eTable 1. Applied keywords and the search results from each database

Database	Keywords	Filter	Date (yyyy/mm/dd)	Result
PubMed	(adhd OR hkd OR add OR Attention deficit hyperactivity disorder OR attention	RCT	2023/04/06	45
	deficit OR hyperactivity OR inattentive OR impulsivity OR neurodevelopmental			
	disorder) and (probiotics OR gut microbiota OR gut-brain axis OR bifidobacteria			
	OR lactobacillus)			
Embase	(adhd OR hkd OR add OR Attention deficit hyperactivity disorder OR attention	RCT	2023/04/06	440
	deficit OR hyperactivity OR inattentive OR impulsivity OR neurodevelopmental			
	disorder) and (probiotics OR gut microbiota OR gut-brain axis OR bifidobacteria			
	OR lactobacillus)			
Cochrane	(adhd OR hkd OR add OR Attention deficit hyperactivity disorder OR attention	Trials	2023/04/06	181
CENTRAL	deficit OR hyperactivity OR inattentive OR impulsivity OR neurodevelopmental			
	disorder) and (probiotics OR gut microbiota OR gut-brain axis OR bifidobacteria			
	OR lactobacillus)			
ScienceDirect	(Attention deficit hyperactivity disorder OR inattentive OR neurodevelopmental	Research	2023/04/06	306

	disorder) and (probiotics)	articles		
ClinicalTrials.gov	(Attention deficit hyperactivity disorder) and (Probiotics)	NA	2023/04/06	6

Abbreviations: NA, not applied; RCT, randomized controlled trial

eTable 2 Reasons for study exclusion

Reason	Number of excluded studies	References
No probiotics treatment group	3	[1-3]
Not targeting children and adolescents	5	[4-8]
No behavioral outcome for symptoms of ADHD	12	[9-20]
Not Randomized	2	[21, 22]
Not clinical trials	3	[23-25]

ADHD Attention deficit hyperactivity disorder

RCT randomized controlled trials

## References

- [1] Wang J, Liu Y, Huang HY, Wu JT, Wang WJ. [Influence of acupuncture on the clinical manifestations and gastrointestinal symptoms of children with autism spectrum disorder]. Zhongguo zhen jiu = Chinese acupuncture & moxibustion. 2022;42(12):1373-6. https://doi.org/10.13703/j.0255-2930.20220111-0004.
- [2] Singh I, Behera DP, T KA, Gupta S. Efficacy and safety of tamsulosin vs its combination with mirabegron in the management of lower urinary tract non-neurogenic overactive bladder symptoms (OABS) because of Benign Prostatic Enlargement (BPE)-An open label randomised controlled clinical study. Int J Clin Pract. 2021;75(7):e14184. https://doi.org/10.1111/ijcp.14184.
- [3] Smits MM, Fluitman KS, Herrema H, Davids M, Kramer MHH, Groen AK, et al. Liraglutide and sitagliptin have no effect on intestinal

- microbiota composition: A 12-week randomized placebo-controlled trial in adults with type 2 diabetes. Diabetes Metab. 2021;47(5):101223. https://doi.org/10.1016/j.diabet.2021.101223.
- [4] Zhang X, Yang H, Zhang K, Zhang J, Lu X, Guo H, et al. Effects of exercise or tai chi on Internet addiction in college students and the potential role of gut microbiota: A randomized controlled trial. Journal of affective disorders. 2023;327:404-15. https://doi.org/10.1016/j.jad.2023.02.002.
- [5] Schaub AC, Schneider E, Vazquez-Castellanos JF, Schweinfurth N, Kettelhack C, Doll JPK, et al. Clinical, gut microbial and neural effects of a probiotic add-on therapy in depressed patients: a randomized controlled trial. Transl Psychiatry. 2022;12(1):227. <a href="https://doi.org/10.1038/s41398-022-01977-z">https://doi.org/10.1038/s41398-022-01977-z</a>.
- [6] Carlos LO, Ramos MRZ, Wagner NRF, Freitas LAC, Felicidade I, Campos ACL. PROBIOTIC SUPPLEMENTATION ATTENUATES BINGE EATING AND FOOD ADDICTION 1 YEAR AFTER ROUX-EN-Y GASTRIC BYPASS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL. Arquivos brasileiros de cirurgia digestiva : ABCD = Brazilian archives of digestive surgery. 2022;35:e1659. https://doi.org/10.1590/0102-672020210002e1659.
- [7] Slykerman RF, Kang J, Van Zyl N, Barthow C, Wickens K, Stanley T, et al. Effect of early probiotic supplementation on childhood cognition, behaviour and mood a randomised, placebo-controlled trial. Acta paediatrica (Oslo, Norway: 1992). 2018;107(12):2172-8. <a href="https://doi.org/10.1111/apa.14590">https://doi.org/10.1111/apa.14590</a>.
- [8] Cardona D, Roman P, Cañadas F, Sánchez-Labraca N. The Effect of Multiprobiotics on Memory and Attention in Fibromyalgia: A Pilot Randomized Controlled Trial. Int J Environ Res Public Health. 2021;18(7). <a href="https://doi.org/10.3390/ijerph18073543">https://doi.org/10.3390/ijerph18073543</a>.
- [9] Yang LL, Stiernborg M, Skott E, Xu J, Wu Y, Landberg R, et al. Effects of a Synbiotic on Plasma Immune Activity Markers and Short-Chain Fatty Acids in Children and Adults with ADHD-A Randomized Controlled Trial. Nutrients. 2023;15(5). https://doi.org/10.3390/nu15051293.
- [10] Yamanbaeva G, Schaub AC, Schneider E, Schweinfurth N, Kettelhack C, Doll JPK, et al. Effects of a probiotic add-on treatment on fronto-limbic brain structure, function, and perfusion in depression: Secondary neuroimaging findings of a randomized controlled trial. Journal of affective disorders. 2023;324:529-38. https://doi.org/10.1016/j.jad.2022.12.142.

- [11] Sherman HT, Liu K, Kwong K, Chan ST, Li AC, Kong XJ. Carbon monoxide (CO) correlates with symptom severity, autoimmunity, and responses to probiotics treatment in a cohort of children with autism spectrum disorder (ASD): a post-hoc analysis of a randomized controlled trial. BMC Psychiatry. 2022;22(1):536. <a href="https://doi.org/10.1186/s12888-022-04151-3">https://doi.org/10.1186/s12888-022-04151-3</a>.
- [12] Li YQ, Sun YH, Liang YP, Zhou F, Yang J, Jin SL. Effect of probiotics combined with applied behavior analysis in the treatment of children with autism spectrum disorder: a prospective randomized controlled trial. Zhongguo Dang Dai Er Ke Za Zhi. 2021;23(11):1103-10. https://doi.org/10.7499/j.issn.1008-8830.2108085.
- [13] Zhang L, Xu Y, Li H, Li B, Duan G, Zhu C. The role of probiotics in children with autism spectrum disorders: A study protocol for a randomised controlled trial. PLoS One. 2022;17(2):e0263109. https://doi.org/10.1371/journal.pone.0263109.
- [14] Park SK, Kang SB, Kim S, Kim TO, Cha JM, Im JP, et al. Additive effect of probiotics (Mutaflor) on 5-aminosalicylic acid therapy in patients with ulcerative colitis. The Korean journal of internal medicine. 2022;37(5):949-57. https://doi.org/10.3904/kjim.2021.458.
- [15] Pärtty A, Kalliomäki M, Wacklin P, Salminen S, Isolauri E. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. Pediatric research. 2015;77(6):823-8.

  <a href="https://doi.org/10.1038/pr.2015.51">https://doi.org/10.1038/pr.2015.51</a>.
- [16] Chou IC, Kuo HT, Chang JS, Wu SF, Chiu HY, Su BH, et al. Lack of effects of oral probiotics on growth and neurodevelopmental outcomes in preterm very low birth weight infants. J Pediatr. 2010;156(3):393-6. https://doi.org/10.1016/j.jpeds.2009.09.051.
- [17] Firmansyah A, Dwipoerwantoro PG, Kadim M, Alatas S, Conus N, Lestarina L, et al. Improved growth of toddlers fed a milk containing synbiotics. Asia Pac J Clin Nutr. 2011;20(1):69-76.
- [18] Akar M, Eras Z, Oncel MY, Arayici S, Guzoglu N, Canpolat FE, et al. Impact of oral probiotics on neurodevelopmental outcomes in preterm infants. J Matern Fetal Neonatal Med. 2017;30(4):411-5. <a href="https://doi.org/10.1080/14767058.2016.1174683">https://doi.org/10.1080/14767058.2016.1174683</a>.
- [19] Billeci L, Callara AL, Guiducci L, Prosperi M, Morales MA, Calderoni S, et al. A randomized controlled trial into the effects of probiotics on electroencephalography in preschoolers with autism. Autism. 2022;27(1):117-32. https://doi.org/10.1177/13623613221082710.
- [20] Sanctuary MR, Kain JN, Chen SY, Kalanetra K, Lemay DG, Rose DR, et al. Pilot study of probiotic/colostrum supplementation on gut

- function in children with autism and gastrointestinal symptoms. PLoS One. 2019;14(1):e0210064. https://doi.org/10.1371/journal.pone.0210064.
- [21] Wang LJ, Yang CY, Kuo HC, Chou WJ, Tsai CS, Lee SY. Effect of Bifidobacterium bifidum on Clinical Characteristics and Gut Microbiota in Attention-Deficit/Hyperactivity Disorder. J Pers Med. 2022;12(2). https://doi.org/10.3390/jpm12020227.
- [22] Wallace CJK, Milev RV. The Efficacy, Safety, and Tolerability of Probiotics on Depression: Clinical Results From an Open-Label Pilot Study. Frontiers in Psychiatry. 2021;12.
- [23] Wang L, Cai Y, Garssen J, Henricks PAJ, Folkerts G, Braber S. The Bidirectional Gut-Lung Axis in COPD. Am J Respir Crit Care Med. 2023. https://doi.org/10.1164/rccm.202206-1066TR.
- [24] Rianda D, Agustina R, Setiawan EA, Manikam NRM. Effect of probiotic supplementation on cognitive function in children and adolescents: a systematic review of randomised trials. Beneficial microbes. 2019;10(8):873-82. <a href="https://doi.org/10.3920/bm2019.0068">https://doi.org/10.3920/bm2019.0068</a>.
- [25] Kalenik A, Kardaś K, Rahnama A, Sirojć K, Wolańczyk T. Gut microbiota and probiotic therapy in ADHD: A review of current knowledge. Prog Neuropsychopharmacol Biol Psychiatry. 2021;110:110277. <a href="https://doi.org/10.1016/j.pnpbp.2021.110277">https://doi.org/10.1016/j.pnpbp.2021.110277</a>.

**eTable 3.** Grading of Recommendations Assessments, Development and Evaluation (GRADE) assessment of the strength of evidence for standard weighted meta-analysis

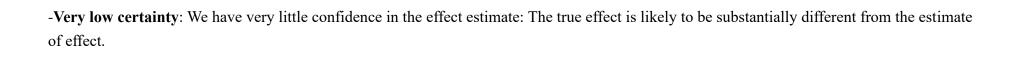
Outcome	Design	Risk of	Indirectness	Inconsistency	Imprecision	Publication bias	Grade Quality
		bias					
Total symptoms	RCT x 7	No	Serious	Serious	No serious	Serious	$\oplus$ OOO <sup>2,3,5</sup>
Total symptoms		serious	indirectness	inconsistency	imprecision	publication bias	
Inattention	RCT x 4	No	Serious	No serious	No serious	Serious	$\oplus \oplus OO^{2,5}$
mattention		serious	indirectness	inconsistency	imprecision	publication bias	
Hymanativity/immylaivity	RCT x 4	No	Serious	No serious	No serious	Serious	$\oplus \oplus OO^{2,5}$
Hyperactivity/impulsivity		serious	indirectness	inconsistency	imprecision	publication bias	
Numbers of dropout	RCT x 5	No	No serious	No Serious	Serious	Serious	$\oplus \oplus OO^{4,5}$
indifficers of dropout		serious	indirectness	inconsistency	imprecision	publication bias	

<sup>\*</sup>Risk of bias was estimated using Cochrane risk of bias, studies were classified as having low risk of bias if none of the domains above was rated as high risk of bias and three or less were rated as unclear risk; moderate if one was rated as high risk of bias or none was rated as high risk of bias but four or more were rated as unclear risk, and all other cases were assumed to pertain to high risk of bias.

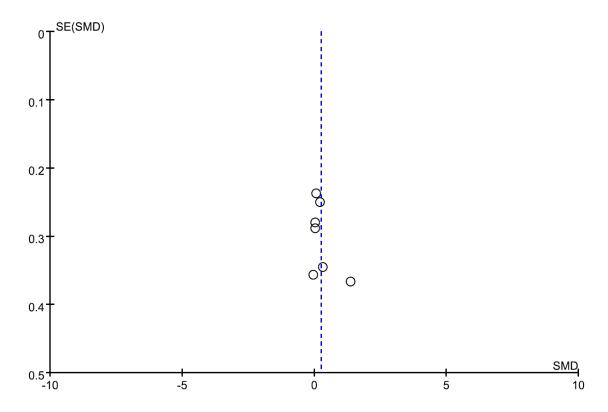
Down-graded due to: <sup>1</sup> risk of bias, <sup>2</sup> indirectness, <sup>3</sup> Inconsistency, <sup>4</sup> Imprecision, <sup>5</sup> publication bias

## **GRADE** Working Group grades of evidence:

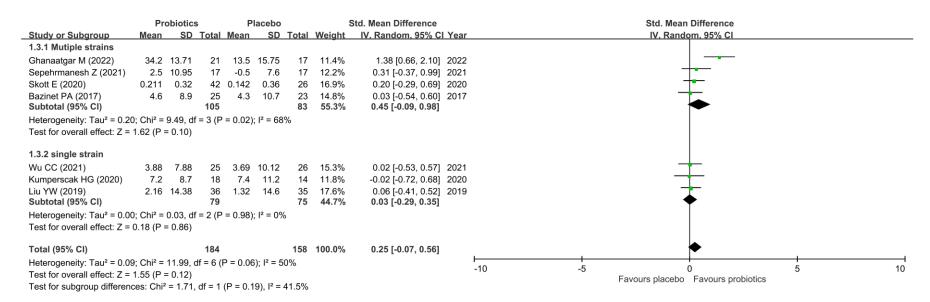
- -High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
- -Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- -Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.



eFigure 1. Funnel plot – ADHD total



eFigure 2. Subgroup analysis - forest plot of effect sizes in subgroup of studies using multiple strains of probiotics versus those using a single strain



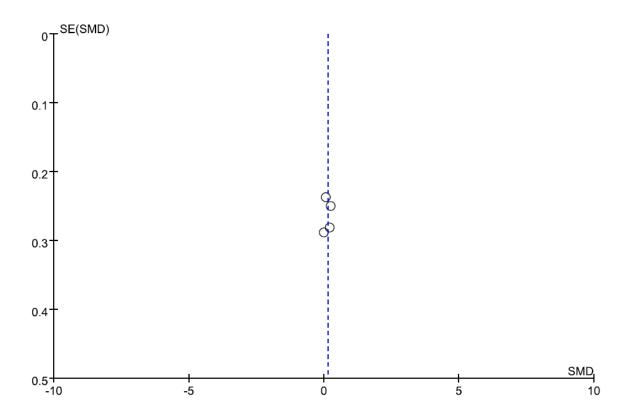
eFigure 3. Forest plot of effect size for comparing the difference in the improvement of inattention between probiotics and placebo groups

	Expe	rimen	tal	С	ontrol		;	Std. Mean Difference		Std. Mear	ı Diffe	rence	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV, Rand	<u>om, 95</u>	5% CI	
Bazinet PA (2017)	-24.1	5.5	25	-24	5.75	23	20.5%	-0.02 [-0.58, 0.55]			<b>†</b>		
Liu YW (2019)	0.79	5.87	36	0.44	4.71	35	30.4%	0.06 [-0.40, 0.53]			•		
Skott E (2020)	0.202	0.31	42	0.115	0.38	26	27.3%	0.25 [-0.24, 0.75]			<b>†</b>		
Wu CC (2021)	2.04	3.27	25	1.27	3.21	26	21.7%	0.23 [-0.32, 0.79]			†		
Total (95% CI)			128			110	100.0%	0.14 [-0.12, 0.39]			•		
Heterogeneity: Tau <sup>2</sup> = Test for overall effect:				3 (P =	0.87);	I <sup>2</sup> = 0%			-10	-5 Favours placebo	0 Favo	5 ours probiotics	10

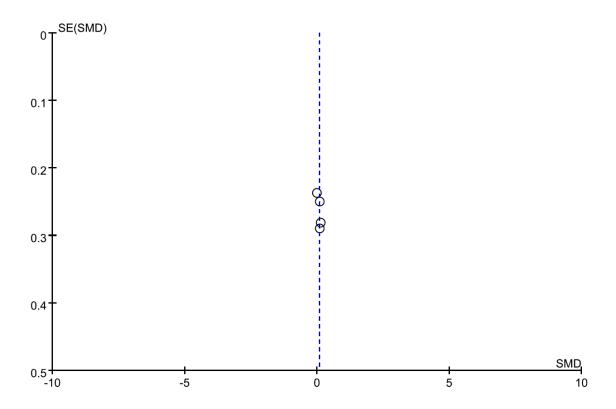
eFigure 4. Forest plot of effect size for comparing the difference in the improvement of hyperactivity/impulsivity between probiotics and placebo groups

	Expe	erimen	ıtal	С	ontrol		,	Std. Mean Difference		Std.	Mean Differe	nce	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV,	Random, 95%	6 CI	
Bazinet PA (2017)	-21.4	4.5	25	-21.9	4.8	23	20.5%	0.11 [-0.46, 0.67]			+		
Liu YW (2019)	0.65	5.38	36	0.72	6.22	35	30.4%	-0.01 [-0.48, 0.45]			*		
Skott E (2020)	0.213	0.42	42	0.172	0.41	26	27.4%	0.10 [-0.39, 0.59]			+		
Wu CC (2021)	1.76	3.74	25	1.12	4.09	26	21.7%	0.16 [-0.39, 0.71]			<b>†</b>		
Total (95% CI)			128			110	100.0%	0.08 [-0.18, 0.34]			•		
Heterogeneity: Tau <sup>2</sup> = Test for overall effect:				: 3 (P =	0.97);	I <sup>2</sup> = 0%	1		-10 Favo	-5 ours [experim	0 ental] Favou	5 rs [control]	10

eFigure 5. Funnel plot – inattention



eFigure 6. Funnel plot – hyperactivity/impulsivity



eFigure 7. Forest plot of odds ratio for comparing the difference in the numbers of dropout between probiotics and placebo groups

Probiotics		s Place	bo	Odds Ratio			Odds Ratio
Study or Subgroup	Events To	otal Events	Total	Weight	M-H, Fixed, 95% CI Yea	ar	M-H, Fixed, 95% CI
Ghanaatgar M (2022)	4	25 8	25	32.6%	0.40 [0.10, 1.58] 202	22 —	
Sepehrmanesh Z (2021)	3	20 3	20	12.4%	1.00 [0.18, 5.67] 202	21	<del></del>
Wu CC (2021)	0	28 0	29		Not estimable 202	21	
Kumperscak HG (2020)	0	21 0	14		Not estimable 202	20	
Skott E (2020)	23	65 7	34	28.8%	2.11 [0.80, 5.60] 202	20	-
Liu YW (2019)	3	39 6	41	26.2%	0.49 [0.11, 2.10] 201	9	•
Total (95% CI)	1	198	163	100.0%	0.99 [0.54, 1.83]		
Total events	33	24					
Heterogeneity: Chi <sup>2</sup> = 4.89,	df = 3 (P = 0)	$(0.18); I^2 = 399$	6			<u> </u>	0.2 0.5 1 2 5 10
Test for overall effect: Z = 0	0.03 (P = 0.98	8)				0.1	0.2 0.5 1 2 5 10 Favours probiotics Favours placebo

eFigure 8. Funnel plot – dropout

