes on studies with high versus low quality, size, location and era (Table 3a and

**Table 2 Quality assessment and risk of bias of the included studies.**

| Trial | | Jadad scoring | | | | Cochrane scoring | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Author | Year | Random | Blinding | Withdrawals Dropouts | Total score | Selection bias | | Performance bias | Detection bias | Attrition bias | Reporting bias | Total score | Total |
| | | | | | | Random generation | Allocation concealment | Blinding (1) | Blinding (2) | Outcome data | | | Trial Quality |
| *Intravenous* | | | | | | | | | | | | | |
| Sidhu SS et al.[14] | 2018 | 2 | 2 | 1 | 5 | Low | Low | Low | Low | Low | Low | Low | High |
| Abid S et al.[15] | 2011 | 1 | 1 | 1 | 3 | Low | Low | Low | UC | Low | Low | Low | High |
| Schmid M et al.[16] | 2010 | 2 | 1 | 1 | 4 | Low | Low | Low | Low | High | Low | Low | High |
| Ahmad I et al.[17] | 2008 | 2 | 0 | 1 | 3 | Low | UC | High | High | Low | Low | High | Low |
| Chen M et al.[18] | 2004 | 1 | 0 | 0.5 | 1.5 | Low | UC | High | High | UC | UC | High | Low |
| Kircheis G et al.[19] | 1997 | 2 | 2 | 1 | 5 | Low | Low | Low | Low | Low | Low | Low | High |
| *Oral* | | | | | | | | | | | | | |
| Alvares-da-Silva MR et al.[20] | 2014 | 2 | 1 | 1 | 4 | Low | Low | Low | Low | Low | UC | Low | High |
| Sharma K et al.[21] | 2014 | 2 | 0 | 1 | 3 | Low | High | High | High | Low | Low | High | Low |
| Mittal VV et al.[22] | 2011 | 2 | 0 | 1 | 3 | Low | Low | High | High | Low | Low | High | Low |
| Stauch S et al.[23] | 1998 | 2 | 2 | 1 | 5 | Low | Low | Low | Low | Low | Low | Low | High |

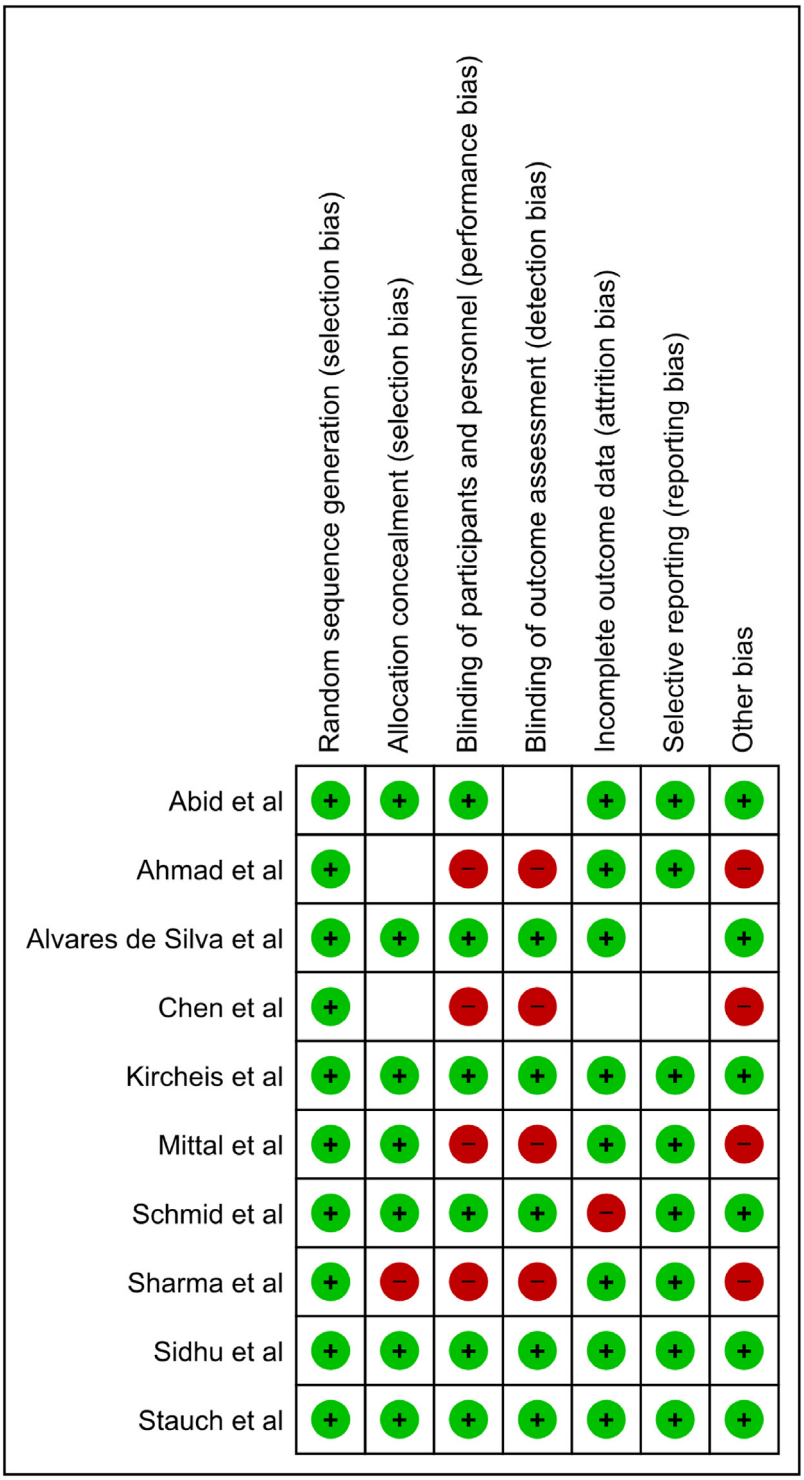


**Figure 2** Cochrane risk of bias summary for the included studies. The (+) symbol indicates low risk, (−) indicates high risk. A blank square represents unclear risk as per Cochrane recommendations.

Table 3b). Interestingly low-quality studies for ammonia reduction had reduced heterogeneity for studies published after 2010, in larger cohorts ($n > 100$ patients) and in Europe. The reduced heterogeneity in later studies or those from Europe may be from more clinical homogeneity in the phenotyping of patients with HE and suggests that studies in future should take into account not only attempts to improve clinical phenotyping and heterogeneity but be of sufficient size. Of note the subgroups with low heterogeneity in the "ammonia-lowering" outcome

**Table 3a Subgroup analysis for the primary outcome of mental state improvement dependent on pre-specified subgroups to determine the impact on heterogeneity and clinical effect.**

| | No. of studies | Risk ratio | Confidence interval | Overall effect *p* value | Heterogeneity *p* value | $I^2$ (%) |
|---|---|---|---|---|---|---|
| **All** | | | | | | |
| *Quality* | | | | | | |
| Low | 4 | 1.46 | 1.03–2.08 | 0.03 | 0.002 | 80% |
| High | 5 | 1.33 | 0.96–1.83 | 0.08 | <0.001 | 85% |
| *Era* | | | | | | |
| Early (pre-2010) | 4 | 1.36 | 1.05–1.78 | 0.02 | 0.01 | 72% |
| Late (2010 and after) | 5 | 1.40 | 0.98–2.02 | 0.07 | <0.001 | 86% |
| *Study size* | | | | | | |
| Small (<100) | 6 | 1.56 | 1.11–2.20 | 0.01 | 0.0001 | 75% |
| Large (>100) | 3 | 1.22 | 0.90–1.64 | 0.20 | 0.001 | 89% |
| *Location* | | | | | | |
| Europe | 2 | 1.89 | 1.32–2.70 | 0.005 | 0.85 | 0% |
| Rest of world | 6 | 1.22 | 1.02–1.48 | 0.003 | 0.0001 | 78% |

**Table 3b Subgroup analysis for the primary outcome of ammonia reduction dependent on pre-specified subgroups to determine the impact on heterogeneity and clinical effect.**

| | No. of studies | Mean difference | Confidence interval | Overall effect *p* value | Heterogeneity *p* value | $I^2$ (%) |
|---|---|---|---|---|---|---|
| **All** | | | | | | |
| *Quality* | | | | | | |
| Low | 2 | −34.31 | −86.5 to 17.9 | 0.20 | <0.001 | 94% |
| High | 6 | −11.47 | −18.26 to −4.71 | 0.0009 | 0.17 | 35% |
| *Era* | | | | | | |
| Early (pre-2010) | 3 | −25.52 | −60.2 to 9.22 | 0.15 | <0.001 | 86% |
| Late (2010 and after) | 5 | −12.75 | −21.50 to −4.00 | 0.004 | 0.11 | 47% |
| *Study size* | | | | | | |
| Small (<100) | 5 | −18.80 | −34.6 to −3.14 | 0.02 | 0.0005 | 80% |
| Large (>100) | 3 | −12.48 | −20.34 to −4.62 | 0.002 | 0.19 | 37% |
| *Location* | | | | | | |
| Europe | 3 | −13.08 | −23.66 to −2.50 | 0.02 | 0.44 | 0% |
| Rest of world | 5 | −20.59 | −35.96 to −5.22 | 0.009 | 0.0002 | 82% |



analysis correlated with overall effect but this was not the case for the mental state improvement outcome.

## Meta-Analysis: LOLA Versus Placebo/No Intervention: Ammonia-Lowering Effect

Figure 3 represents Forest plots indicating the pooled effect in 709 patients of LOLA compared to control for the lowering of blood ammonia in (A) all patients with HE treated with LOLA, (B) subgroup analysis of the efficacy of intravenous formulations and (C) oral formulations of LOLA.

Assessment of the pooled data from all included trials revealed that LOLA was consistently effective for the lowering of blood ammonia compared to placebo/no intervention (MD = −17.50, 95% CI: −27.73 to −7.26), test for overall effect: $Z = 3.35$, $p = 0.0008$.

LOLA was effective in trials where the intravenous and oral formulations were assessed individually (intravenous formulation, 520 patients: MD: −27.16, 95% CI: −44.77 to −9.56, test for overall effect: $Z = 3.02$, $p = 0.002$, oral formulation, 189 patients: MD: −8.44, 95% CI: −12.42 to −4.46, test for overall effect: $Z = 4.16$, $p < 0.0001$). Details

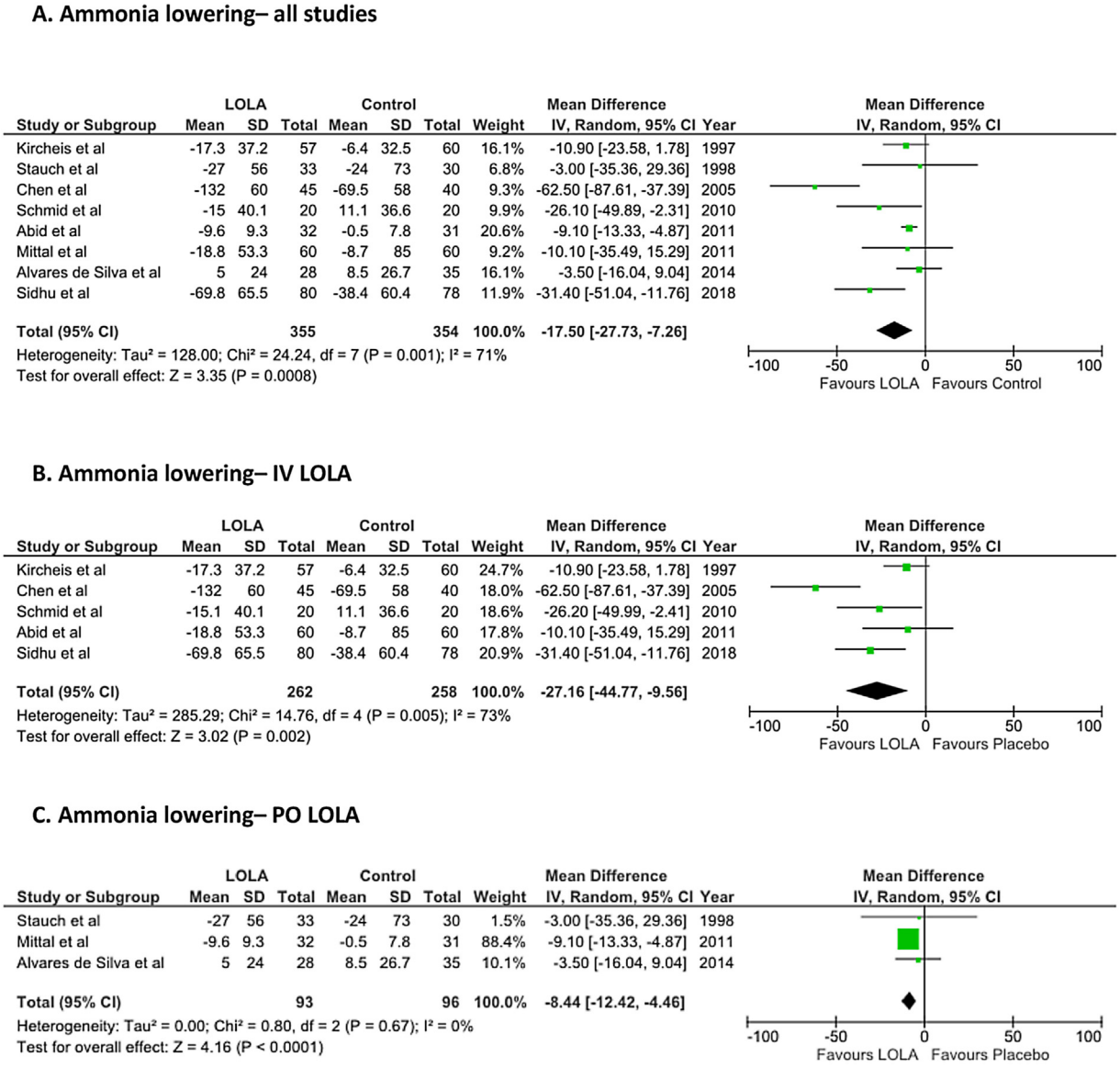


**Figure 3** Forest plots indicating the pooled effect of LOLA versus placebo/no intervention controls for the lowering of blood ammonia in (A) all patients in trials selected according to inclusion/exclusion criteria irrespective of the type of LOLA formulation, (B) and (C) patients selected according to the LOLA formulation (intravenous, oral). LOLA: L-Ornithine L-Aspartate, RR: Risk Ratio, CI: Confidence Interval.

including individual trial data are provided in Figure 3B and C.

## Meta-Analysis: LOLA Versus Placebo/No Intervention: Effect on Improvement in Mental State, All HE Groups

Figure 4 represents Forest plots indicating the pooled effect in 843 patients for the improvement in mental state in (A) all trials of patients with HE (regardless of HE type) treated with LOLA compared to placebo/no intervention (Figure 4A) where RR was 1.36 with 95% CI of 1.10–1.69, test for overall effect: $Z = 2.82$, $p = 0.005$.

Both intravenous and oral formulations of LOLA were effective when assessed independently (intravenous formulation, 573 patients, RR: 1.17, 95% CI: 1.00–1.37, test for overall effect: $Z = 1.98$, $p = 0.05$, oral formulation, 270 patients: RR: 2.33, 95% CI: 1.55–3.49, test for overall effect: $Z = 4.07$, $p < 0.0001$), Figure 4B and C.

## Meta-Analysis: LOLA Versus Placebo/No Intervention: Effect on Improvement in Mental State in OHE

Figure 5 represents Forest plots indicating the pooled effect in 551 patients for the improvement in mental state in patients diagnosed with OHE according to Westhaven criteria. Treatment with LOLA led to significantly greater improvement compared to control with RR of 1.19, 95% CI of 1.01–1.39, test for overall effect: $Z = 2.14$, $p = 0.03$.

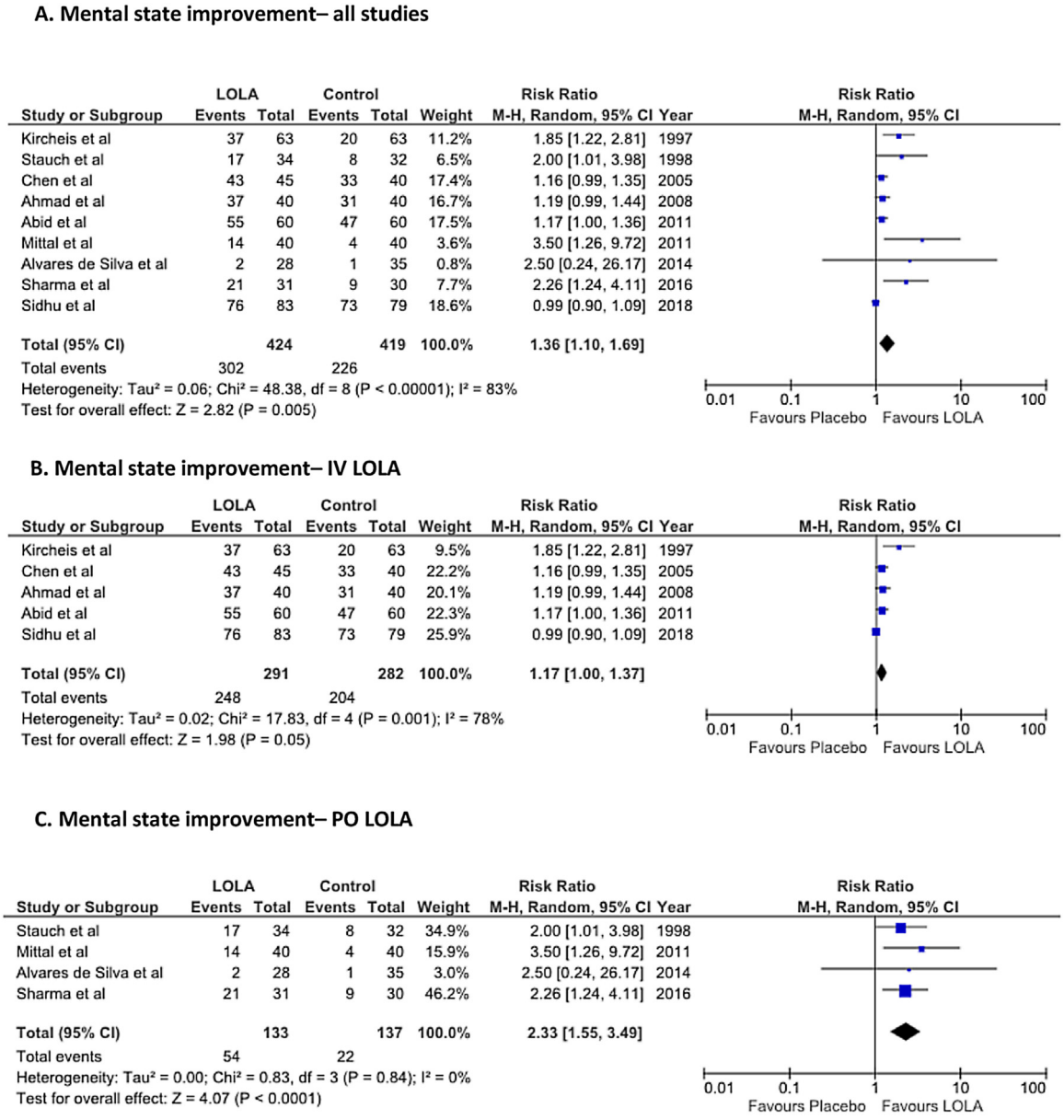


**Figure 4** Forest plots indicating the pooled effect of LOLA versus placebo/no intervention controls for the improvement in mental state for all forms of HE in (A) all patients in trials selected according to inclusion/exclusion criteria irrespective of the type of LOLA formulation, (B) and (C) patients selected according to the LOLA formulation (intravenous, oral). LOLA: L-Ornithine L-Aspartate, RR: Risk Ratio, CI: Confidence Interval, HE: Hepatic Encephalopathy.

Subgroup analysis of intravenous and oral formulations was not feasible for OHE since only a single trial using the oral formulation was available.

## Meta-Analysis: LOLA Versus Placebo/No Intervention: Effect on Improvement in Mental State in MHE

Compared to placebo/no intervention controls, there was a significantly greater improvement in mental state assessed by psychometric testing in 292 LOLA-treated patients with MHE (RR: 2.15, 95% CI: 1.48–3.14, test for overall effect: $Z = 3.98$, $p < 0.0001$), Figure 6A.

Although subgroup analysis showed that intravenous LOLA treatment tended to result in improvement of MHE the degree of improvement did not reach statistical significance (RR: 1.67, 95% CI: 0.90–3.08, test for overall effect: $Z = 1.64$, $p = 0.10$) (Figure 6B). In contrast, significant beneficial effects of LOLA were evident in trials of the oral formulation of LOLA assessed in 227 patients with MHE (RR: 2.54, 95% CI: 1.54–4.18, test for overall effect: $Z = 3.67$, $p = 0.0002$), Figure 6C.

Importantly the overall heterogeneity statistic for the MHE pooled RR was 0%.

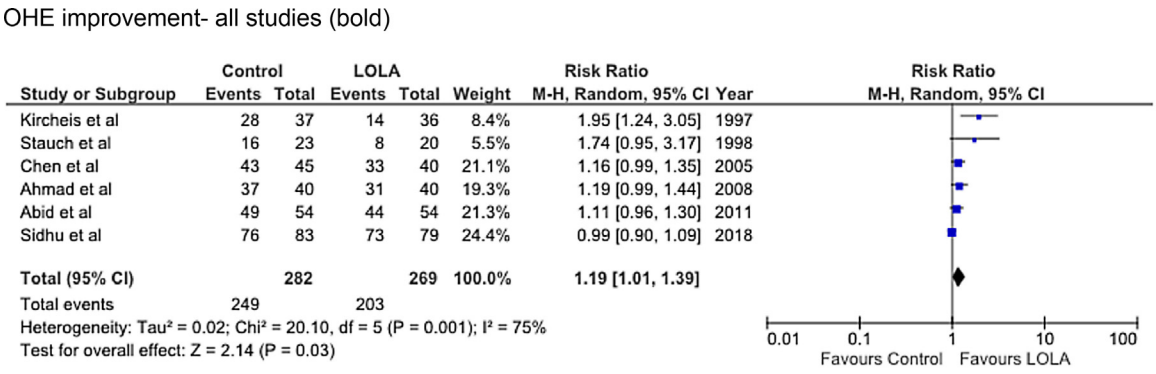


**Figure 5** Forest plot indicating the pooled effect of LOLA versus placebo/no intervention controls for the improvement in mental state for OHE in trials selected according to inclusion/exclusion criteria irrespective of the type of LOLA formulation (intravenous, oral). LOLA: L-Ornithine L-Aspartate, RR: Risk Ratio, CI: Confidence Interval, OHE: Overt Hepatic Encephalopathy.

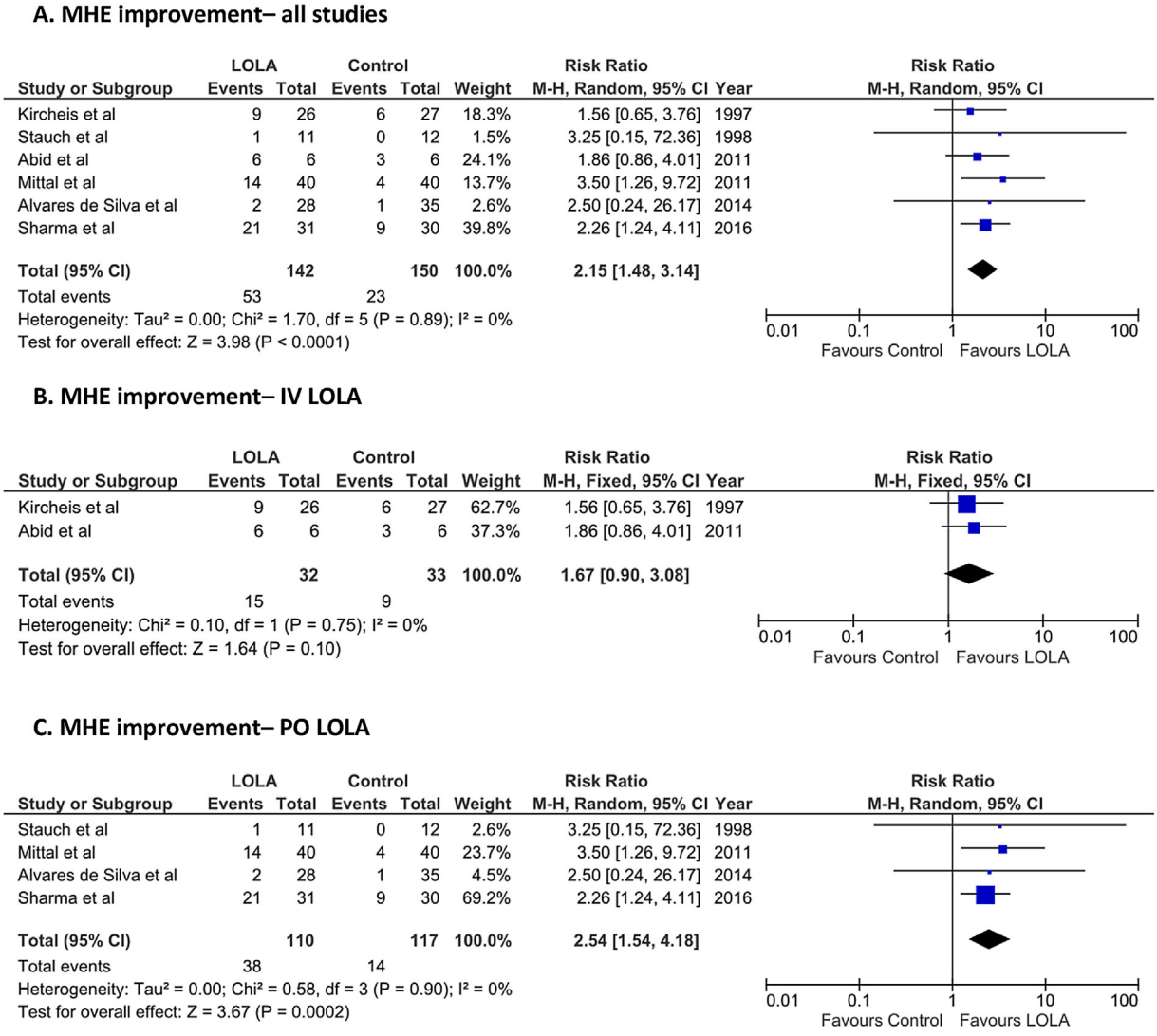


**Figure 6** Forest plots indicating the pooled effect of LOLA versus placebo/no intervention controls for the improvement in mental state for minimal HE in (A) all patients in trials selected according to inclusion/exclusion criteria irrespective of the type of LOLA formulation, (B) and (C) patients selected according to the LOLA formulation (intravenous, oral). LOLA: L-Ornithine L-Aspartate, RR: Risk Ratio, CI: Confidence Interval, MHE: Minimal Hepatic Encephalopathy.



 

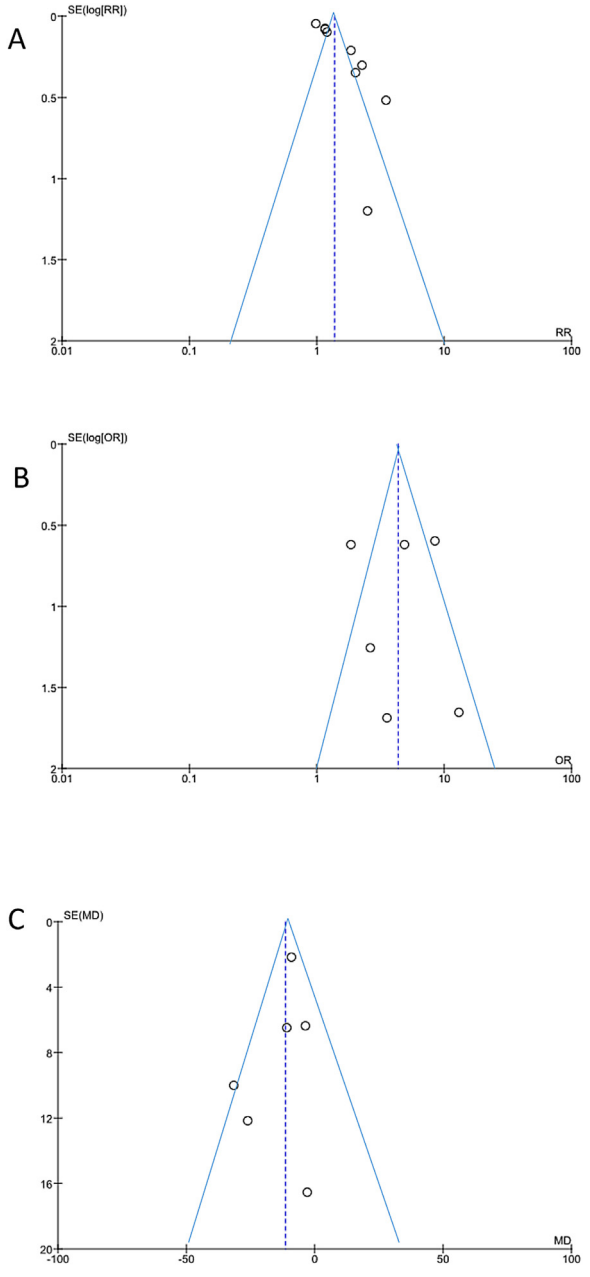


**Figure 7** Funnel plots for the meta-analysis data for (A) any mental state improvement (B) minimal hepatic encephalopathy (MHE) improvement and (C) ammonia reduction. In the funnel plots, the *Y* parameter is the standard error associated with the particular measure and the *X*-axis is the relative risk (for categorical outcomes) and mean difference (for continuous outcomes). In the figures, both arms of the funnel plots are shown.

Funnel plots for the primary outcomes did not demonstrate any significant asymmetry for (A) Mental state improvement, (B) MHE improvement and (C) ammonia lowering, Figure 7.

## DISCUSSION

*Hyperammonemia*: Results of the present systematic review and meta-analysis significantly extend the findings of a number of individual RCTs namely that LOLA is effective in lowering blood ammonia in patients with cirrhosis. Pooled data from the eight RCTs in which blood ammonia was measured in all patients with HE revealed that blood ammonia was significantly reduced although the degree of reduction was variable from trial-to-trial, the overall effect was statistically significant (MD: −17.50, 95% CI: −27.73, −7.26, test for overall effect: $Z = 3.35$, $p = 0.0008$). Moreover the results demonstrate, for the first time in a meta-analysis, that both intravenous and oral formulations are superior to placebo/no intervention.

Mechanisms generally considered to underpin the effective lowering of blood ammonia by LOLA in cirrhosis include the synthesis of urea and glutamine by residual hepatocytes and skeletal muscle respectively.[5] In addition, there is a growing body of evidence in favor of LOLA of a direct hepatoprotective mechanism.[32] The concept was originally proposed[33] following the report of an observational study in 378 patients with cirrhosis with attenuation of liver enzymes and bilirubin by LOLA treatment. Improvement of hepatic function was subsequently confirmed based upon changes in liver enzymes and bilirubin, improvements in prothrombin time, Child–Pugh and MELD scores. These findings are described in RCTs included in the present meta-analysis.[15,18,20,34] These interesting findings suggest that lowering of blood ammonia due to LOLA may result from increased ammonia removal via urea or glutamine as a result of the provision of key optimization of hepatocyte function by virtue of diminished cellular damage. Further studies are ongoing to assess these possibilities.

*Mental state improvement in all types of hepatic encephalopathy:* When the 9 RCTs involving 843 patients in which the effects of LOLA compared to placebo/no intervention were assessed with regard to mental state improvement either by Westhaven criteria or by psychometric testing, a benefit of LOLA was evident (RR: 1.36: 95% CI: 1.10, 1.69, Test for overall effect: $Z = 2.82$, $p = 0.005$). Reports of benefit in a smaller numbers of similar trials have appeared previously.[11,12] However, no attempt was made in these studies to assess independently the effects of the intravenous and oral formulations. Furthermore, these previous meta-analyses were conducted using the Fixed Effects paradigm rather than the more conservative Random Effects paradigm used exclusively in the present meta-analysis making comparison of findings difficult. In the present study, intravenous LOLA was effective for improvement of mental state in 5 RCTs (573 patients) with RR of 1.17: 95% CI: 1.00, 1.37, test for overall effect: $Z = 1.98$, $p = 0.05$. Efficacy of the oral formulation appeared to be superior to intravenous with RR of 2.33, 95% CI: 1.55, 3.49, Test for overall effect: $Z = 4.07$, $p < 0.0001$. These results are novel and challenge the viewpoint that the oral formulation of LOLA is ineffective.[2]

*Mental state improvement in overt hepatic encephalopathy*: When the 6 trials (551 patients) in the OHE subgroup identified by Westhaven criteria were assessed, treatment with LOLA showed significant improvement of HE grading with RR of 1.19 95% CI: 1.01–1.39, test for overall effect: $Z = 2.14$, $p = 0.03$. Similar findings were previously reported.[12] Unfortunately the availability of only a single trial employing the oral formulation, subgrouping according to LOLA formulation for improvement of mental state by LOLA in OHE was not feasible and further trials are clearly required.

*Mental state improvement in minimal hepatic encephalopathy*: RCTs in which 292 patients with MHE were treated with LOLA demonstrated clear improvements in psychometric test performance scores with RR of 2.15: 95%CI: 1.48, 3.14, Test for overall effect: $Z = 3.98$, $p < 0.0001$. Similar, though less extensive, findings were previously reported[12] but a contrasting report has also appeared.[11] The reasons for these inconstancies are unclear at the present time. In the present meta-analysis, both formulations led to improvements of psychometric test scores. However in the case of intravenous LOLA, improvements failed to reach statistical significance, a finding that could be attributed to very low patient numbers and available RCTs.

The oral formulation of LOLA in the 4 trials in which it was assessed (total of 227 patients), resulted in the novel finding of a clear and consistent improvement of psychometric test scores with RR of 2.54, 95% CI: 1.54, 4.18, Test for overall effect: $Z = 3.67$, $p = 0.0002$.

### Comparison With Other Current Treatments for Hepatic Encephalopathy in Cirrhosis

The primary objective of the present systematic review and meta-analysis was an assessment of evidence in support of the efficacy of LOLA for the treatment of HE in cirrhosis. Consequently only these RCTs in which placebo or no intervention controls were included and trials using a comparator agent such as lactulose, probiotics or antibiotics were not included in the literature search. Several previous RCTs of efficacy of these agents have been investigated in patients with MHE and results published. In all cases, LOLA was found to be equivalent or superior in efficacy to lactulose,[34,35] probiotics[21] or rifaximin.[21] Network meta-analyses have so far largely confirmed these findings.[36,37]

While results of the present systematic review and meta-analysis provide a significant level of evidence in support of the efficacy of LOLA for the treatment of HE in cirrhosis, there remain important areas in which evidence remains incomplete. Studies involving patients with cirrhosis and severe grades of OHE (HE-3 and HE-4) are still insufficient in number to provide data for an accurate evidence-based analysis. Likewise, evidence in favor of LOLA as an effective agent for the prevention of post-TIPS HE are limited to two RCTs the results of which are equivocal.[34,38] Few studies have addressed the efficacy of LOLA for the prevention of recurrence of OHE in patients with cirrhosis. In one such study, significant improvements in hyperammonemia as well as psychometric test scores and CFF values were reported.[39] Further studies assessing the efficacy of LOLA for the treatment of HE relating to the above clinical sub-groups are clearly now required.

In summary, results of the present systematic review and meta-analysis provide evidence in support of the thesis that LOLA is an effective agent for the lowering of blood ammonia and for improving mental state in patients with cirrhosis and HE. However, the relative efficacy of LOLA is dependent on the type of HE (overt or minimal) on the nature of the LOLA formulation (intravenous or oral) as well as the quality and risk of bias of the included trials. Both the intravenous and oral forms appear to be effective for ammonia lowering and for general improvements of mental state. MHE appears to benefit from the preferential use of the oral formulation. Further analyses are now required to confirm these results and assess the efficacy of LOLA for the treatment of high grade overt HE, post-TIPS HE and for primary and secondary prophylaxis of HE. Additional studies addressing the treatment of covert HE in cirrhosis are also required.

## CONFLICTS OF INTEREST

The authors have none to declare.

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