Review

Efficacy of probiotics on cognition, and biomarkers of inflammation and oxidative stress in adults with Alzheimer's disease or mild cognitive impairment — a meta-analysis of randomized controlled trials

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ABSTRACT

Probiotics are live microbes that confer health benefits to the host. Preliminary animal evidence supports the potential role of probiotics in ameliorating cognitive health, however, findings from clinical trials in Alzheimer's disease (AD) or mild cognitive impairment (MCI) subjects are controversial. Thus, a meta-analysis is needed to clarify the efficacy of probiotics on cognition in AD or MCI patients. EMBASE, PubMed, Web of Science and Cochrane library were systematically searched and manually screened for relevant published randomized controlled trials (RCTs). Among the 890 citations identified, 5 studies involving 297 subjects met eligibility. There was a significant improvement in cognition (SMD = 0.37; 95% CI, 0.14, 0.61; P = 0.002; I^2 = 24%), while a significant reduction in malondialdehyde (SMD = −0.60; 95% CI, −0.91, −0.28; P = 0.000; I^2 = 0.0%) and high-sensitivity C-reactive protein (SMD = −0.57; 95% CI, −0.95, −0.20; P = 0.003; I^2 = 0.0%) post-intervention levels between the probiotics and control group. This meta-analysis indicated that probiotics improved cognitive performance in AD or MCI patients, possibly through decreasing levels of inflammatory and oxidative biomarkers. However, current evidence is insufficient, and more reliable evidence from large-scale, long-period, RCT is needed.

INTRODUCTION

The incidence of Alzheimer's disease (AD) is increasing globally and has reached the point of being a costly public health issue [1]. AD, featured by impaired cognition and memory loss, is hidden onset and possesses a long incubation period [2], during which mild cognitive impairment (MCI) constitutes the typical prodromal stage [3]. The *World Alzheimer Report 2019* [4] showed that 50 million people are living with dementia worldwide. Remarkably, there will be one new case every 3 seconds, and the number is predicted to be 152 million persons by 2050 [1, 4], resulting in a huge lifestyle and economic burden to both the patients and their families. The total estimated worldwide cost of dementia was US$1 trillion and will rise to US$2 trillion by 2030 [1, 4]. Unfortunately, there is currently no curative treatment for cognitive impairment and dementia [5].

The gut microbiota (GM) consists of a vast bacterial community that resides primarily in the lower gut and

lives in a symbiotic relationship with the host [6]. Emerging experimental and clinical evidence has demonstrated the close interconnection between the gastrointestinal tract and the brain, known as the gut-brain axis [7]. Recently, the GM has been found to regulate brain development and behavior via the gut-brain axis, and this has been called the microbiota-gut-brain (MGB) axis [8]. It has been found that dysfunction in behavior and cognition is associated with GM dysbiosis [9–13]. Activation of gut inflammation has been regarded as a possible pathogenic cofactor in cognitive deterioration and dementia [14, 15]. Moreover, the most distinctive alterations in the GM of AD patients are decreased abundance of anti-inflammatory bacterial species (e.g. *Bifidobacterium breve*strain A1) and increased abundance of pro-inflammatory flora phyla (e.g. *Firmicutes* and *Bacteroidetes*) [16–18]. And restoring GM homeostasis could slow down the progression of AD [18, 19]. Therefore, the GM has been proposed as a key player in the pathogenesis of AD and might be a new potential therapeutic target for the prevention and treatment of AD [20, 21].

Probiotics are live microbes that confer health benefits to the host when administered in adequate amounts, possibly through their anti-inflammatory or anti-oxidative effects [22–24]. Recently, some probiotics have been shown to influence the central nervous system (CNS) and behavior via the MGB axis [25]. Moreover, eleven preclinical studies have shown that neither single strains nor multi-strain probiotics were beneficial for improving cognitive function in animal models [26]. These preclinical results have indicated that probiotics might be an effective dietary intervention to ameliorate age-associated cognitive deficits. Nevertheless, findings from available clinical trials focusing on the effects of probiotics in patients with AD or MCI are inconsistent [27–31]. Additionally, the previous relevant reviews are focused on the effect of probiotics on neurodegenerative and neurodevelopmental disorders in both animal models and human trials. And they found that probiotics showed efficacy in improving psychiatric disorder-related behaviors including anxiety, depression, autism spectrum disorder, and memory abilities [26, 32]. However, the evidence for the effects of psychobiotics on mental and neurological conditions/disorders remains limited. Further studies of psychobiotics are needed in order to determine their effectiveness and mechanism as treatments for various psychiatric disorders. Accordingly, this meta-analysis was the first to quantitatively examine the potential effect of probiotics on cognitive performance and inflammatory and oxidative biomarkers, which might be related to the underlying mechanisms in AD or MCI subjects.

RESULTS

Literature search and screening

A total of 883 records were obtained after the initial search of the electronic databases, and 7 studies were identified through manual searching of the reference lists of relevant published reviews (for a detailed description of the literature search and results, please see the supplementary materials). Of these, 203 trials were removed as duplicates, and 667 publications were excluded after screening the title and abstract. The remaining 20 articles were scrutinized with a full-text screening, of which 15 were excluded for reasons detailed in the PRISMA flow chart (Figure 1). Then, 5 studies were considered eligible and finally included in the quantitative meta-analysis.

Study characteristics

As shown in Table 1, the publication years of the five included studies ranged from 2016 to 2019, with an aggregated sample of 297 individuals. All studies were randomized, double-blind and controlled trials. Among which, three studies [27–29] recruited subjects diagnosed with AD and another two [30, 31] included MCI adults. Most studies included a higher ratio of women except for two studies (one [30] had a balanced proportion and the other [28] did not report the sex ratio of the recruited subjects).

The intervention duration of the involved studies was all 12 weeks. Two studies [30, 31] used a sole strain of probiotics, while the other three studies applied multiple strains. The study from Azadeh Agahi et al. [29] had two active probiotics groups which were regarded as one group for the present analysis. All studies used a matched placebo that was indistinguishable from the probiotics in terms of packaging, form, appearance, size, taste, smell, and so forth.

With regard to the main findings, three studies [27, 28, 31] found significant difference in improving cognition between the probiotics and control groups, whereas one study [29] found no significant difference, and another study [30] reported mixed findings. Similarly, the results regarding changes in various inflammatory and oxidative metabolites were inconsistent across studies.

Risk of bias assessment

Although all five included studies were RCT designs, one study [29] failed to provide information on the random sequence generation, and three studies [28–30] had no information on allocation concealment procedures. All studies described the blindness of the

participants and implementing personnel, whereas only two studies [27, 30] reported the blindness of outcome assessments. No risk of incomplete outcome data was found for any of the recruited studies. Overall, the assessment of bias generally reported a low to moderate risk of bias across all domains. The risks of bias assessment across the recruited studies are summarized in Figure 2.

Meta-analysis: Main results

Five studies included 154 subjects in the probiotics group and 143 subjects in the control group. A fixed-effects model was selected for quantitative synthesis. In general, the result of meta-analysis revealed a significant difference between the probiotics and control group regarding improvement in cognition (SMD = 0.37; 95% CI, 0.14, 0.61; P = 0.002; I^2= 24%). The forest plot of the meta-analysis is shown in Figure 3.

Meanwhile, fixed-effects models were applied to meta-analyses of malondialdehyde (MDA), high-sensitivity C-reactive protein (hs-CRP), total glutathione (GSH) and nitric oxide (NO) on account of acceptable heterogeneity among studies, while a random-effects model was selected for analysis of total anti-oxidant capacity (TAC). The results showed significant decrease in levels of MDA (SMD = −0.60; 95% CI, −0.91, −0.28; P = 0.000; I^2 = 0.0%; n = 3 studies; n = 82 subjects) and hs-CRP (SMD = −0.57; 95% CI, −0.95, −0.20; P = 0.003; I^2 = 0.0%, n = 2 studies, n = 57 subjects). And no significant differences were observed in TAC (SMD = 0.04; 95% CI, −0.75, 0.83; P = 0.919; I^2 = 83.9%, n = 3 studies; n = 82 subjects), GSH (SMD = 0.04; 95% CI, −0.28, 0.35; P = 0.822; I^2 = 44.1%; n = 3 studies; n = 82 subjects), and NO (SMD = −0.16; 95% CI, −0.47, 0.15; P = 0.316; I^2 = 4.4%; n = 3 studies; n = 82 subjects) between the probiotics and control group. The forest plots of these meta-analyses are shown in Figure 4.

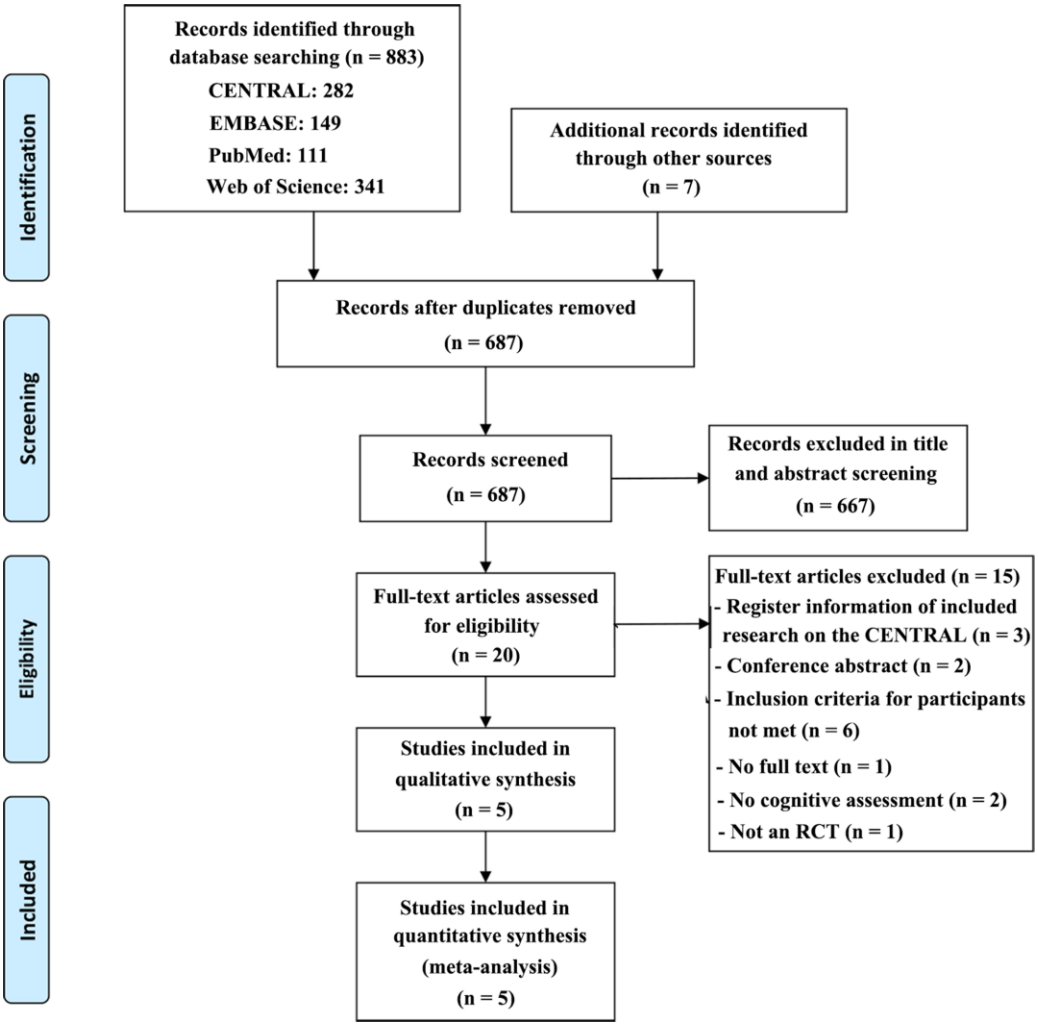

Figure 1. PRISMA flow diagram of the literature search and abstraction process.

Table 1. Main characteristics of the included studies.

Study	Study design	Participants		Age (M±SD)		Sex ratio (M/F)		Intervention			Outcome assessments		Main findings
				PRO	CON	PRO	CON						
Elmira Akbari (2016)	Randomized, double-blind, controlled trial	60	AD (NINCDS-ADRDA criteria)	77.67 ± 2.62	82.00 ± 1.69	6/24	6/24	Multiple (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Lactobacillus fermentum*)	12	8×10^9 (CFU/g)	MMSE	TAC GSH MDA hs-CRP NO	Probiotic consumption for 12 weeks positively affected cognitive function and some metabolic statuses in the AD patients
Omid Reza Tamtaji (2018)	Randomized, double-blind, controlled trial	90	AD (NINCDS-ADRDA criteria)	76.2 ± 8.1	78.8 ± 10.2			Multiple (*Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Bifidobacterium longum*)	12	6×10^9 (CFU/day)	MMSE	TAC GSH MDA hs-CRP NO	Probiotic and selenium co-supplementation for 12 weeks to patients with AD improved cognitive function and some metabolic profiles.
Azadeh Agahi (2018)	Randomized, double-blind, controlled trial	48	AD (NINCDS-ADRDA criteria)	79.70 ± 1.72	80.57 ± 1.79	7/18	10/13	Multiple (*Lactobacillus fermentum*, *Lactobacillus plantarum*, *Bifidobacterium lactis* *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Bifidobacterium longum*)	12	3×10^9 (CFU/day)	TYM	TAC GSH MDA NO	Cognitive and biochemical indications in the patients with severe AD were insensitive to the probiotic supplementation.
Y. Kobayashi (2019)	Randomized, double-blind, controlled trial	121	Subjective memory complaints (MMSE, 22-27)	61.5 ± 6.83	61.6 ± 6.37	30/31	30/30	Sole (*Bifidobacterium breve* A1)	12	$>2.0\times10^{10}$ (CFU/day)	MMSE	hs-CRP	No significant intergroup difference was observed in terms of changes in scores from the baseline scores
		44	MCI (RBANS <41)					Sole (*Bifidobacterium breve* A1)	12	$>2.0\times10^{10}$ (CFU/day)	MMSE		Significant difference between *B. breve* A1 and placebo groups in terms of MMSE total score in the subjects with MCI
Yun-Ha Hwang (2019)	Multi-center, randomized, double-blind, controlled trial	100	MCI (DSM-5)	68.0 ± 5.12	69.2 ± 7.00	20/30	14/36	Sole (*Lactobacillus plantarum* C29)	12	$>1.0\times10^{10}$ (CFU/day)	VLT ACPT DST		DW2009 can be safely administered to enhance cognitive function in individuals with MCI.

Abbreviations: PRO, probiotics group; CON, control group; CFU, colony-forming units; AD, Alzheimer's disease; MCI, mild cognitive impairment; NINCDS-ADRDA criteria, National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA); MMSE, Mini-Mental State Examination; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; TAC, total anti-oxidant capacity; GSH, total glutathione; MDA, malondialdehyde; hs-CRP, high-sensitivity C-reactive protein; NO, nitric oxide; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, 5th edition; VLT, verbal learning test; ACPT, auditory continuous performance test; DST, digit span test; DW2009, *Lactobacillus plantarum* C29-fermented soybean.

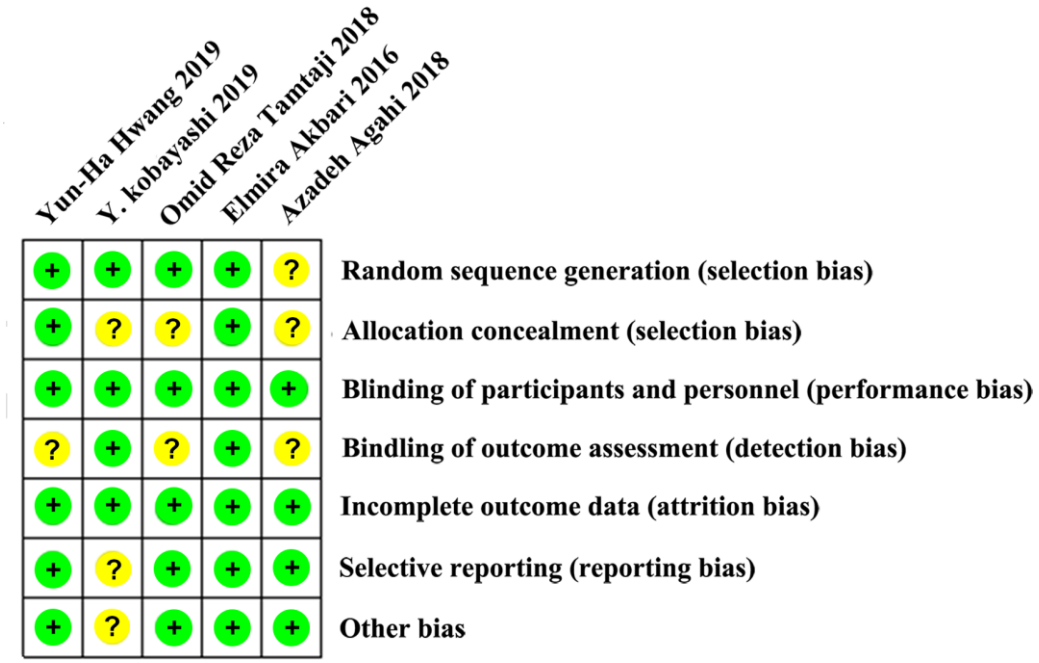

Figure 2. Summary of risk of bias assessment: judgments of the review authors on each risk of bias item for the included studies (n = 5). One study was rated as "low risk of bias", and the other four were assessed as "moderate risk of bias." No study was judged as "high risk of bias".

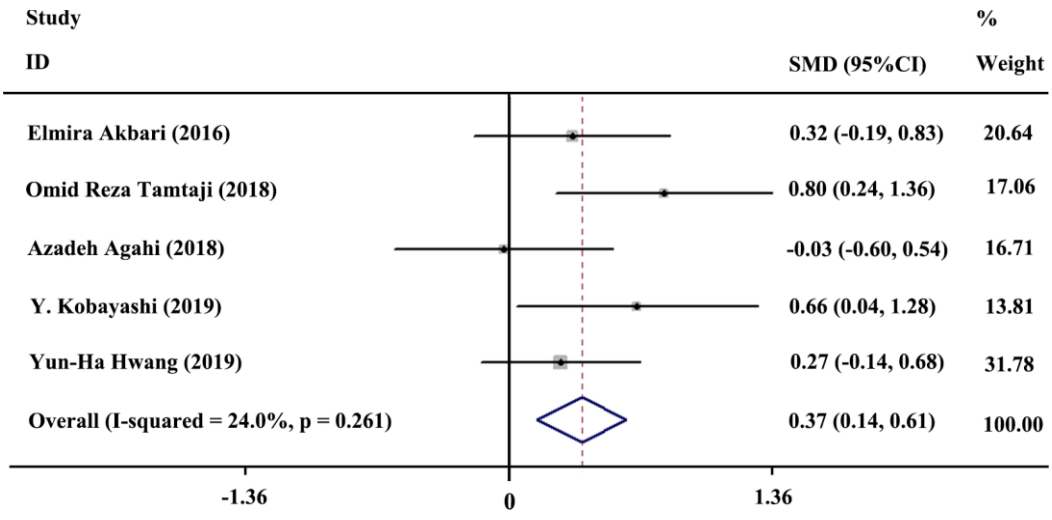

Figure 3. Forest plot showing the standardized mean difference (SMD) in cognitive enhancement, comparing the probiotics group versus the control group. Weights were assigned according to the number of subjects and SD using STATA 12. A fixed-effects model was applied to the meta-analysis. The sizes of the data markers represent the weight of each study, and the diamond indicates the overall estimated effect.

Subgroup analyses

A total of three subgroup analyses were conducted, and the results were summarized in Table 2 (for forest plots of the subgroup analyses, please see the supplementary materials). The heterogeneity obviously declined only in the subgroup analysis of "MMSE versus non-MMSE" after stratification by cognitive rating scales. Additionally, the meta-analysis results from the subgroups for disease type (AD versus MCI) and strains of flora (multiple versus sole) were consistent with the overall pooled results, while there was no effect on cognition in the subset of studies with the non-MMSE rating scales (SMD = 0.16; 95% CI: −0.17, 0.50; P = 0.33; I^2 = 0.0%).

Publication bias assessment and sensitivity analysis

We quantitatively assessed the publication bias through Egger's test and Begg's test. The results demonstrated no significant publication bias on cognition (Egger's test: P = 0.54, Begg's test: P = 0.81; see supplementary materials). The sensitivity analysis was performed to test the reliability of the meta-analysis results by omitting studies one by one. Systematically removing each trial did not significantly influence the overall effects of probiotics on cognition (see supplementary materials). Thus, the findings between the probiotics and control groups regarding cognitive enhancement were considered reliable.

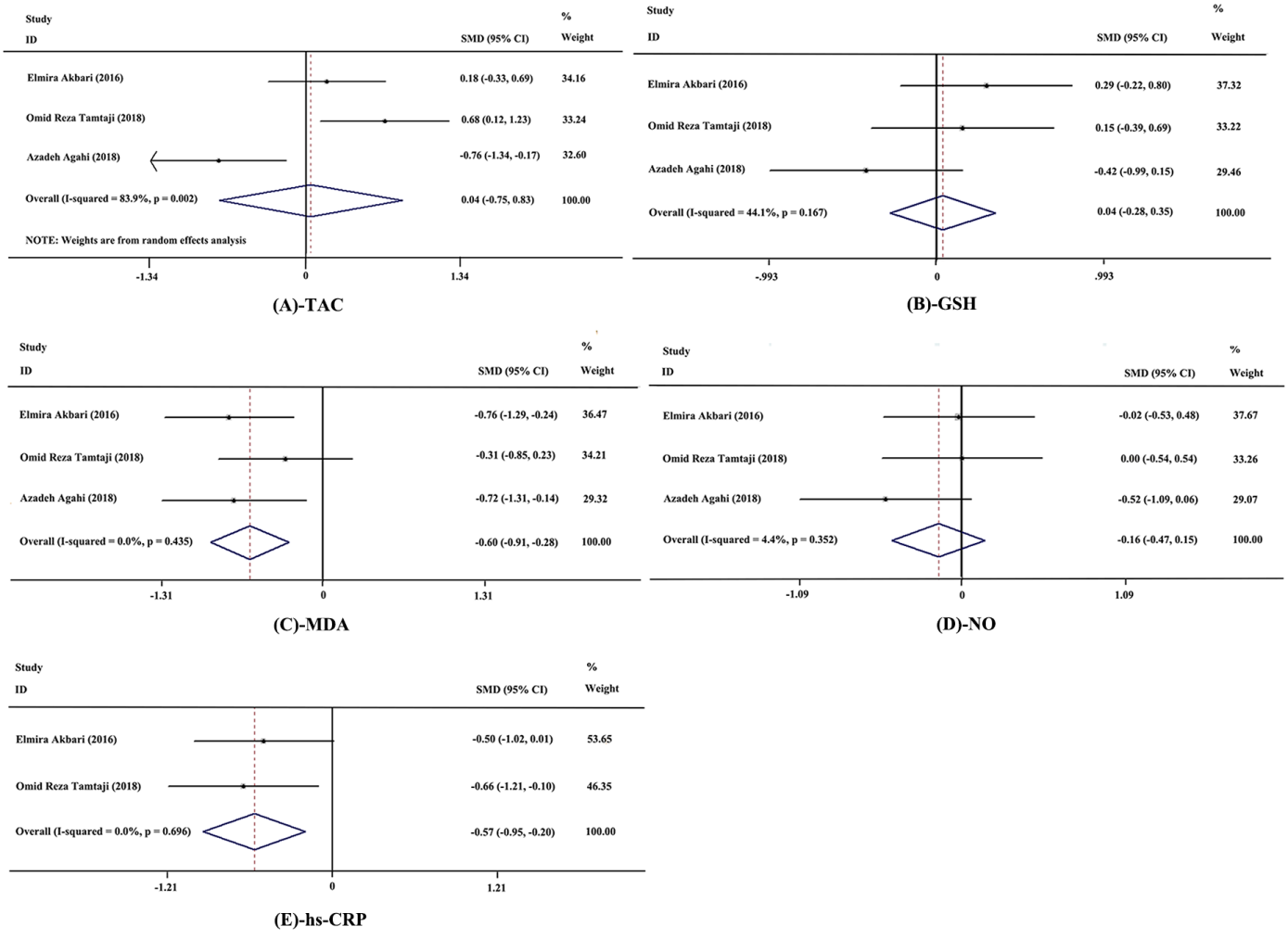

Figure 4. Forest plot showing the effects in the probiotics group versus the control group on biomarkers of inflammation and oxidative stress. (**A**) Meta-analysis of the effects of probiotics on total anti-oxidant capacity (TAC). (**B**) Meta-analysis of the effects of probiotics on total glutathione (GSH). (**C**) Meta-analysis of the effects of probiotics on malondialdehyde (MDA). (**D**) Meta-analysis of the effects of probiotics on nitric oxide (NO). (**E**) Meta-analysis of the effects of probiotics on high-sensitivity C-reactive protein (hs-CRP). Weights were assigned according to the number of subjects and SD using STATA 12. A random-effects model was applied to the meta-analysis of TAC, while other biomarkers used a fixed-effects model. The sizes of data markers represent the weight of each study, and the diamonds indicate the overall estimated effect. SMD, standardized mean difference.

Table 2. Summary of meta-analysis and subgroup analyses on cognition.

Outcome or Subgroup	No. of trials	Participants	Estimated Effect	Test of heterogeneity		P^2
				P^1	I^2 (%)	
1.0 Probiotics versus control group	5	297	0.37 (0.14, 0.61)	0.26	24	0.002
1.1 Subgroup by type of disease						
1.1.1 AD	3	161	0.36 (0.05, 0.68)	0.12	52.2	0.023
1.1.2 MCI	2	136	0.39 (0.04, 0.73)	0.30	6.4	0.028
1.2 Subgroup by cognitive rating scale						
1.2.1 MMSE	3	157	0.57 (0.25, 0.89)	0.44	0	0.001
1.2.2 Non-MMSE	2	140	0.16 (-0.17, 0.50)	0.41	0	0.33
1.3 Subgroup by strains of flora						
1.3.1 Multiple	3	161	0.36 (0.05, 0.68)	0.12	52.2	0.023
1.3.2 Sole	2	136	0.39 (0.04, 0.73)	0.30	6.4	0.028

Note: P^1 for heterogeneity: $P < 0.1$ was considered to indicate significant heterogeneity across studies. I^2 for heterogeneity: $I^2 > 50\%$ was considered to indicate significant heterogeneity across studies. P^2 for meta-analysis: $P < 0.05$ was considered to indicate a significant effect of probiotics on cognition by using a fixed-effects model.

DISCUSSION

To the best of our knowledge, this study is the first meta-analysis to elucidate the pronounced beneficial effects of probiotics on improving cognition in individuals with AD or MCI, which might be attributed to the effective decrease in some inflammatory and oxidative related biomarkers (MDA and hs-CRP).

Over the past decades, many studies have focused on effective pharmaceutical strategies to control AD; however, none have yet yielded a satisfying therapy. Recently, it has been demonstrated that modification of lifestyle factors such as dietary patterns plays an important role in alleviating cognitive decline [33]. This indicates that it is imperative to explore potential dietary interventions that may ameliorate age-associated cognitive deficits. Probiotics intervention could be a novel and feasible dietary strategy for managing dementia and other related diseases [34]. Remarkably, our findings provide evidence that relatively low-cost, widely available, and well-tolerated probiotics could be potential candidates for the control or prevention of AD and MCI. However, the specific underlying mechanisms remain unknown.

Although the exact pathogenesis of AD is not completely understood, accumulating evidence suggests that the oxidative stress and inflammatory pathways may play a critical role in the underlying mechanisms of cognitive deficits and AD [35]. Increased neuro-inflammation and oxidative stress are frequently observed complications in participants with MCI and AD [36–38]. Amyloid-beta peptide (Aβ) oligomers has been considered as the major pathogenic factor associated to AD, which tend to accumulate extracellularly as amyloid deposits [39]. Neuroinflammation in AD is thought to be triggered by Aβ and/or by the substances released by dying neurons, causing microglia activation [40, 41]. Activated microglia triggers the recruitment and proliferation of astrocytes that actively bolster the inflammatory response to extracellular Aβ deposits. This neuro-inflammatory component of AD is further characterized by a local cytokine (e.g. TNF-α, IL-1β, IL-6) accumulation mediating acute-phase response, activation of the complement cascade and induction of inflammatory enzyme systems such as inducible nitric oxide synthase (iNOS) and COX-2 [42, 43]. Meanwhile, Aβ is also shown to trigger oxidative stress by increasing ROS production through damage in mitochondrial structure and function [44, 45], or the activation of NADPH oxidase in microglia and astrocytes [46, 47], which in turn promotes further Aβ production, triggering a vicious cycle. All these factors, either alone or in concert, can contribute to neuronal dysfunction and death that occur in AD. Accordingly, huge numbers of potential anti-inflammatory and anti-oxidative agents have been explored to develop effective therapeutic strategies for AD.

GM has been reported to play an important role in the pathogenesis of AD through the MGB axis [21], indicating that restoring GM homeostasis may induce beneficial effects on the progression of AD [48]. Probiotics are live microorganisms intended to provide health benefits, generally by improving or restoring the GM [22–24, 49]. Moreover, Athari Nik

Azm et al. [50] pointed out that reduction in the number of amyloid plaques, inflammation and oxidative stress were observed in an Alzheimer-probiotics group. Kobayashi et al. [51] also revealed that probiotics administration suppressed hippocampal gene expression of inflammation and oxidative stress related genes in an AD mouse model. These results suggest that probiotics might exert health benefits through their anti- inflammatory and anti-oxidative effects. Herein, we found that some inflammatory and oxidative biomarkers (hs-CRP and MDA) were significantly decreased by probiotics, which might be related to the underlying mechanisms of their beneficial effects in AD or MCI subjects. Meanwhile, some inflammatory and oxidative biomarkers (TAC, GSH, and NO) were not significantly different in the meta-analysis. Apart from diverse interventions conditions, such as strains of flora, total intervention duration, dosage, and so forth, it is worthwhile to infer that inflammatory and oxidative stress pathways are not specific to AD and MCI, but potentially play an important role in various kinds of diseases, especially age-related diseases [11]. Additionally, the inflammatory and oxidative biomarkers enrolled in the current study were only a part of the pathophysiology in AD and MCI patients. Therefore, more comprehensive inflammatory and oxidative metabolites need to be tested in future clinical trials in order to clarify the exact mechanism of the beneficial effect of probiotics on AD and MCI patients.

Furthermore, a systematic review reveals that neither single nor multi-strain probiotics are beneficial for improving cognitive function according to eleven animal studies [26]. Many studies demonstrate that probiotics, such as *Bifidobacterium* and *Lactobacillus* strains, ameliorate cognitive and memory deficits in animal models of AD [50, 52, 53]. In the current study, the strains used in the involved clinical trials were also from *Bifidobacterium* and *Lactobacillus* (Table 3). We found that sole or multi-strain probiotics were both beneficial for improving cognitive function, suggesting that *Bifidobacterium* and *Lactobacillus* strains would be the most potential candidates. However, AD patients were insensitive to a mixture of six type of strains in the study from Azadeh Agahi et al. [29], indicating that the effectiveness of probiotics may be influenced by multifactors such as the severity of patients, proportion of each strains, dosage and so forth.

Given the differences exist across species, the appropriate dosing of probiotics should be demonstrated for the specific target host [54]. The definition of probiotics requires the administration of an "adequate amount" in order to obtain a health benefit. However, the definition does not specify the size of this "adequate" dose [55, 56]. The commonly dose of probiotics used are ranging from 10^8 to 10^{11} CFU in related studies. Some researches [57–59] appear to support a dose-response for probiotics in reducing the risk of antibiotic associated diarrhoea, suggesting that a dose greater than 10^{10} CFU is most effective. In contrast, there is no dose-response for probiotics in *Clostridium difficile* associated diarrhoea, necrotising enterocolitis, atopic dermatitis and slow intestinal transit [60–67]. Compiled evidence indicates that the effective dose of probiotics is influenced by a multitude of variables, including health endpoint, the specific probiotic used, delivery vehicle and route of administration [68]. Therefore, it seems difficult to epitomize one optimal dose for probiotic intervention. At present, we failed to provide a dose-response of probiotics in ameliorating cognitive deficits for the information on the intervention dose of probiotics was not enough in the involved studies. Nevertheless, the absence of evidence for a dose-effect does not imply evidence of absence of a dose-effect [67]. More reliable evidence from various dosages especially outside the common concentration (10^8 ~ 10^{11} CFU) of probiotics trials is needed.

Additionally, the safety of probiotics should also be considered. People have been eating fermented foods for centuries without suffering any harmful side effects. Probiotics are generally accepted in the medical community as a harmless and very efficient, natural remedy. However, the United States Food and Drug

Administration requires phase I safety studies for probiotics when the intended use of the product is as a drug. Researchers [69] have performed 10^8 to 10^{11} CFU/day doses of *Bifidobacterium animalis*subsp *lactis* (BB-12) and *Lactobacillus paracasei* subsp *paracasei* (CRL-431) in healthy young adults, and found that the increasing doses of probiotic bacteria were well tolerated and no volunteers reported adverse side effects during the intervention. Moreover, Patricia L. Hibberd et al. [70] reported that *Lactobacillus rhamnosus* GG ATCC 53103 (LGG)(1×10^{10} CFU), one of the most studied probiotic, is safe and well tolerated in healthy adults aged 65 years and older. Among the five studies included in our present study, two [28, 29] did not report on the incidence of side effects; two [27, 30] reported no side effects and only one [31] reported mild adverse events (e.g. stomach aches, headaches, gastritis, erectile dysfunction). These results indicate that probiotics are considered generally safe to consume, but may cause bacteria-host interactions and unwanted side effects in rare cases. Accordingly, the *Bifidobacterium* and *Lactobacillus* strains used in the involved studies were safe and well tolerated, however, the specific strain still need phase I safety studies before using as a drug.

Table 3. Components of the probiotics in each RCTs.

Type of strains	Elmira Akbari (2016)	Omid Reza Tamtaji (2018)	Azadeh Agahi (2018)	Y. Kobayashi (2019)	Yun-Ha Hwang (2019)
Bifidobacterium bifidum	√	√	√		
Bifidobacterium breve A1				√	
Bifidobacterium lactis			√		
Bifidobacterium longum		√	√		
Lactobacillus acidophilus	√	√	√		
Lactobacillus casei	√				
Lactobacillus fermentum	√		√		
Lactobacillus plantarum			√		√

Note: √ represents containing this type of strain in the component of probiotics.

The main limitations are as follows: 1) First, the cognitive rating scales varied across the recruited studies. The widely accepted cognitive rating scale, the MMSE, was selected in three studies [27, 28, 30], and one trial chose the Test Your Memory (TYM) test. Remarkably, AD patients assessed by the MMSE [27, 28] revealed a positive effect of the probiotics, while the TYM test in patients with AD found the opposite result. The main reason for this discrepancy may arise from the difference in the outcome indexes among studies; furthermore, the TYM test has been reported to be more sensitive in detecting dementia than the MMSE [71]. In addition, Yun-Ha Hwang et al. [31] assessed three cognitive domains combined using the verbal learning test (VLT), auditory continuous performance test (ACPT), and digit span test (DST). Cognitive domains of this combined one are incomprehensive enough compared to the MMSE or TYM. The subgroup analysis showed that cognitive rating scales, i.e., "MMSE versus non-MMSE", could partly explain the source of heterogeneity, and the imbalanced assessment methods may have discounted the meta-analysis outcome. 2) Second, the inclusion criteria for AD patients were all in accordance with the NINCDS-ADRDA criteria. However, the criteria for recruiting MCI subjects were different: Y. Kobayashi et al. [30] discriminated MCI and normal individuals using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), while Yun-Ha Hwang et al. [31] diagnosed MCI according to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5). This may have generated low comparability of these two trials attributable to inconsistent inclusion criteria. 3) Third, three of the five clinical trials used in the cognitive meta-analysis are from the same region. Though the participants' characteristics, intervention conditions, cognitive rating scales were varied from each other and the main findings were inconsistent. There is still concern that the relatively low heterogeneity might be partially attributed to the clinical trials from the same region. Additionally, two of the three studies also had concerns regarding allocation bias and evaluation bias. These limitations might discount the outcomes of our meta-analysis. 4) Fourth, some other key variables, including the age, sex, and BMI of the samples, specific strain of the flora, dosage of probiotics, and drug form, may have exerted an influence on the results, but the relevant information obtained on the results was limited in this study. Moreover, possible dose-response of probiotics in ameliorating cognitive deficits also should be further explored. 5) Last but not least, we concluded that the potential mechanism was partially related to the anti-inflammatory and anti-oxidative property of probiotics in only 12 weeks study duration. However, many clinical trials of AD drugs (i.e, β-amyloidantibody, β-secretase inhibitors, tau aggregation inhibitor), long-term interventions of several years have failed [72–74]. The beneficial efficacy of probiotics for AD and MCI in only 12 weeks study duration might be attributed to multi-directional mechanism regulation, including alterations in the levels of certain neurotransmitters, increasing neuroprotective molecules, such as brain-derived neurotrophic factor, reduction of inflammation and so forth [21, 75, 76]. In addition, there might be existence of sponsor bias in part of the included studies. Therefore, longer study durations are needed to determine the overall net benefits of probiotics. With a view to the above limitations, future RCTs that control these confounding factors are needed to clarify the moderators of probiotics effects on cognition.

CONCLUSIONS

The meta-analysis indicated that probiotics consumption enhanced cognition in subjects with AD or MCI, possibly through decreasing the levels of inflammatory and oxidative biomarkers. However, the current RCT evidence is insufficient and limited, the results should be cautiously interpreted, and more reliable evidence from large-scale, long-period, randomized, controlled trials is needed.

METHODS

Literature search

A literature search was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The preliminary search was performed through the EMBASE, PubMed, Web of Science and the Cochrane Central Register of Controlled Trials (CENTRAL) electronic databases without time restrictions, up to June 10, 2019. A snowball search was manually carried out by searching reference lists from relevant published reviews and the retrieved papers. We searched the first-group-term (AD or MCI-related term) with "OR". Then used the same way for the second-group-term (human-related term) search and the third-group-term (probiotic-related term) search, followed by combing the number of records generated from the three groups of search term with "AND". The following terms were searched in "all fields" for each electronic database: (Alzheimer's disease OR dementia OR mild cognitive impairment OR cognitive dysfunction OR cognitive defect OR cognition OR memory OR mental capacity) AND (adult OR human) AND (probiotic OR yeast OR yoghurt OR fermented product OR lactobacillus OR bifidobacterium OR fermented dairy product OR synbiotics OR cultured milk products). Articles were limited to randomized controlled trials (RCTs).

Study selection

Eligible studies had to meet the following criteria: (1) The study was an RCT and published in peer-reviewed journal in English; (2) Adult human participants who had a diagnosis of AD or MCI (aged over 18 y); (3) Significant difference in form, appearance, taste, and smell of probiotics and placebo should not occur at baseline; (4) Any validated measure of cognitive assessment was acceptable; (5) Continuous data of cognitive outcomes, inflammatory and oxidative biomarkers at baseline and post-intervention, or the change from baseline, and the number of participants at baseline and post-intervention were reported or could be calculated from the data reported in the article.

Studies were excluded if they met any one of the following criteria: (1) The publications were abstracts, reviews, conference papers, study protocols, cross-sectional studies, nonhuman (in vitro and animal) studies or papers that did not report on any outcome of interest; (2) No post-intervention or change from baseline on scale scores of cognition was reported, and these data could not be calculated based on the information in the article; (3) The study reported on a sample that overlapped the sample in another study. In this case, only the study with the larger sample size was included.

Articles were initially and independently screened for eligibility by two investigators based on titles and abstracts. Duplicate and irrelevant papers were excluded. For the relevant candidates, the full articles were retrieved, reviewed and the references of each document were checked to find out potential candidates. Disagreements were resolved by discussion between the two researchers, or with a third reviewer.

Data extraction

Data were extracted independently by two authors (H-Y.D, M-L.C), using a predetermined form in accordance with the guidance of the Cochrane Handbook for Systematic Reviews of Interventions. Parameters collected including basic information of the RCTs (first author, published year, and study design), characteristics of the participants (sample size, disease type, age, and sex ratio), intervention-related variables (type of strains, duration, and dosage), outcome assessments (cognitive outcomes, inflammatory and oxidative biomarkers) and the main findings of each included study. To perform the meta-analysis, the following data of outcome assessments were extracted: the mean change score along with the associated variance (standard deviation [SD] or standard error of the mean [SEM]). When change scores were not available, the scores (mean±SD or mean±SEM) and the number of participants at baseline and post-intervention were extracted.

Risk of bias assessment

All studies were independently assessed for risk of bias by two authors, with disagreements resolved by discussion to reach consensus. The tool of the Cochrane Handbook for Systematic Reviews of Interventions was used, which included criteria for the following six aspects: random sequence generation, allocation concealment, blindness of participants and personnel, blindness of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias. The results of the risk of bias assessment were pooled into Revman 5.3, and a "summary of risk of bias assessment" table was generated.

Statistical analysis

The primary outcomes of this study were the standardized mean differences (SMDs) of change from baseline between probiotics and placebo group. The SMD was tested by a Z statistic, and a two-tailed $P < 0.05$ was regarded as statistically significant. The inter-study heterogeneity was examined by chi-square ($\chi 2$) statistics and I^2 statistics. The heterogeneity among the different studies was considered high if $P < 0.1$ for the $\chi 2$ statistic or $I^2 > 50\%$ [77]. SMDs were calculated by

fixed-effects or random-effects models. A sensitivity analysis was conducted to test the reliability of the findings using the leave-one-out method, while publication bias was assessed by Egger's test and Begg's test. Subgroup analyses were performed to examine the possible source of heterogeneity within these studies, and the subtypes involved cognitive rating scales (Mini-Mental State Examination, MMSE, versus non-MMSE), type of disease (AD versus MCI) and strains of flora (multiple versus sole). The statistical analyses of forest plots, sensitivity analysis, Egger's test, and Begg's test were performed in STATA software (version 12; StataCorp), while Revman 5.3. was used to generate the summary of risk of bias assessment.

Abbreviation

AD: Alzheimer's disease; MCI: mild cognitive impairment; GM: gut microbiota; MGB: microbiota-gut-brain axis; CNS: central nervous system; CENTRAL: Cochrane Central Register of Controlled Trials; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCTs: randomized controlled trials; SMD: standardized mean difference; CIs: confidence intervals; SD: standard deviation; SEM: standard error of the mean; MMSE: Mini-Mental State Examination; TYM: Test Your Memory; VLT: verbal learning test; ACPT: auditory continuous performance test; DST: digit span test; NINCDS-ADRDA: National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA); RBANS: Repeatable Battery for the Assessment of Neuropsychological Status; DSM-5: Diagnostic and Statistical Manual of Mental Disorders, 5th edition; MDA: malondialdehyde; hs-CRP: high-sensitivity C-reactive protein; TAC: total anti-oxidant capacity; GSH, total glutathione; NO: nitric oxide; ROS: reactive oxygen species; BBB: blood-brain barrier

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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PMID:27429978

74. Gautam RRG. Pipeline assessment of beta-secretase inhibitors for alzheimer's disease: hopes or gloom for a trillion dollar market. Value Health. 2017; 20:A887.
https://doi.org/10.1016/j.jval.2017.08.2654

75. Dinan TG, Cryan JF. The Microbiome-Gut-Brain Axis in Health and Disease. Gastroenterol Clin North Am. 2017; 46:77–89. https://doi.org/10.1016/j.gtc.2016.09.007 PMID:28164854

76. Angelucci F, Cechova K, Amlerova J, Hort J. Antibiotics, gut microbiota, and Alzheimer's disease. J Neuroinflammation. 2019; 16:108. https://doi.org/10.1186/s12974-019-1494-4 PMID:31118068

77. Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions, Version 5.1.0. 2013.

SUPPLEMENTARY MATERIALS

Details of searching strategy and screening process

Literature search

The specific searching strategy and results for each database were described as follows:

EMBASE

Search strategy: ('alzheimer disease'/exp OR 'alzeimer disease' OR 'alzeimer`s disease' OR 'alzeimers disease' OR 'alzheimer dementia' OR 'alzheimer disease' OR 'alzheimers disease' OR 'alzheimer fibrillary change' OR 'alzheimer fibrillary lesion'OR 'alzheimer neurofibrillary change' OR 'alzheimer neurofibrillary degeneration' OR 'alzheimer neuron degeneration' OR 'alzheimer perusini disease' OR 'alzheimer sclerosis' OR 'alzheimer syndrome' OR 'alzheimer`s disease' OR 'cortical sclerosis, diffuse' OR 'dementia, alzheimer' OR 'diffuse cortical sclerosis' OR 'late onset alzheimer disease' OR 'dementia'/exp OR 'amentia' OR 'dementia' OR 'demention' OR 'mild cognitive impairment'/exp OR 'amnestic mild cognitive impairment' OR 'mild cognitive impairment' OR 'cognitive defect'/exp OR 'cognition disorder' OR 'cognition disorders' OR 'cognitive defect' OR 'cognitive defects' OR 'cognitive deficit' OR 'cognitive disability' OR 'cognitive disorder' OR 'cognitive disorders' OR 'cognitive impairment' OR 'delirium, dementia, amnestic, cognitive disorders' OR 'overinclusion' OR 'response interference' OR 'cognition'/exp OR 'cognition' OR 'cognitive accessibility'OR 'cognitive balance' OR 'cognitive dissonance' OR 'cognitive function' OR 'cognitive structure' OR 'cognitive symptoms' OR 'cognitive task' OR 'cognitive thinking' OR 'neurobehavioural manifestations' OR 'volition' OR 'memory'/exp OR 'item recall' OR 'memory' OR 'memory function' OR 'nonspatial memory' OR 'remembering' OR 'reminiscence' OR 'mental capacity'/exp OR 'ability, mental' OR 'attainment' OR 'capacity, mental' OR 'fitness, mental' OR 'mental ability' OR 'mental capacity' OR 'mental competency' OR ad OR mci OR 'cognitive fail*' OR 'cognitive decline*' OR 'cognitive impair*' OR alzheimer* OR dement* OR 'cognitive dysfunction' OR 'cognitive performance' OR cognitive) AND ('probiotic agent'/exp OR 'probiotic' OR 'probiotic agent' OR 'probiotics' OR 'yeast'/exp OR 'flora, yeast' OR 'fungi, yeast' OR 'yeast' OR 'yeast fermentation' OR 'yeast flora' OR 'yeast fungus' OR 'yeast germination' OR 'yeast metabolism' OR 'yeasts' OR 'yoghurt'/exp OR 'yoghourt' OR 'yoghurt' OR 'yogurt' OR 'zabadi' OR 'lactobacillus'/exp OR 'betabacterium' OR 'lactobacileae' OR 'lactobacilleae' OR 'lactobacillus' OR 'lactobacteria' OR 'lactobacilli' OR 'bifidobacterium'/exp OR 'bifidobacterium' OR 'fermented dairy product'/exp OR 'cultured dairy foods' OR 'cultured dairy product' OR 'cultured milk foods' OR 'cultured milk product' OR 'cultured milk products' OR 'fermented dairy foods' OR 'fermented dairy product' OR 'fermented milk' OR 'fermented milk product' OR 'fermented product'/exp OR 'fermented food' OR 'fermented foods' OR 'fermented product' OR 'synbiotic agent'/exp OR 'synbiotic' OR 'synbiotic agent' OR 'synbiotics' OR probiotic*) AND ('placebo'/exp OR 'placebo' OR 'placebo gel' OR 'placebos') AND ('randomized controlled trial'/exp OR 'controlled trial, randomized' OR 'randomised controlled study' OR 'randomised controlled trial' OR 'randomized controlled study' OR 'randomized controlled trial' OR 'trial, randomized controlled' OR 'controlled clinical trial'/exp OR 'clinical trial, controlled' OR 'controlled clinical comparison' OR 'controlled clinical drug trial' OR 'controlled clinical experiment' OR 'controlled clinical study' OR 'controlled clinical test' OR 'controlled clinical trial' OR rct OR random* OR control* OR trial*) AND 'human'/de AND ([adult]/lim OR [aged]/lim OR [middle aged]/lim OR [very elderly]/lim OR [young adult]/lim)

Search results: 149 items

PubMed

Search strategy: ((((("Adult"[Mesh]) AND "Humans"[Mesh]) AND ((((((((((((((((((("Alzheimer Disease"[Mesh]) OR Alzheimer*) OR AD) OR "Dementia"[Mesh]) OR Dement*) OR mild cognitive impairment) OR MCI) OR ("Cognition"[Mesh] OR "Cognition Disorders"[Mesh])) OR ("Memory"[Mesh] OR "Memory Disorders"[Mesh])) OR "Cognitive Dysfunction"[Mesh]) OR cognitive defect) OR Mental capacity) OR Cognitive) OR cognitive performance) OR cognitive impair*) OR cognitive decline*) OR cognitive fail*)) AND ("Placebos"[Mesh]) AND ((((((((((" Probiotics"[Mesh]) OR "Cultured Milk Products"[Mesh]) OR "Yogurt"[Mesh]) OR (("Yeast, Dried"[Mesh]) OR "Yeasts"[Mesh])) OR "Bifidobacterium"[Mesh]) OR "Lactobacillus"[Mesh]) OR "Synbiotics"[Mesh]) OR probiotic*) OR fermented product) OR fermented dairy product)) AND (((((((((("Randomized Controlled Trial"[Publication Type] OR "Controlled Clinical Trial"[Publication Type])) OR randomized controlled trials) OR Controlled Clinical Trials) OR RCT) OR random*) OR control*) OR trial*)))))

Search results: 111 items

Cochrane Central Register of Controlled Trials (CENTRAL)

Search strategy:

ID	Search	Hits
#1	MeSH descriptor: [Lactobacillus] explode all trees	1487
#2	MeSH descriptor: [Yogurt] explode all trees	329
#3	MeSH descriptor: [Probiotics] explode all trees	1796
#4	MeSH descriptor: [Bifidobacterium] explode all trees	655
#5	MeSH descriptor: [Yeasts] explode all trees	711
#6	MeSH descriptor: [Cultured Milk Products] explode all trees	499
#7	MeSH descriptor: [Synbiotics] explode all trees	118
#8	#1 or #2 or #3 or #4 or #5 or #6 or #7	3356
#9	"probiotic*" or "fermented product" or "fermented dairy product"	4357
#10	#8 or #9	6286
#11	adult and humans	351364
#12	MeSH descriptor: [Alzheimer Disease] explode all trees	3015
#13	MeSH descriptor: [Dementia] explode all trees	5224
#14	MeSH descriptor: [Cognitive Dysfunction] explode all trees	922
#15	MeSH descriptor: [Cognition] explode all trees	9641
#16	MeSH descriptor: [Memory] explode all trees	7470
#17	MeSH descriptor: [Cognition Disorders] explode all trees	4475
#18	MeSH descriptor: [Memory Disorders] explode all trees	1148
#19	Alzheimer* or AD or Dement* or "cognitive" or "mild cognitive impairment" or MCI or "cognitive fail*" or "cognitive performance" or "cognitive impair*" or "cognitive decline*" or "memory function" or "Mental capacity" or "cognitive defect"	99308
#20	#12 or #13 or #14 or #15 or #16 or #17 or #18 or #19	107156
#21	MeSH descriptor: [Randomized Controlled Trial] explode all trees	124
#22	MeSH descriptor: [Controlled Clinical Trial] explode all trees	132
#23	RCT or "randomized controlled trials" or "Controlled Clinical Trials" or "random*" or "control*" or "trial*"	1159526
#24	#21 or #22 or #23	1159526
#25	#10 and #20 and #24	302
#26	#25 and trails	282

Search results: 282 items

Web of Science

Search strategy: ('alzheimer disease'/exp OR 'alzeimer disease' OR 'alzeimer`s disease' OR 'alzeimers disease' OR 'alzheimer dementia' OR 'alzheimer disease' OR 'alzheimers disease' OR 'alzheimer fibrillary change' OR 'alzheimer fibrillary lesion'OR 'alzheimer neurofibrillary change' OR 'alzheimer neurofibrillary degeneration' OR 'alzheimer neuron degeneration' OR 'alzheimer perusini disease' OR 'alzheimer sclerosis' OR 'alzheimer syndrome' OR 'alzheimer`s disease' OR 'cortical sclerosis, diffuse' OR 'dementia, alzheimer' OR 'diffuse cortical sclerosis' OR 'late onset alzheimer disease' OR 'dementia'/exp OR 'amentia' OR 'dementia' OR 'demention' OR 'mild cognitive impairment'/exp OR 'amnestic mild cognitive impairment' OR 'mild cognitive impairment' OR 'cognitive defect'/exp OR 'cognition disorder' OR 'cognition disorders' OR 'cognitive defect' OR 'cognitive defects' OR 'cognitive deficit' OR 'cognitive disability' OR 'cognitive disorder' OR 'cognitive disorders' OR 'cognitive impairment' OR 'delirium, dementia, amnestic, cognitive disorders' OR 'overinclusion' OR 'response interference' OR 'cognition'/exp OR 'cognition' OR 'cognitive accessibility'OR 'cognitive balance' OR 'cognitive dissonance' OR 'cognitive function' OR 'cognitive structure' OR 'cognitive symptoms' OR 'cognitive task' OR 'cognitive thinking' OR 'neurobehavioural manifestations' OR 'volition' OR 'memory'/exp OR 'item recall' OR 'memory' OR 'memory function' OR 'nonspatial memory' OR 'remembering' OR 'reminiscence' OR 'mental capacity'/exp OR 'ability, mental' OR 'attainment' OR 'capacity, mental' OR 'fitness, mental' OR 'mental ability' OR 'mental capacity' OR 'mental competency' OR ad OR mci OR 'cognitive fail*' OR 'cognitive decline*' OR 'cognitive impair*' OR alzheimer* OR dement* OR 'cognitive dysfunction' OR 'cognitive performance' OR cognitive) AND ('probiotic agent'/exp OR 'probiotic' OR 'probiotic agent' OR 'probiotics' OR 'yeast'/exp OR 'flora, yeast' OR 'fungi, yeast' OR 'yeast' OR 'yeast fermentation' OR 'yeast flora' OR 'yeast fungus' OR 'yeast germination' OR 'yeast metabolism' OR 'yeasts' OR 'yoghurt'/exp OR 'yoghourt' OR 'yoghurt' OR 'yogurt' OR 'zabadi' OR 'lactobacillus'/exp OR 'betabacterium' OR 'lactobacileae' OR 'lactobacilleae' OR 'lactobacillus' OR 'lactobacteria' OR 'lactobacilli' OR

'bifidobacterium'/exp OR 'bifidobacterium' OR 'fermented dairy product'/exp OR 'cultured dairy foods' OR 'cultured dairy product' OR 'cultured milk foods' OR 'cultured milk product' OR 'cultured milk products' OR 'fermented dairy foods' OR 'fermented dairy product' OR 'fermented milk' OR 'fermented milk product' OR 'fermented product'/exp OR 'fermented food' OR 'fermented foods' OR 'fermented product' OR 'synbiotic agent'/exp OR 'synbiotic' OR 'synbiotic agent' OR 'synbiotics' OR probiotic*) AND ('randomized controlled trial'/exp OR 'controlled trial, randomized' OR 'randomised controlled study' OR 'randomised controlled trial' OR 'randomized controlled study' OR 'randomized controlled trial' OR 'trial, randomized controlled' OR 'controlled clinical trial'/exp OR 'clinical trial, controlled' OR 'controlled clinical comparison' OR 'controlled clinical drug trial' OR 'controlled clinical experiment' OR 'controlled clinical study' OR 'controlled clinical test' OR 'controlled clinical trial' OR rct OR random* OR control* OR trial*) AND (Human AND adult)

Search results: 341 items

Literature screening

All studies were imported to Endnote X6. After discarding the duplicates, 687studies were remained. The title and abstract screening excluded 667 papers with 20 papers remained. The full-text screening further excluded 15 studies, with the remaining 5 studies included in the meta-analysis. The 15 studies excluded according reasons as follows:

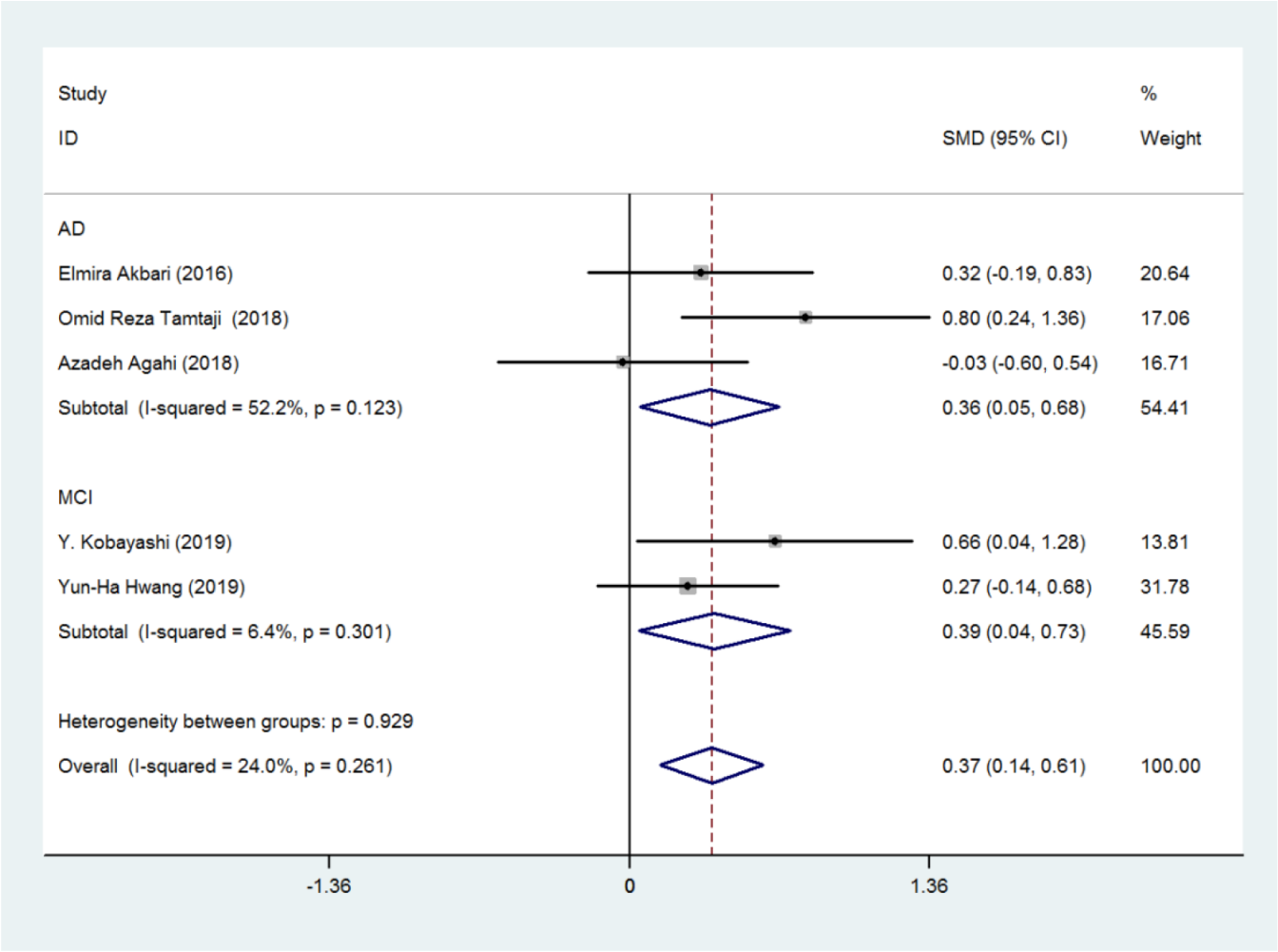
No.	Author	Year	Title	Source	Decision	Notes
1	Irct201511305623N.	2015	Effect of supplementation in treatment of patients with Alzheimer's disease.	https://www.cochranelibrary.com/central/doi/10.1002/central/CN-01814566/full	Exclude: register information of included research (Akbari, 2016)	IRCT201511305623N60
2	Irct2017061534549N.	2017	The effect of probiotic supplementation on cognitive function and inflammatory markers in patients with early and late phases of Alzheimer's disease.	https://www.cochranelibrary.com/central/doi/10.1002/central/CN-01892678/full	Exclude: register information of included research (Aghi.A, 2018)	IRCT2017061534549N1
3	Irct2017061203 4497N.	2018	Effect of combined probiotic and selenium supplementation in treatment of patients with Alzheimer's disease.	https://www.cochranelibrary.com/central/doi/10.1002/central/CN-01896860/full	Exclude: register information of included research (Omid Reza Tamtaji, 2018)	IRCT20170612034497N5
4	Jicha, G. A.	2015	A phase I, randomized, double-blind, placebo-controlled, multiple ascending dose study of AT-001 yeast selenium for the prevention of Alzheimer's disease.	Alzheimer's and Dementia, 11(7), P471.	Exclude: Conference abstract	Alzheimer's Association International Conference 2015
5	Jung Park, H.	2019	A randomized, double-blind, placebo-controlled study on the memory-enhancing effect of lactobacillus fermented Saccharina japonica extract.	European Journal of Integrative Medicine, 28, 39-46. doi: 10.1016/j.eujim.2019.04.006	Exclude: Inclusion criteria for participants not met	
6	Kim KY	2018	Association between diets and mild cognitive impairment in adults aged 50 years or older.	Nutrition Research and Practice, 12(5), 415-425. doi: 10.4162/nrp.2018.12.5.415	Exclude: not an RCT	

7	Louzada, E. R.	2018	Synbiotic supplementation, systemic inflammation, and symptoms of brain disorders in elders: A secondary study from a randomized clinical trial.	Nutritional Neuroscience, 1-8. doi: 10.1080/1028415X.2018.1477349	Exclude: Inclusion criteria for participants not met	
8	Nct.	2013	Cognitive and Metabolic Effects of a Probiotic Supplement.	https://clinicaltrials.gov/ct2/show/nct02005003	Exclude: Inclusion criteria for participants not met	Accepts Healthy Volunteers: Yes; Healthy (self-reported) and not on medication
9	Nct.	2017	Probiotic on Psychological and Cognitive Effects.	https://clinicaltrials.gov/show/nct03080818.	Exclude: Inclusion criteria for participants not met	Accepts Healthy Volunteers: Yes
10	Nct.	2018	Examining the Effects of One-Month Probiotic Treatment on Mental Fatigue.	https://clinicaltrials.gov/show/nct03611478.	Exclude: Inclusion criteria for participants not met	The aim of this study is to demonstrate the effects of 28-days supplementation with a novel probiotic formulation on mental fatigue following a cognitive load in healthy adults
11	Nct.	2018	Understanding gut feelings: probiotics and cognition.	https://www.cochranelibrary.com/central/doi/10.1002/central/CN-01906153/full	Exclude: Inclusion criteria for participants not met	Inclusion criteria: Healthy adult participants
12	Owen, L.	2014	A double blind, placebo controlled, randomised pilot trial examining the effects of probiotic administration on mood and cognitive function.	Proceedings of the Nutrition Society, 73(OCE1), E29-E29. doi: 10.1017/s0029665114000433	Exclude: Conference abstract	Winter Meeting, 11–12 December 2013, Diet, gut microbiology and human health
13	Pelka, R. B.	1995	Pre-Alzheimer study. Effect of a plant yeast extract (Bio-Strath®) in a randomized double-blind study.	Ars Medici, 85(1), 54-61.	Exclude: No full text form	
14	Friedrich Leblhuber	2018	Probiotic Supplementation in Patients with Alzheimer's Dementia - An Explorative Intervention Study	Current Alzheimer Research, 2018, 15, 1106-1113	Exclude: no cognition assessment	
15	M. CARLSSON	2009	The feasibility of serving liquid yoghurt supplemented with probiotic bacteria, Lactobacillus rhamnosus LB 21, and Lactococcus lactis L1A - A pilot study among old people with dementia in a residential care facility	The Journal of Nutrition, Health & Aging©Volume 13, Number 9, 2009	Exclude: no cognition assessment	

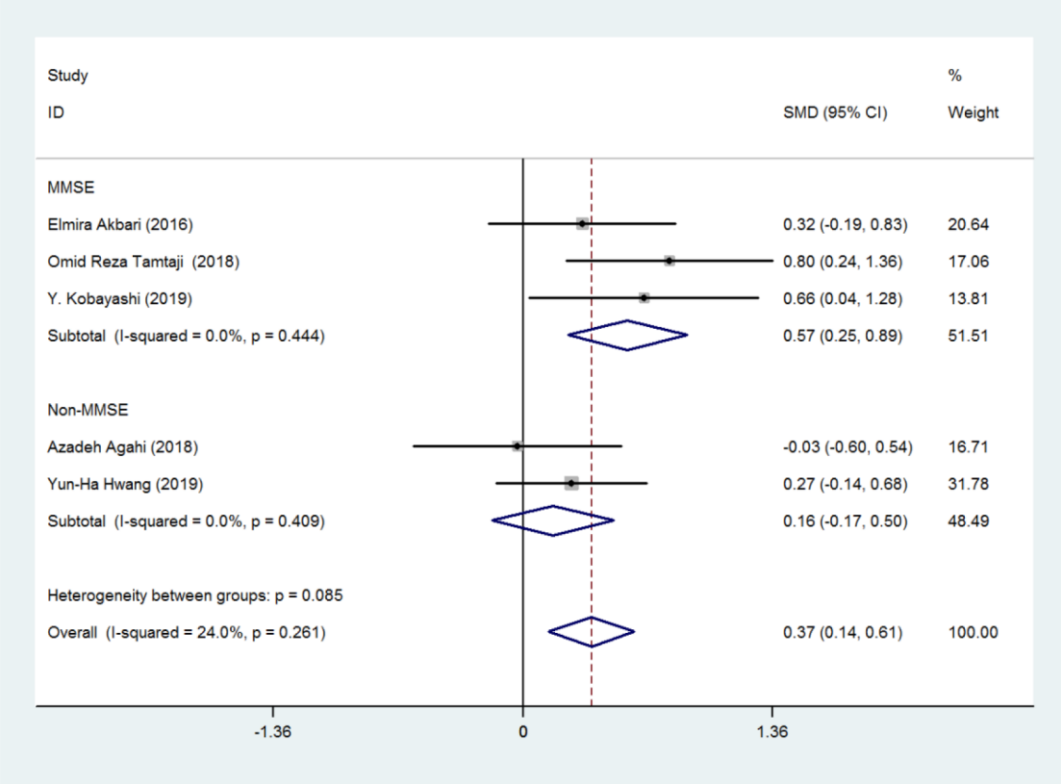
Detailed results of subgroup analyses, publication bias and sensitivity analysis

Subgroup analyses

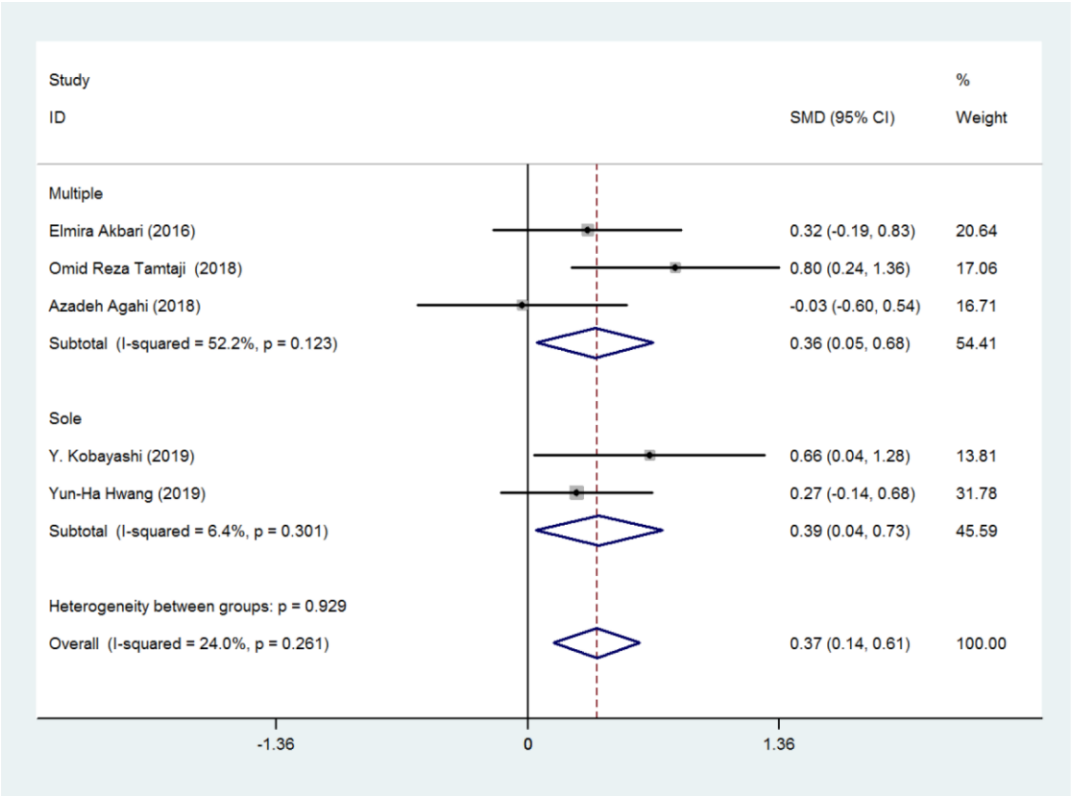
Subgroup by type of diseases:



Subgroup by the cognitive rating scales:

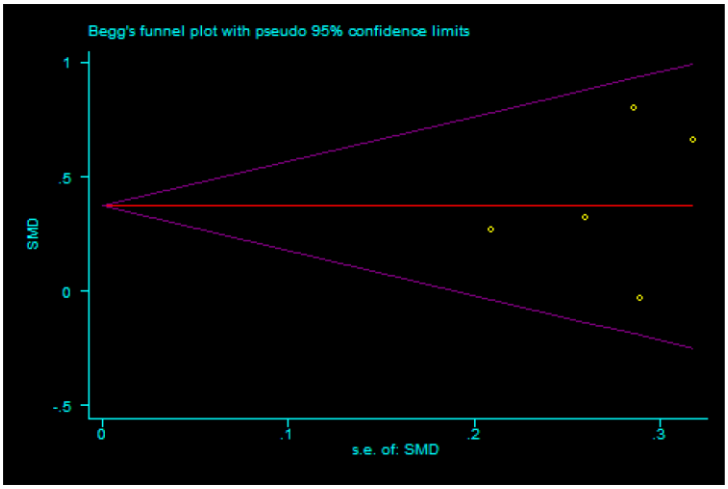


Subgroup by the strains of flora:



Publication bias

Begg's funnel plot and test

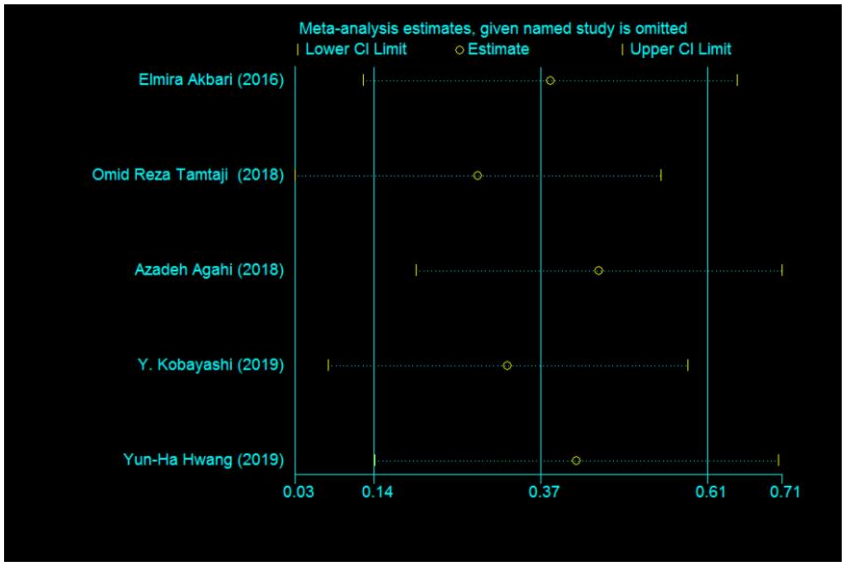


adj. Kendall's Score (P-Q) = 2
Std. Dev. of Score = 4.08
Number of Studies = 5
z = 0.49
Pr > |z| = 0.624
z = 0.24 (continuity corrected)
Pr > |z| = 0.806 (continuity corrected)

Egger's test

Std_Eff	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
slope	-.3018107	.9814531	-0.31	0.779	-3.425232	2.821611
bias	2.586162	3.716345	0.70	0.537	-9.240906	14.41323

Sensitivity analysis (Leave-one-out influence analysis)



Study omitted |	Estimate	[95% Conf. Interval]	
Elmira Akbari (2016)|	.3868553	.1270016	.64670902
Omid Reza Tamtaji (2018)|	.28621155	.03203233	.54039079
Azadeh Agahi (2018)|	.45443958	.2008017	.70807743
Y. Kobayashi (2019)|	.3278729	.07854173	.57720405
Yun-Ha Hwang (2019)|	.42384291	.14358303	.70410275
Combined |	.37365879	.14217712	.60514046

A Research Protocol to Guide the Meta-Analysis of Efficacy of Probiotics in Alzheimer's Disease or Mild Cognitive Impairment Patients Studies

INTRODUCTION

Rationale

The incidence of Alzheimer's disease (AD) is increasing globally and has reached the point of being a costly public health issue [1]. Mild cognitive impairment (MCI) is a syndrome defined as cognitive deterioration that does not compromise daily functioning; however, amnestic MCI has a high risk of progression to AD and has been considered as a typical prodromal stage of AD [2]. Unfortunately, there is currently no curative treatment for cognitive impairment and dementia [3].

The gut microbiota (GM) consists of a vast bacterial community that resides primarily in the lower gut and lives in a symbiotic relationship with the host [4]. Accumulating evidence has demonstrated the close interconnection between the gastrointestinal tract and the brain, known as the gut-brain axis [5]. Recently, the GM has been found to regulate brain development and behavior via the gut-brain axis, and this has been called the microbiota-gut-brain axis (MGB) [6]. It has been found that dysfunction in behavior and cognition are associated with GM dysbiosis [7], and further activation of gut inflammation has been regarded as a possible pathogenic cofactor in cognitive deterioration and dementia [8]. Moreover, decreased abundance of anti-inflammatory bacterial species such as *Bifidobacterium breve* strain A1 and increased abundance of pro-inflammatory flora phyla such as *Firmicutes* and *Bacteroidetes* are the most distinctive alterations in the GM observed in AD patients [9]. Therefore, the GM has been proposed as a key player in the pathogenesis of AD and might be a new potential therapeutic target for the prevention and treatment of AD [10].

Probiotics are live microbes that confer health benefits to the host when administered in adequate amounts [11], possibly through their anti-inflammatory or anti-oxidative effects [12, 13]. Recently, some probiotics have been shown to influence the central nervous system (CNS) and behavior via modulation of the MGB [14]. Moreover, eleven preclinical studies have shown that neither single strains nor multi-strain probiotics were beneficial for improving cognitive function in animal models [15]. These preclinical results have indicated that probiotics supplementation might be an effective dietary intervention to ameliorate age-associated cognitive deficits. Nevertheless, findings from available clinical trials focusing on the effects of probiotics in patients with AD or MCI are inconsistent [16–20]. Additionally, previous relevant reviews mainly focused on the effect of probiotics in neurodegenerative and neurodevelopmental disorders with both animal models and human trials [15, 21]. The evidence for the effects of psychobiotics on mental and neurological conditions/disorders remains limited. Thus, a meta-analysis is needed to clarify the efficacy of probiotics on cognition in adults suffering from AD or MCI, as well as the possible underlying mechanisms. Given the potential importance of such a meta-analysis, this protocol provides an in-depth description of the research objectives as well as the methodological and analytical approaches that will be used to identify, appraise, and synthesize the relevant studies.

Objectives

(1) Conduct a meta-analysis to clarify the efficacy of probiotics on cognition in adults suffering from AD or MCI.
(2) To examine the effect of probiotics on inflammatory and oxidative biomarkers in adults with AD or MCI.

METHODS

Study selection

Eligibility criteria
(1) Adult human participants who had a diagnosis of AD or MCI (aged over 18 y);
(2) The study was an RCT and published in peer-reviewed journals in English;
(3) No constraint on dosage, strain, or form of probiotics

(either stand alone or in combination with other compounds) was applied;
(4) Probiotic interventions had no significant difference in form, appearance, taste, and smell compared to the control interventions;
(5) No restrictions were applied on validated measures of cognitive assessment;
(6) Continuous data at baseline and post-intervention, or the change from baseline, were reported or could be calculated from the data reported in the article.

Exclusion criteria

(1) The publications were abstracts, reviews, conference papers, study protocols, cross-sectional studies, nonhuman (in vitro and animal) studies or papers that did not report on any outcome of interest;

(2) No post-intervention or change from baseline on scale scores of cognition was reported, and these data could not be calculated based on the information in the article; (3) The study reported on a sample that overlapped the sample in another study. In this case, only the study with the larger sample size was included.

Information sources

The preliminary search will be performed through electronic databases including the EMBASE, PubMed, Web of Science and the Cochrane Central Register of Controlled Trials (CENTRAL). And a snowball search will be manually carried out by searching reference lists from relevant published reviews and the retrieved papers.

Search strategy

The search strategy was developed, finalized and adapted for each database with a combination of free text and controlled vocabulary keywords, including the following terms: (Alzheimer's disease OR dementia OR mild cognitive impairment OR cognitive dysfunction OR cognitive defect OR cognition OR memory OR mental capacity) AND (adult OR human) AND (probiotic OR yeast OR yoghurt OR fermented product OR lactobacillus OR bifidobacterium OR fermented dairy product OR synbiotics OR cultured milk products). Predefined search strategy for each electronic databases are as follows:

EMBASE

Search strategy: ('alzheimer disease'/exp OR 'alzeimer disease' OR 'alzeimer`s disease' OR 'alzeimers disease' OR 'alzheimer dementia' OR 'alzheimer disease' OR 'alzheimers disease' OR 'alzheimer fibrillary change' OR 'alzheimer fibrillary lesion'OR 'alzheimer neurofibrillary change' OR 'alzheimer neurofibrillary degeneration' OR 'alzheimer neuron degeneration' OR 'alzheimer perusini disease' OR 'alzheimer sclerosis' OR 'alzheimer syndrome' OR 'alzheimer`s disease' OR 'cortical sclerosis, diffuse' OR 'dementia, alzheimer' OR 'diffuse cortical sclerosis' OR 'late onset alzheimer disease' OR 'dementia'/exp OR 'amentia' OR 'dementia' OR 'demention' OR 'mild cognitive impairment'/exp OR 'amnestic mild cognitive impairment' OR 'mild cognitive impairment' OR 'cognitive defect'/exp OR 'cognition disorder' OR 'cognition disorders' OR 'cognitive defect' OR 'cognitive defects' OR 'cognitive deficit' OR 'cognitive disability' OR 'cognitive disorder' OR 'cognitive disorders' OR 'cognitive impairment' OR 'delirium, dementia, amnestic, cognitive disorders' OR 'overinclusion' OR 'response interference' OR 'cognition'/exp OR 'cognition' OR 'cognitive accessibility'OR 'cognitive balance' OR 'cognitive dissonance' OR 'cognitive function' OR 'cognitive structure' OR 'cognitive symptoms' OR 'cognitive task' OR 'cognitive thinking' OR 'neurobehavioural manifestations' OR 'volition' OR 'memory'/exp OR 'item recall' OR 'memory' OR 'memory function' OR 'nonspatial memory' OR 'remembering' OR 'reminiscence' OR 'mental capacity'/exp OR 'ability, mental' OR 'attainment' OR 'capacity, mental' OR 'fitness, mental' OR 'mental ability' OR 'mental capacity' OR 'mental competency' OR ad OR mci OR 'cognitive fail*' OR 'cognitive decline*' OR 'cognitive impair*' OR alzheimer* OR dement* OR 'cognitive dysfunction' OR 'cognitive performance' OR cognitive) AND ('probiotic agent'/exp OR 'probiotic' OR 'probiotic agent' OR 'probiotics' OR 'yeast'/exp OR 'flora, yeast' OR 'fungi, yeast' OR 'yeast' OR 'yeast fermentation' OR 'yeast flora' OR 'yeast fungus' OR 'yeast germination' OR 'yeast metabolism' OR 'yeasts' OR 'yoghurt'/exp OR 'yoghourt' OR 'yoghurt' OR 'yogurt' OR 'zabadi' OR 'lactobacillus'/exp OR 'betabacterium' OR 'lactobacileae' OR 'lactobacilleae' OR 'lactobacillus' OR 'lactobacteria' OR 'lactobacilli' OR 'bifidobacterium'/exp OR 'bifidobacterium' OR 'fermented dairy product'/exp OR 'cultured dairy foods' OR 'cultured dairy product' OR 'cultured milk foods' OR 'cultured milk product' OR 'cultured milk products' OR 'fermented dairy foods' OR 'fermented dairy product' OR 'fermented milk' OR 'fermented milk product' OR 'fermented product'/exp OR 'fermented food' OR 'fermented foods' OR 'fermented product' OR 'synbiotic agent'/exp OR 'synbiotic' OR 'synbiotic agent' OR 'synbiotics' OR probiotic*) AND ('placebo'/exp OR 'placebo' OR 'placebo gel' OR 'placebos') AND ('randomized controlled trial'/exp OR 'controlled trial, randomized' OR 'randomised controlled study' OR 'randomised controlled trial' OR 'randomized controlled study' OR 'randomized controlled trial' OR 'trial, randomized controlled' OR 'controlled clinical trial'/exp OR 'clinical trial, controlled' OR 'controlled clinical comparison' OR 'controlled clinical drug trial' OR

'controlled clinical experiment' OR 'controlled clinical study' OR 'controlled clinical test' OR 'controlled clinical trial' OR rct OR random* OR control* OR trial*) AND 'human'/de AND ([adult]/lim OR [aged]/lim OR [middle aged]/lim OR [very elderly]/lim OR [young adult]/lim)

PubMed

Search strategy: ((((("Adult"[Mesh]) AND "Humans"[Mesh]) AND ((((((((((((((((("Alzheimer Disease"[Mesh]) OR Alzheimer*) OR AD) OR "Dementia"[Mesh]) OR Dement*) OR mild cognitive impairment) OR MCI) OR ("Cognition"[Mesh] OR "Cognition Disorders"[Mesh])) OR ("Memory"[Mesh] OR "Memory Disorders"[Mesh])) OR "Cognitive Dysfunction"[Mesh]) OR cognitive defect) OR Mental capacity) OR Cognitive) OR cognitive performance) OR cognitive impair*) OR cognitive decline*) OR cognitive fail*)) AND ("Placebos"[Mesh]) AND (((((((((("Probiotics"[Mesh]) OR "Cultured Milk Products"[Mesh]) OR "Yogurt"[Mesh]) OR (("Yeast, Dried"[Mesh]) OR "Yeasts"[Mesh])) OR "Bifidobacterium"[Mesh]) OR "Lactobacillus"[Mesh]) OR "Synbiotics"[Mesh]) OR probiotic*) OR fermented product) OR fermented dairy product)) AND (((((((("Randomized Controlled Trial" [Publication Type] OR "Controlled Clinical Trial" [Publication Type])) OR randomized controlled trials) OR Controlled Clinical Trials) OR RCT) OR random*) OR control*) OR trial*)))))

Cochrane Central Register of Controlled Trials (CENTRAL)

Search strategy:

#1 MeSH descriptor: [Lactobacillus] explode all trees
#2 MeSH descriptor: [Yogurt] explode all trees
#3 MeSH descriptor: [Probiotics] explode all trees
#4 MeSH descriptor: [Bifidobacterium] explode all trees
#5 MeSH descriptor: [Yeasts] explode all trees
#6 MeSH descriptor: [Cultured Milk Products] explode all trees
#7 MeSH descriptor: [Synbiotics] explode all trees
#8 #1 or #2 or #3 or #4 or #5 or #6 or #7
#9 "probiotic*" or "fermented product" or "fermented dairy product"
#10 #8 or #9
#11 adult and humans
#12 MeSH descriptor: [Alzheimer Disease] explode all trees
#13 MeSH descriptor: [Dementia] explode all trees
#14 MeSH descriptor: [Cognitive Dysfunction] explode all trees
#15 MeSH descriptor: [Cognition] explode all trees
#16 MeSH descriptor: [Memory] explode all trees
#17 MeSH descriptor: [Cognition Disorders] explode all trees
#18 MeSH descriptor: [Memory Disorders] explode all trees
#19 Alzheimer* or AD or Dement* or "cognitive" or "mild cognitive impairment" or MCI or "cognitive fail*" or "cognitive performance" or "cognitive impair*" or "cognitive decline*" or "memory function" or "Mental capacity" or "cognitive defect"
#20 #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19
#21 MeSH descriptor: [Randomized Controlled Trial] explode all trees
#22 MeSH descriptor: [Controlled Clinical Trial] explode all trees
#23 RCT or "randomized controlled trials" or "Controlled Clinical Trials" or "random*" or "control*" or "trial*"
#24 #21 or #22 or #23
#25 #10 and #20 and #24
#26 #25 and trails

Web of Science

Search strategy: ('alzheimer disease'/exp OR 'alzeimer disease' OR 'alzeimer`s disease' OR 'alzeimers disease' OR 'alzheimer dementia' OR 'alzheimer disease' OR 'alzheimers disease' OR 'alzheimer fibrillary change' OR 'alzheimer fibrillary lesion'OR 'alzheimer neurofibrillary change' OR 'alzheimer neurofibrillary degeneration' OR 'alzheimer neuron degeneration' OR 'alzheimer perusini disease' OR 'alzheimer sclerosis' OR 'alzheimer syndrome' OR 'alzheimer`s disease' OR 'cortical sclerosis, diffuse' OR 'dementia, alzheimer' OR 'diffuse cortical sclerosis' OR 'late onset alzheimer disease' OR 'dementia'/exp OR 'amentia' OR 'dementia' OR 'demention' OR 'mild cognitive impairment'/exp OR 'amnestic mild cognitive impairment' OR 'mild cognitive impairment' OR 'cognitive defect'/exp OR 'cognition disorder' OR 'cognition disorders' OR 'cognitive defect' OR 'cognitive defects' OR 'cognitive deficit' OR 'cognitive disability' OR 'cognitive disorder' OR 'cognitive disorders' OR 'cognitive impairment' OR 'delirium, dementia, amnestic, cognitive disorders' OR 'overinclusion' OR 'response interference' OR 'cognition'/exp OR 'cognition' OR 'cognitive accessibility'OR 'cognitive balance' OR 'cognitive dissonance' OR 'cognitive function' OR 'cognitive structure' OR 'cognitive symptoms' OR 'cognitive task' OR 'cognitive thinking' OR 'neurobehavioural manifestations' OR 'volition' OR 'memory'/exp OR 'item recall' OR 'memory' OR 'memory function' OR 'nonspatial memory' OR 'remembering' OR 'reminiscence' OR 'mental capacity'/exp OR 'ability, mental' OR 'attainment' OR 'capacity, mental' OR 'fitness, mental' OR 'mental ability' OR 'mental capacity' OR 'mental competency' OR ad OR mci OR 'cognitive fail*' OR 'cognitive decline*'

OR 'cognitive impair*' OR alzheimer* OR dement* OR 'cognitive dysfunction' OR 'cognitive performance' OR cognitive) AND ('probiotic agent'/exp OR 'probiotic' OR 'probiotic agent' OR 'probiotics' OR 'yeast'/exp OR 'flora, yeast' OR 'fungi, yeast' OR 'yeast' OR 'yeast fermentation' OR 'yeast flora' OR 'yeast fungus' OR 'yeast germination' OR 'yeast metabolism' OR 'yeasts' OR 'yoghurt'/exp OR 'yoghourt' OR 'yoghurt' OR 'yogurt' OR 'zabadi' OR 'lactobacillus'/exp OR 'betabacterium' OR 'lactobacileae' OR 'lactobacilleae' OR 'lactobacillus' OR 'lactobacteria' OR 'lactobacilli' OR 'bifidobacterium'/exp OR 'bifidobacterium' OR 'fermented dairy product'/exp OR 'cultured dairy foods' OR 'cultured dairy product' OR 'cultured milk foods' OR 'cultured milk product' OR 'cultured milk products' OR 'fermented dairy foods' OR 'fermented dairy product' OR 'fermented milk' OR 'fermented milk product' OR 'fermented product'/exp OR 'fermented food' OR 'fermented foods' OR 'fermented product' OR 'synbiotic agent'/exp OR 'synbiotic' OR 'synbiotic agent' OR 'synbiotics' OR probiotic*) AND ('randomized controlled trial'/exp OR 'controlled trial, randomized' OR 'randomised controlled study' OR 'randomised controlled trial' OR 'randomized controlled study' OR 'randomized controlled trial' OR 'trial, randomized controlled' OR 'controlled clinical trial'/exp OR 'clinical trial, controlled' OR 'controlled clinical comparison' OR 'controlled clinical drug trial' OR 'controlled clinical experiment' OR 'controlled clinical study' OR 'controlled clinical test' OR 'controlled clinical trial' OR rct OR random* OR control* OR trial*) AND (Human AND adult)

Study records

Data management

All electronic databases citations retrieved using the above search strategy will be imported into Endnote (Endnote X6, Thomson Reuters, San Francisco, CA) to manage and delete duplicate records. Studies retrieved from reference lists of published reviews and retrieved articles will be entered into Microsoft Excel spread sheet for de-duplication and screening.

Selection process

Screening will be performed by screening the titles and abstracts followed by the retrieval and screening of full text articles using the inclusion and exclusion criteria. We will contact the study authors for full text articles with the maximum of three attempts for any articles that we are unable to retrieve. Articles were initially and independently screened for eligibility by two investigators. Any disagreements that arise between the reviewers will be resolved through discussion, or with a third reviewer. Moreover, duplicate and non-eligible articles will be excluded and reasons for exclusion will be recorded. A PRISMA flow chart will be used to trace the overall process.

Data collection process

Two reviewers will extract data from the selected studies independently and crosscheck all articles for accuracy. Disagreements will be resolved by discussion between the reviewers or with a third reviewer.

Data items

We will extract data according to our predetermined form(as shown in Supplementary Table 1), containing five main categories: 1) Basic information of the included studies (e.g., study title, name of first author, year of publication, whether single-center or multicenter, country of study, total sample size); 2) Characteristics of the participants (e.g., gender, age, disease type, diagnostic criteria); 3) Intervention-related variables (e.g., type of strains, dosage, duration); 4) Outcome measurements (e.g., MMSE, TAC, GSH, MDA, hs-CRP, NO); 5) Main findings of each included studies. To perform the meta-analysis, the following data form of outcome assessments are to be extracted: the mean change score along with the associated variance (standard deviation [SD] or standard error of the mean [SEM]). When change scores are not available, the scores (mean ± SD or mean ± SEM) and the number of participants at baseline and post-intervention will be extracted.

Outcomes and prioritization

Primary outcomes

The primary outcomes of this study are the standardized mean differences (SMDs) of change from baseline between probiotics and placebo group, including pooled estimate of cognition, malondialdehyde (MDA), high-sensitivity C-reactive protein (hs-CRP), total glutathione (GSH) and nitric oxide (NO).

Secondary outcomes

Where available, secondary outcomes for this review will include subgroups such as cognitive rating scales (MMSE versus non-MMSE), disease type (AD versus MCI), strains of flora (multiple versus sole).

Risk of bias in individual studies

An assessment of risk of bias will be incorporated into our analysis. By assessing the quality of the studies that will be included in the meta-analysis, we can assess the strength of the body of evidence. In particular, information relating to bias will be extracted from each study during the data extraction process. This assessment will follow the same procedure with the data collection process where disagreements will be resolved by

discussion between the reviewers or with a third reviewer. To facilitate the appraisal of possible risk of bias, paired reviewers will evaluate independently the risk of bias of included RCTs using the tool of the Cochrane Handbook for Systematic Reviews of Interventions, containing the following six criteria: random sequence generation, allocation concealment, blindness of participants and personnel, blindness of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias. Furthermore, to evaluate the risk of bias, reviewers will assess and rate each of the above items into three ratings: "low risk of bias" or "high risk of bias" or "uncertain-risk". In particular, "low risk" indicates that further research is very unlikely to change the confidence of the estimates whereas "high risk" indicates that further research is very likely to change the estimate. The results of the risk of bias assessment will be pooled into Revman 5.3, and generate a "summary of risk of bias assessment" table.

Data synthesis

We will use STATA software (version 12; StataCorp) to perform the statistical analyses. The primary outcomes of this study are the standardized mean differences (SMDs) of change from baseline between probiotics and placebo group. The SMDs will be tested by a Z statistic, and a two-tailed $P < 0.05$ will be regarded as statistically significant. To determine the extent of variation between the selected studies, tests of heterogeneity will be performed. The inter-study heterogeneity was examined by chi-square (χ^2) statistics and I^2 statistics. Higgins and Colleagues provided tentative benchmarks for I^2 where values below 25% might be considered as low, 50-75% as moderate and above 75% as high. In our study, the heterogeneity among the different studies was considered high if $P < 0.1$ for the χ^2 statistic or $I^2 > 50\%$ [22]. A random-effects model was used if significant heterogeneity was shown among trials. Otherwise, the results were obtained from a fixed-effects model. Subgroup analyses will be performed to examine the possible source of heterogeneity within these studies.

Meta-bias(es)

We will use STATA software (version 12; StataCorp) to perform sensitivity analysis and publication bias. A sensitivity analysis will be conducted to test the reliability of the findings using the leave-one-out method, while publication bias will be assessed by Egger's test and Begg's test.

AUTHOR CONTRIBUTIONS

(1) Research planning and design: Mingliang Chen and Haoyue Deng; (2) Feasibility testing of the study: Mingliang Chen, Haoyue Deng, Xunhu Dong and Zhongmin Zou; (3) Conceptualization: Mingliang Chen and Haoyue Deng; (4) Methodology: Mingliang Chen, Haoyue Deng, Xunhu Dong; (5) Writing – original draft: Haoyue Deng; (6) Writing – review & editing: Mingliang Chen, Zhongmin Zou; (7) Supervision: Mingliang Chen, Zhongmin Zou.

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Supplementary Table

Please browse Full Text version to see the data of Supplementary Table 1.

Supplementary Table 1. Main characteristics of the included studies.