Regular Article

Review Manuscript: N-acetylcysteine in the Treatment of Craving in Substance Use Disorders: Systematic Review and Meta-Analysis[1]

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Running head: N-acetylcysteine for Craving

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[1] This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi:10.1111/ajad.12620

Abstract

Background and objectives: Recent neurobiological evidences along with clinical observations justify the use of N-acetylcysteine (NAC) as a medication for craving. The objective of our study was to assess the evidence of efficacy of NAC for craving in substance use disorders in randomized clinical trials (RCTs).

Methods: Systematic review of the RCTs literature (PROSPERO number 56698) until February, 2017, using MEDLINE, Cochrane Library and clinicaltrials.gov. We included seven RCTs (n=245); most with small-to-moderate sample sizes. The main outcome was the Hedges' g for continuous scores in a random-effects model. Heterogeneity was evaluated with the I^2 and the χ^2 test. Publication bias was evaluated using the Begg's funnel plot and the Egger's test. Meta-regression was performed using the random-effects model.

Results: Comparing NAC vs. placebo, NAC was significantly superior for craving symptoms (Hedges' g = 0.94; 95% CI 0.55 – 1.33). The funnel plot showed the risk of publication bias was low and between-study heterogeneity was not significant (I^2=44.4%, p=0.07 for the χ^2 test). A subgroup analysis performed using meta-regression showed no particular influence.

Discussion and Conclusions: NAC was superior to placebo for craving reduction in SUDs. The relatively small number of trials and their heterogeneous methodology were possible limitations; however, these positive thrilling results stimulate further studies for clarifying the potential impact of NAC for craving symptoms in SUDs.

Scientific Significance: The safety profile of NAC and favorable tolerability, in addition to being an over-the-counter medication, presents with an interesting potential clinical use for craving in SUDs.

Key words: Addiction; Substance-Related Disorders; Dependence; Craving; Acetylcysteine; Cocaine; Opioid; Cannabis; Nicotine; Alcohol; Systematic Review; Meta-analysis.

1. Introduction:

Craving is an intermittent state of intense desire for substance use. The inclusion of this symptom in the diagnostic criteria of the DSM-5 made it a key clinical construct for assessment and treatment of substance use disorders (SUDs)[1]. Recent neurobiological evidences and clinical observations have justified new scientific investigation into therapeutic approaches for craving control[2].

Altered dopamine release, unbalanced prefrontal control and hyperactive striatal-limbic responses are the main biological alterations associated with drug seeking elicited by cues[3]. Neuroimaging studies have also corroborated these findings and shown the activation of the amygdala and prefrontal regions along with hippocampus, insula and ventral tegmental area (VTA) in different clinical craving scenarios[4]. Altered neurotransmitter pathways (mainly dopamine and glutamate) are critically involved in these changes. Restoration of these glutamatergic and dopaminergic pathways are therapeutic targets for medication development[5].

N-acetylcysteine (NAC) has potential as a medication for craving reduction. Animal studies have shown that NAC can serve as a source of cystine that can promote glutamate exchange through the cystine–glutamate antiporter in glial cells located within the nucleus accumbens[2,6,7]. This normalizes glutamate functioning within the extracellular space of the accumbens by activating inhibitory metabotropic glutamatergic receptors[5]. Additionally, glutamate uptake via the Glial Glutamate transporter (GLT1), which is downregulated after long-term drug use, is restored by NAC. Restoration of the GLT1 prevents glutamate overflow in the synaptic cleft in the accumbens. This in turn prevents subsequent excessive stimulation of postsynaptic mGluR5 and N-methyl-D-aspartate (NMDA) receptors, which would otherwise increase signaling, potentiate synaptic activity, and increase drug-seeking behaviors. Therefore, NAC normalizes synaptic potentiation, and reduces subsequent drug-seeking behaviors. In addition to restoration of glutamate function in the accumbens, increased glutathione levels and interactions with inflammatory

mediators (also promoted by NAC administration) are also associated with increased neurotrophic factors and neurite sprouting[4,5,7]. All of the studied mechanisms have been correlated with clinical models of craving and drug seeking models[1,8,9].

The preclinical findings justify phase II clinical trials in humans. Since 2006, randomized controlled studies in addictions have shown diverse results for NAC therapy. One systematic review explored these studies and suggested a positive result for NAC therapy on craving[10]. However, to our best knowledge, no quantitative analysis was conducted. Therefore, in the present study we performed the first meta-analysis assessing the impact of NAC on craving.

3. Methods:

3.1 Overview

A systematic review was conducted in MEDLINE, Cochrane databases and clinicaltrials.gov by two authors (MD and APT) independently and any discrepancy was resolved by consensus. We followed the recommendations from the Cochrane group and the PRISMA guidelines[11]. The following Boolean terms were reviewed: ("Acetylcysteine"[Mesh] OR ("acetylcysteine"[MeSH Terms] OR "acetylcysteine"[All Fields] OR "n acetylcysteine"[All Fields])) AND ("Substance-Related Disorders"[Mesh] OR "Marijuana Abuse"[Mesh] OR "Tobacco Use Disorder"[Mesh] OR "Morphine Dependence"[Mesh] OR "Heroin Dependence"[Mesh] OR "Opioid-Related Disorders"[Mesh] OR "Cocaine-Related Disorders"[Mesh] OR "Amphetamine-Related Disorders"[Mesh] OR "Alcoholism"[Mesh]).

3.2 Eligibility criteria

We adopted the following inclusion criteria: (1) manuscript written in English, Spanish or Portuguese; (2) randomized, placebo-controlled trials; (3) data provided by authors of the original studies (upon request) for the estimation of the main outcomes, i.e., mean (SD) values and response

and remission rates; (4) standardized assessment of craving, using validated instruments. We excluded pre-clinical research, case reports and series of cases, non-controlled trials and trials assessing conditions other than addiction/craving end points or interventions other than NAC.

3.3 Data extraction

The following variables were extracted, according to a structured checklist previously elaborated by the authors: (1) metadata (i.e., authorship, publication date, etc.); (2) specific type of addiction; (3) study design (cross-over, double arm, triple-arm); (4) duration of intervention (in weeks); (5) dosage of NAC intervention (in mg per day); (6) presence of add-on therapy; (7) craving symptoms scale; (8) study sample size; and (9) study results. Data were extracted from studies published prior to February, 2017.

The primary outcome was based on the craving symptoms scales that were continuous or discrete. We chose to interpret trial results in a continuous manner considering that continuous effect size would provide a standard measure facilitating comparison across studies. We extracted data corresponding to the study definition of the craving outcome. Studies could differ as to craving assessment and evaluation but trial design had to be consistent to the question in study. When a paper reported scores at more than one time-point we used the scores corresponding to the longest time period prior to unblinding.

For studies in which three groups were compared, two separate datasets were considered in two different analyses. Thus, the same study was imputed twice in the statistical analysis, each in a different arm. We also highlighted how each study handled missing data and if intention-to-treat analysis was conducted.

3.4 Statistical Analysis

All analyses were performed using the statistical packages for meta-analysis of Stata 13.1 for Mac OSX. For craving symptoms scales assessment, we calculated the standardized mean difference, the pooled standard deviation of each comparison and the 95% confidence interval (CI). The Hedges' g was used as the measure of effect size. The pooled effect size was weighted by the inverse variance method and measured using the random-effects model. Heterogeneity was assessed using I^2 index. The random effects model was chosen due to the need to represent all effect sizes in the final summary estimate derived from small sampled trials. We further used the Funnel Plot to test for the presence of publication bias. Sensitivity analysis was also performed, which assesses the impact of each study in the overall results by excluding one study at a time. Meta-regressions were also performed for possible confounders such as baseline severity scores, age, sample size, NAC doses, type of addiction and augmentation strategy.

4. Results:

4.1 Qualitative analysis

Our systematic search yielded 448 studies (Figure 1). Among them, 426 were excluded; 27 papers for not having original data, 397 for not being SUD-related and two for using interventions other than NAC[12]. The remaining 22 studies were fully assessed for eligibility. From those, seven were excluded for not assessing craving symptoms, four for being open label studies, one was excluded for assessing NAC plus naltrexone[13], one for using animal models[14] and two for including data previously published by the authors[15,16] (Figure 1). The quantitative analysis incorporated the remaining seven articles that met all the inclusion criteria (Table 1)[15–23].

Although NAC is approved by the FDA since 1963 and is known for its safety profile, all the included studies performed an adverse effects evaluation. No serious adverse event was registered and mild to moderate adverse effects did not differ between active and placebo groups.

Gastrointestinal discomfort, cutaneous rash and mild sleep disturbances were the most common events reported. Adverse events were unrelated to dropout rates (12-20).

As to publication bias, the systematic review published by Asevedo et al. (2013)[10] addressed three out of the seven articles included in our study. As to main biases found, underpowered studies, little description of blinding process and moderate attrition rates were the main limitations. Among the resting four articles, La Rowe et al. (2013)[20] reported low patient compliance to treatment as a source of bias; Roten et al. (2013)[18] main outcome was assessed by an adapted scale; Mousavi et al. (2015)[17] showed significant periodic effect due to low patient compliance; and Froelinger et al. (2015)[23] used craving as a secondary outcome.

4.2 Quantitative analysis.

Within the seven articles that were included in our analysis (n=245), most patients were diagnosed with cocaine and methamphetamine dependence. A handful of studies that involved patients diagnosed with nicotine and cannabis dependence were also included (Table 1).

Outcome evaluation included all questionnaires that assessed cravings or urges for drugs in the period during and after therapy. NAC dosage ranged from 1,200 mg per day to 3,600mg per day; most of the sample received 2,400 mg per day. Time of intervention was also heterogeneous; some studies were shorter (three or four days of intervention) and others longer (four, eight or 12 weeks).

Regarding crossover designs, the study conducted by Mousavi et al. (2015) reported the results from both phases. We imputed the data separately under the headings “Mousavi A” and “Mousavi B”[17]. Also, the study conducted by LaRowe et al. (2013)[20] was a triple-arm clinical trial comparing different doses of NAC (1,200 mg/day and 2,400 mg/day). We included the craving reports from the time-to-relapse sub-analysis that involved those individuals who were abstinent at

initiation of the trial. Data were included separately under the headings as “LaRowe A” and “LaRowe B” comparing each treatment arm twice in comparison to the placebo group[20].

Considering continuous outcomes, i.e, rated reductions for craving symptoms, we found significant clinical difference between active and placebo groups (Figure 2). The Hedge’s g showed an effect size of 0.94 with a 95% CI ranging from 0.55 to 1.33. Statistical analysis underscored small heterogeneity among studies, not statistically significant (I^2: 44.4%, p=0.07). Meta-regression showed no association with baseline severity scores, age, sample size, NAC doses, type of addiction and augmentation strategy.

All studies were within limits in the Begg’s funnel plot (Figure 3). The funnel plot didn’t show apparent signs of publication bias, with studies evenly distributed, but due to some asymmetry and the small sample sizes, we performed the Egger’s test to confirm low publication bias (p>0.05). Moreover, sensitivity analysis suggested that no study individually influenced the pooled effect size as assessed by the “metainf” command in Stata.

5. Discussion:

In the present study, we performed a systematic review and meta-analysis focused on the impact of NAC on craving symptoms across different SUDs. A previous systematic review of NAC for craving symptoms showed contradictory results among clinical trials, but with interesting trends towards positive outcomes regarding craving symptoms[10]. Authors emphasized the different administration schemes, methodological differences among trials and main outcomes, stimulating further studies on the matter. As stated before, among the seven trials considered in the present work two examined cocaine dependence, one methamphetamine dependence, three nicotine dependence and one cannabis dependence (Table 1).

Our results indicated that NAC therapy was superior than placebo for craving symptoms (Figure 2). Sensitivity analysis with the exclusion of each trial individually explored the

independent influence of trials in the pooled effect size (Figure 4). No study influenced the final results. Subgroup analysis was also conducted for baseline severity scores, age, sample size, NAC doses, type of addiction and augmentation strategy. There was no association between any of these possible confounders and the main outcome.

No phase III trials were found in our search. Regarding triple-arm and crossover trial designs, our methodology transformed these to conform as close as possible to a two-arm design. We chose to separate each arm and compare it independently to the placebo group. Though this approach may increase type I error, duplicating the placebo sample in the study would negatively influence the final hedge's g, underestimating it, what would be a more conservative analysis. Hedge's g was chosen because studies had small sample sizes, different subsets of scales and some heterogeneity. We analyzed each study endpoint result in comparison to baseline and compared the standardized mean difference.

As to the dataset limitations, heterogeneity was not evident (I^2= 44.4%; p=0.07 for the χ^2 test), representing the potential use of our results as stimulating for further studies on the subject. The funnel plot and the Egger's test assessed publication bias, showing no important variability among studies ($p > 0.05$).

Our positive findings in the clinical setting support the evidence that NAC may act on the neuropathophysiology of craving symptoms, what could be important for relapse prevention. NAC, a cysteine prodrug, is involved in restoring intracellular and extracellular glutamate concentrations in the nucleus accumbens, which would be associated with craving symptoms and, consequently, relapse[24–27]. Preclinical studies consistently suggest that NAC may ameliorate cellular neuropathologies induced by chronic cocaine administration, establishing an enduring protection from conditioned cocaine-induced reinstatement of cocaine-seeking behavior for up to three weeks after the last daily NAC administration in studies murine subjects[28,29] Baker et al.[25] demonstrated in

rats that relapse to cocaine-seeking behavior was linked to decreased basal concentrations of extracellular glutamate followed by reduced activation of the group II metabotropic glutamate (mGluR2/3) receptors, which inhibit presynaptic glutamate release. Stimulating the mGluR2/3 receptors, which inhibits synaptic glutamate release, reduces the rewarding effect[30]. Consistent with these findings, human positron emission tomography (PET) studies reported an elevated glutamate receptor occupancy in the medial prefrontal cortex (mPFC), that could result from reduced receptor density or changes in affinity of the glutamate binding site[31]. NAC would be responsible for restoring the concentrations of extracellular glutamate (increasing activation of the mGluR2/3 receptors) as a primary mechanism[24,32]. Secondary mechanisms rely on NAC antioxidant effects. NAC serves as a source of cysteine, which can promote glutamate exchange through the cystine–glutamate antiporter in glial cells located within the nucleus accumbens. Cysteine would then increase cellular production of gluthatione, which is an intracellular antioxidant[33], preventing cellular damage from reactive oxygen species.

Studies on the use of NAC for post-traumatic stress disorders and obsessive compulsive and related disorders have been performed, given glutamate's role in mediating fear related memory and its abnormalities in the poor cognitive processing and doubt in OCD and the positive results of glutamate-modulating agents to regulate impulse control[34]. Promising results were obtained from clinical trials for obsessive compulsive-disorder in a 12-week clinical trial, in which patients were allocated to either placebo (n=19) or 2,400 mg of NAC (n=20)[35]. Grant and colleagues published their findings from a randomized, double blind, placebo controlled trial with 44 subjects with trichotillomania, demonstrating the superiority of 2,400mg of NAC from the ninth week on[36]. Similarly, results on NAC for skin picking in Prader-Willi Syndrome, a prevalent and difficult to treat condition that affects 80-95% of this population, have been reported by Miller et al in a 12-week duration open-label clinical trial, with doses ranging from 450 to 1,200mg[37]. All participants

had a reduction in the compulsive picking behavior and 71% of participants showed a complete recovery of skin lesions.

The positive results from the present meta-analytical study, in addition to its safety profile, low cost, tolerability even to high doses and easy access as an over-the-counter medication, stimulate the possible use of NAC for craving symptoms, especially for patients that frequently hesitate the use of conventional psychotropic medications.

To the best of our knowledge, this is the first systematic review and meta-analysis evaluating the use of NAC in the treatment of craving symptoms in SUDs.

6. Conclusion:

The safety profile of NAC and favorable tolerability, in addition to being an over-the-counter medication, presents with an interesting potential clinical use for craving in SUDs. In our study, NAC was superior to placebo for craving reduction in SUDs. Notwithstanding, the relatively small number of trials and their heterogeneous methodology, these positive thrilling results stimulate further studies for clarifying the potential impact of NAC for craving symptoms in SUDs.

Acknowledgements:

MB is supported by a NHMRC Senior Principal Research Fellowship 1059660.

Authors declare no conflict of interest related to the present manuscript.

Declaration of Interest:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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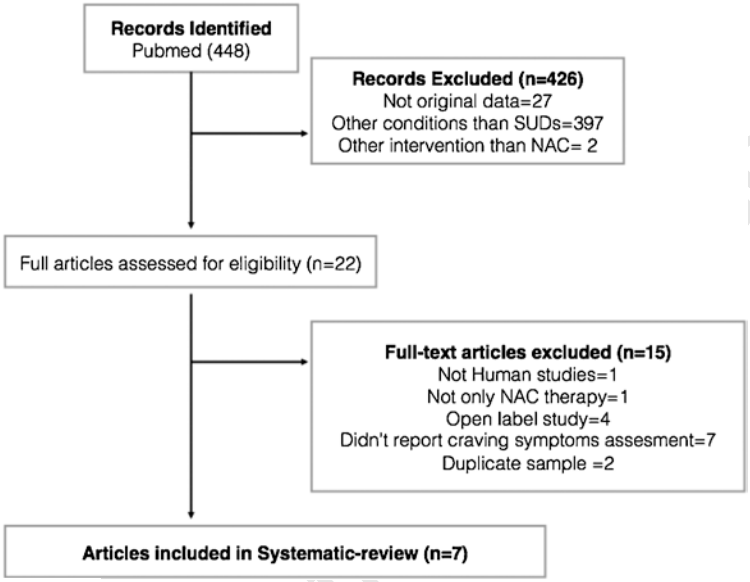

Figure1

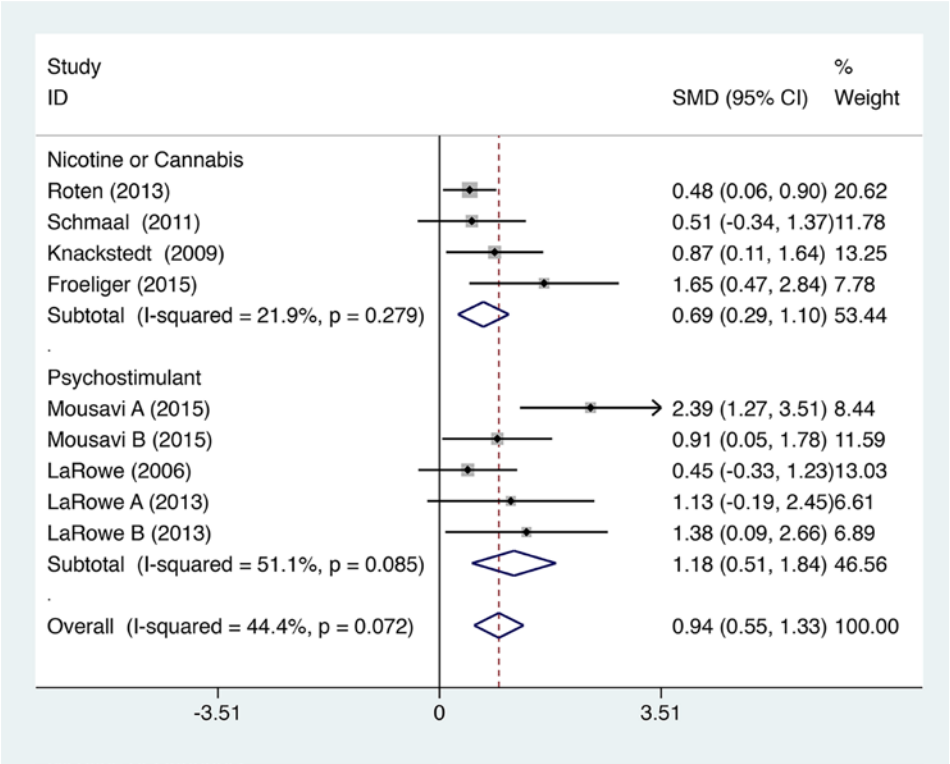

Figure 1

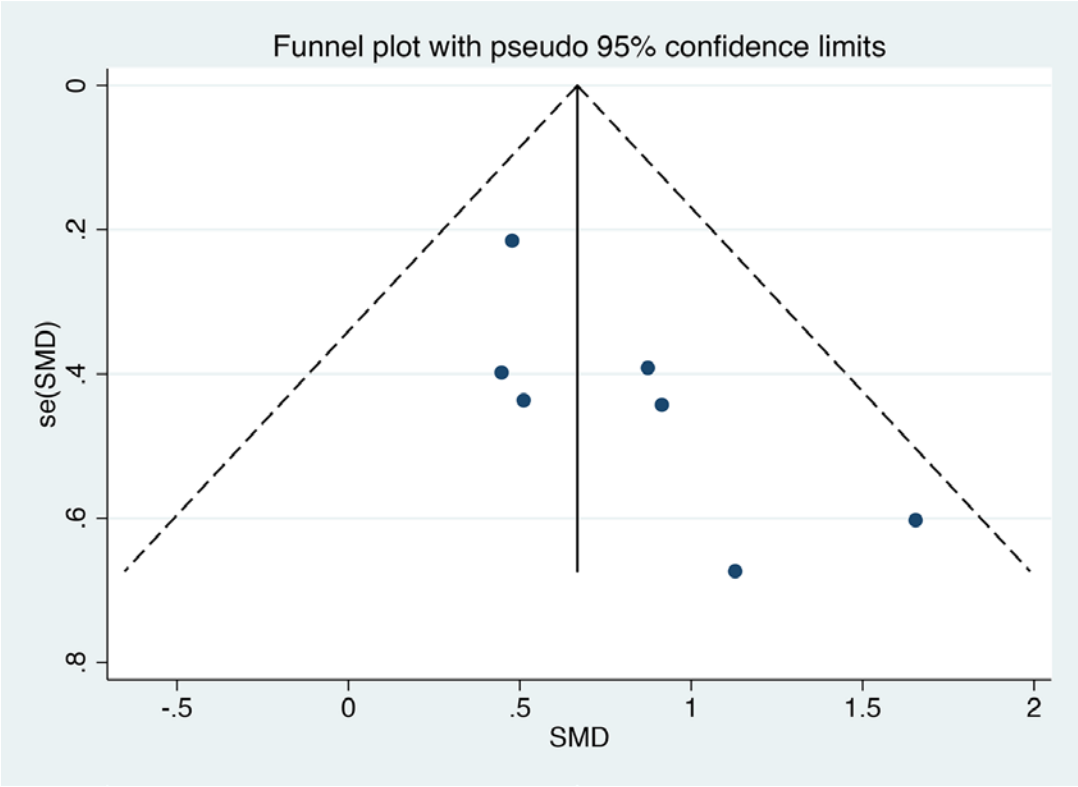

Figure 2

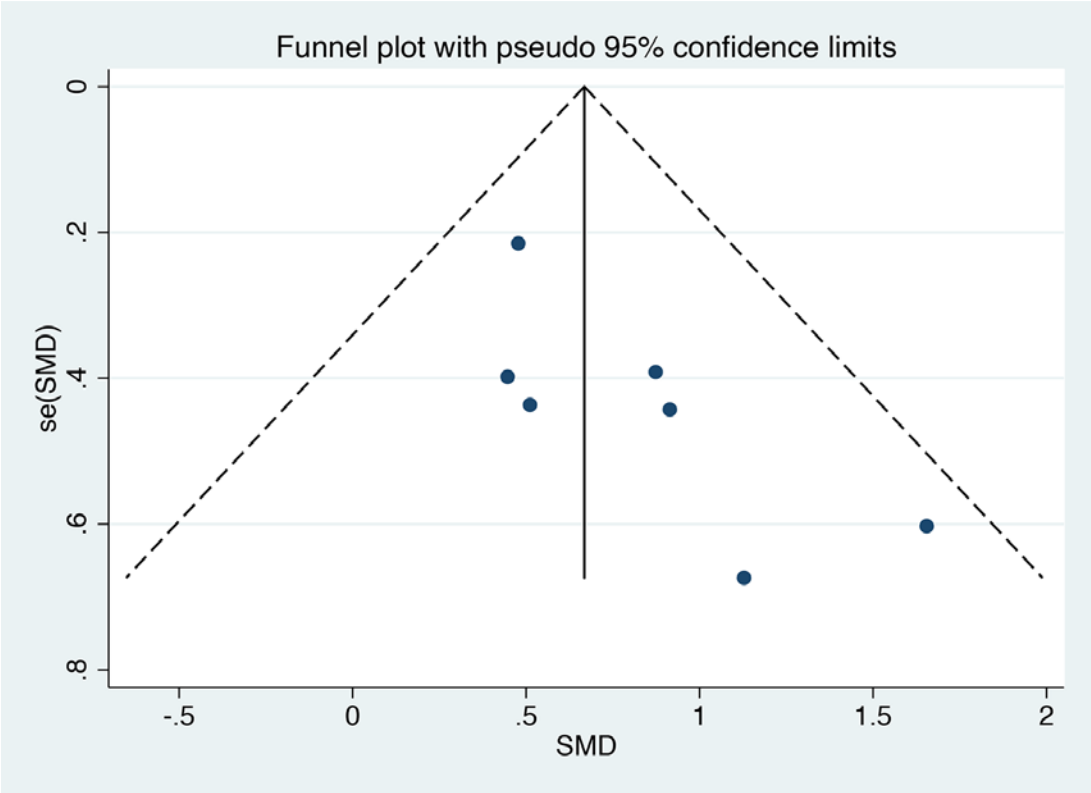

Figure 3

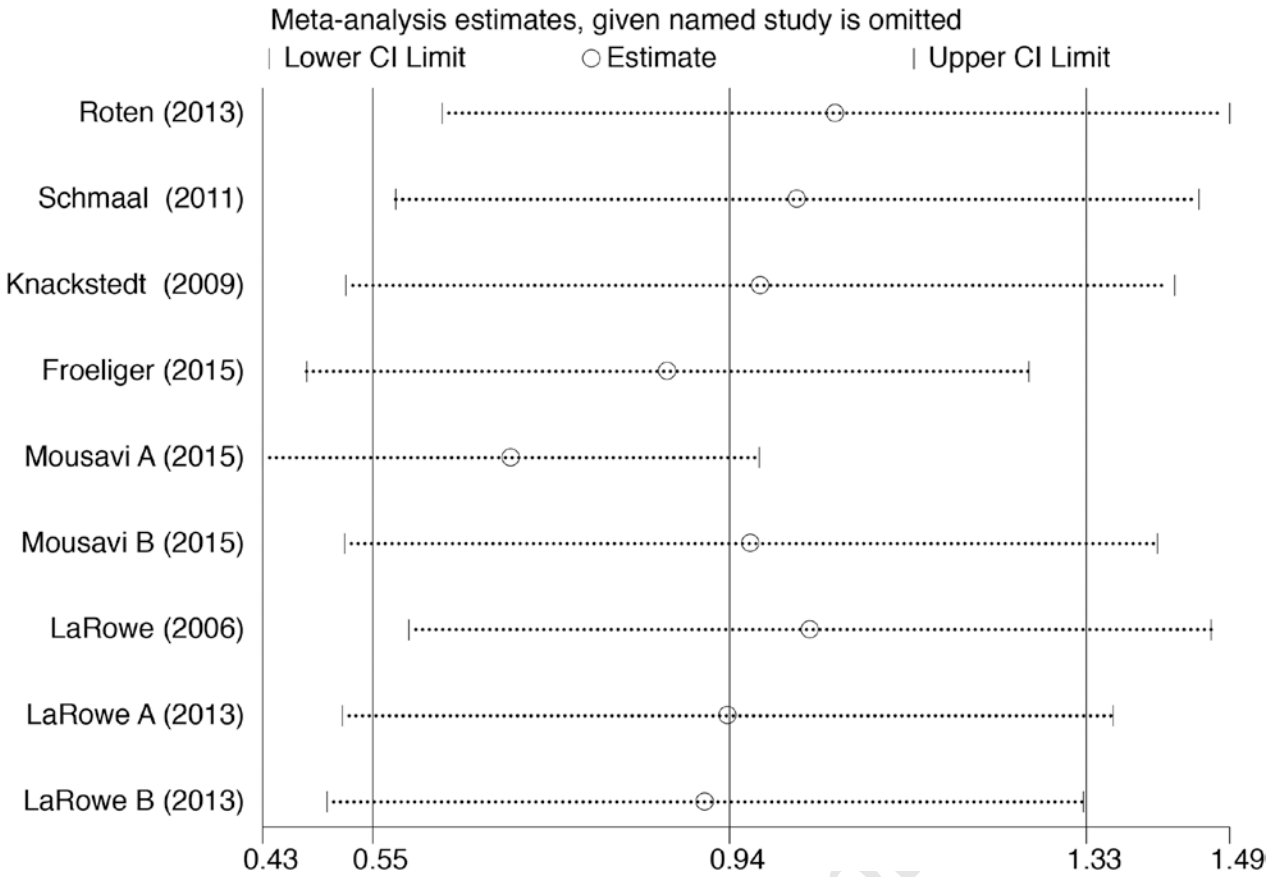

Figure 4